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Uterine blood flow in sows: Effects of pregnancy stage and litter size

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Abstract — Female pigs were assigned to three groups at 94 days of age: a control group (CTR), a group undergoing the ligation and severing of the left oviduct (LIG), and a group undergoing right hysteroovariectomy (HHO). They were inseminated at 307 days of age. At 35 days of pregnancy, an ultrasonic transit time flow probe was implanted around the middle artery of one uterine horn in 33 sows and uterine blood flow was measured during thirteen 24-h periods between 44 and 111 days. Despite large differences in ovulation rate per uterine horn (4.8, 8.3 and 16.9 in the LIG, CTR and HHO groups, respectively), variation of litter size was considerably reduced with advancement of pregnancy (3.0, 6.6 and 10.8 foetuses per uterine horn at 35 days, and 3.0, 5.8 and 4.9 at 112 days (slaughter), respectively). Uterine blood flow increased linearly during pregnancy. It was lower in the LIG sows (0.82 to 1.74 $L{\cdot}min^{-1}{\cdot}horn^{-1}$ from 44 to 111 days) than in the CTR and HHO sows (1.22 to 2.84 and 1.09 to 2.63 $L{\cdot}min^{-1}{\cdot}horn^{-1}$, respectively). It was more closely related to litter weight than to litter size and amounted to 0.42 $L{\cdot}min^{-1}{\cdot}kg\ foetus^{-1}$ at 111 days. Uterine blood flow per foetus decreased when litter size increased. It increased from 0.31 to 0.72, 0.26 to 0.60 and 0.20 to 0.43 $L{\cdot}min^{-1}{\cdot}foetus^{-1}$ from 44 to 111 days when there were 2 to 3, 4 to 5, and 6 to 8 foetuses in the uterine horn, respectively. This explains why piglets from large litters are lighter at birth.

sow / pregnancy / uterus / blood flow / foetus / litter size

Résumé — **Débit sanguin dans l'utérus chez la truie : effets du stade de gestation et de la taille de la portée.** Trois lots de cochettes ont été constitués à 94 jours d'âge : CTR, lot témoin ; LIG, ligature de l'oviducte gauche ; HHO, hémi-hystéro-ovariectomie droite. Elles ont été inséminées à 307 jours. À 35 jours de gestation, une sonde de mesure du débit par ultrasons a été implantée sur l'artère principale d'une corne utérine de 33 d'entre elles, et le débit sanguin utérin a été mesuré pendant 13 périodes de 24 h entre 44 et 111 jours de gestation. En dépit de différences importantes dans le nombre d'ovulations (4,8, 8,3 et 16,9 corps jaunes par corne utérine respectivement dans les lots LIG, CTR et HHO), la variabilité de la taille de la portée se réduit considérablement pendant la gestation (respectivement 3,0, 6,6 et 10,8 fœtus par corne utérine à 35 jours, et 3,0, 4,8 et 4,9

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à l'abattage à 112 jours). Le débit sanguin utérin augmente de façon linéaire pendant la gestation. Il est plus faible chez les truies LIG (0,82 à 1,74 $L \cdot min^{-1} \cdot corne^{-1}$ entre 44 et 111 jours) que chez les truies CTR et HHO (respectivement 1,22 à 2,84 et 1,09 à 2,63 $L \cdot min^{-1}$·corne utérine^{-1}). Il est plus étroitement lié au poids de la portée qu'à sa taille, et s'élève à 0,42 $L \cdot min^{-1} \cdot kg$ fœtus^{-1} à 111 jours. Le débit sanguin utérin par fœtus diminue lorsque la taille de la portée augmente. Il s'accroît de 0,31 à 0,72, de 0,26 à 0,60 et de 0,20 à 0,43 $L \cdot min^{-1}$·fœtus^{-1} entre 44 et 111 jours respectivement pour des effectifs de 2 à 3, 4 à 5, et 6 à 8 fœtus dans la corne utérine. Ces résultats expliquent que les porcelets de portées importantes soient plus légers à la naissance.

truie / gestation / utérus / débit sanguin / fœtus / taille de portée

1. INTRODUCTION

Sows seem to be able to ensure the development of only a limited number of foetuses until term, and most of the supernumerary foetuses die. The limitation of litter size, called uterine capacity [3], is characteristic of the genotype and of the animal. For instance, the efficiency of the uterus is higher in hyperprolific Large-White × Meishan than in standard Large White sows [32]. It is also higher in multiparous than in primiparous sows. According to Knight et al. [31], the foetuses that exceed uterine capacity die after day 25 of pregnancy. Available space in the uterus [44] and competition between foetuses for some biochemical factors [4] were proposed as mechanisms involved in the uterine capacity.

All the substances essential for foetal development are carried by maternal blood and taken up by the pregnant uterus. The amount of substrates reaching the foetuses depends on several factors including their concentration in maternal blood and uterine blood flow (UBF). Some experiments show that the restriction of blood flow to the uterus reduces foetal development and (or) survival [27, 38, 43]. The blood supplied to the uterus was found to limit litter size in mice [53]. The present experiment was undertaken to determine to what extent UBF is involved in uterine capacity in sows. UBF has been determined in few experiments and during relatively short periods in sows [24, 26, 48]. Variation of UBF with pregnancy stage and litter size was then studied.

2. MATERIALS AND METHODS

2.1. Animals and diets

The experiment was a part of a larger study involving 114 Large White gilts in which the effect of the number of pig embryos on their survival and development was studied [40]. Gilts were fed a diet containing 3.1 Mcal of $DE \cdot kg^{-1}$, 17.6% crude protein, and 0.85% lysine during the growth period. The animals were housed in groups of five and fed individually. Feed allowance progressively increased from 2 $kg \cdot day^{-1}$ between 35 and 40 kg live weight to a maximum of 2.7 $kg \cdot day^{-1}$ between 65 and 100 kg live weight, and 2.5 $kg \cdot day^{-1}$ thereafter. During pregnancy, gilts were fed 2.5 $kg \cdot day^{-1}$ of a diet based on cereals and soybean oil meal containing 3.0 Mcal of $DE \cdot kg^{-1}$, 13.1% crude protein, and 0.65% lysine.

2.2. Experimental procedure and measurements

The gilts were assigned to the experiment in three replicates. They were allotted to three treatments according to litter origin and live weight. Littermates were split between treatments. The treatments were applied in order to have different potential numbers of embryos per uterine horn. The experimental groups were as follows: control group (CTR); a group in which the left oviduct was ligated and severed (LIG); a group in which a unilateral, right-side hysteroovariectomy was performed (HHO). Surgeries were performed at 94 ± 6 days of age and 38 ± 6 kg live weight (mean ± sd) under general anaesthesia maintained with 2 to 5% halothane (Fluothane, Pitman-Moore, 77100 Meaux, France).

The gilts were inseminated with semen from Large White boars at 307 ± 12 days of age and 161 ± 6 kg live weight after oestrous synchronisation (Regumate, provided by Roussel-Uclaf, Paris, France). At 35 ± 1 days of gestation, a median laparotomy was performed under general anaesthesia on 25 LIG, 32 CTR, and 27 HHO gilts. They were premedicated with atropine (20 $mg \cdot kg^{-1}$ live weight given i.v.), and anaesthesia was then induced with sodium thiopenthal (10 $mg \cdot kg^{-1}$ live weight given i.v.). Anaesthesia was maintained with 2 to 5% halothane in oxygen (3 $L \cdot min^{-1}$). A 20-cm midline incision was made through the abdomen, and the uterine horns were gently exteriorised. Ovulation rate was determined, and foetuses were counted by palpation of the uterine horns. Thirty-three gilts were selected according to the number of foetuses in one of the uterine horns: 2 to 4 in the LIG group ($n = 11$), 8 to 15 in the HHO group ($n = 13$), and an intermediary number in the CTR group ($n = 9$). An ultrasonic transit time flow probe (8 RS probe, Transonic Systems Inc., Ithaca, NY, USA) was implanted around the middle artery irrigating one of the two uterine horns. With wide-beam ultrasonic illumination, the receiving transducers of these probes integrate the blood velocity over the vessel's full width and yield volume flow. Unlike the ultrasonic Doppler flow velocity technique, the ultrasonic transit time technique then directly measures true flow and not blood velocity. It was shown that blood flow measured with this technique is independent of vessel diameter, vessel shape, flow profile, or vessel alignment within the probe [15, 16]. This technique then allows the measurement of blood flow in growing vessels as is the case in the present experiment. The 8 RS flow probes are adapted to chronic measurements of blood flow lower than 10 $L \cdot min^{-1}$ in vessels with diameters contained between 4.4 and 8 mm. The cable of the flow probe (2 m length) was covered with silicone tubing. A 2-cm segment of the middle uterine artery supplying one uterine horn was dissected to separate it from the uterine vein. The flow probe was implanted around the artery and secured within the broad ligament with a decimal 2 polypropylene suture (Ethnor, 92200 Neuilly, France). The peritoneum was then sutured with plaited decimal 3 polyester (Braun, 92107 Boulogne, France). Oxytetracyclin (5 $mg \cdot kg^{-1}$ live weight, Pfizer, 94107 Orsay, France) was injected into the peritoneal cavity. Decimal 8 plaited polyester (Braun) was used to suture the opening in the muscles. The cable of the probe (2 m long) was then tunnelled under the skin using a stainless steel trocar 50 cm long, and externalised through the skin of the back, at the level of the lumbar vertebrae. In order to prevent infection along the cable, a 5 × 5 cm piece of Dacron® material was rolled around the silicone tubing of the cable at the level of the exit site. It was fixed to the cable with sterile tissue adhesive (Histoacryl®, Braun) and two silicone rings, and introduced under the skin. The external part of the cable was wrapped up in a tissue bag sutured on the skin. The skin was sutured with decimal 3 plaited polyester (Braun). Postsurgical antibiotic therapy consisted of daily i.m. injections of Ampicillin (10 $mg \cdot kg$ live $weight^{-1} \cdot d^{-1}$) during three days.

Blood flow measurements began about one week after surgery. 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 flow meter (model T206, Transonic Systems Inc.)

which allows for about 170 measurements per second. The flowmeter was connected to a microcomputer with an application program that integrates and stores the measurements during 5-min periods. UBF was measured during 24-h periods once per week from 44 to 98 days of pregnancy and twice per week thereafter. Sows were slaughtered at 112 ± 1 days of pregnancy. Genital tracts were recovered and dissected. Ovulation rate, position in the uterine horns and weight of each foetus and placenta were recorded. Age at death of mummified foetuses was also estimated. The arteries of the broad ligament were dissected in order to ensure that the flow probe was correctly implanted and that the total blood flow supplying the uterine horn was measured. Length and weight of the uterine horns were determined after dissection of the broad ligament.

2.3. Calculations and statistical analysis

All calculations were made on the uterine horn bearing a flow probe. Number of foetuses in the uterine horn, litter weight and mean piglet weight at slaughter were determined. The mean weight of the foeto-placental unit (FPU) corresponding to the mean weight of the foetus plus uteroplacenta, and the ratio between uterine horn weight or length and number of foetuses in the uterine horn were calculated.

The mean blood flow was calculated for each 24-h measurement. Blood flow at reference stages (44, 50, 57, 64, 71, 78, 84, 91, 98, 101, 105, 108 and 111 days) was calculated through linear interpolation between the real measurement stages. The reference stages corresponded to the more frequent real stages of measurement. The results were expressed according to two classifications: the experimental group, and the litter size at slaughter in the uterine horn bearing a flow probe. Three classes of litter size were defined in order to include similar numbers of sows: less than four foetuses (2–3 class), four or five foetuses (4–5 class) and more than five foetuses (6–8 class).

Blood flow was calculated per uterine horn and per foetus found at term at all stages of pregnancy, and per kg foetus or FPU at slaughter. Statistical effects of treatment and litter size on UBF were tested at each reference stage as well as the evolution of blood flow during gestation. Data were analysed by analysis of variance, using the GLM procedure of SAS [52]. The model used for the effects on the measurements made at slaughter after dissection of the reproductive tract and on UBF at each pregnancy stage included class (group or litter size) and replicate as the main effects. Means of the classes were then separated by F-protected LSD. The variation of UBF during gestation was analysed according to the repeated measurement procedure of SAS [52] including the effects of class, replicate, and class × replicate interaction. Rates of variation of blood flow with pregnancy stage were calculated by covariance analysis with sow and class as main effects and pregnancy stage as a covariate, according to SAS [52] specifications.

3. RESULTS

Flow probes were implanted in 33 gilts. Two gilts broke the cable and three others aborted within a few days after surgery. As a result, uterine blood flow was measured in 28 gilts (8 CTR, 10 LIG, and 10 HHO).

3.1. Reproductive performance, uterus and uterine contents

Reproductive performance and characteristics of the uterine horns bearing flow probes at 112 days of gestation are presented in Table I. The group × replicate interaction was never significant. Ovulation rate and number of foetuses at 35 days of pregnancy differed significantly between the three groups. They were higher in the HHO group,

Table I. Reproductive performance and characteristics of the uterine horn bearing a flowprobe.

Item	Group[a] LIG	CTR	HHO	RMSE[b]	Statistical significance[c]	
Ovulation rate per uterine horn	4.75[d]	8.31[e]	16.91[f]	1.66	G^{**}	R+
Number of foetuses at 35 days	3.00[d]	6.63[e]	10.80[f]	1.61	G^{***}	
Number of foetuses at 112 days	3.00[d]	5.75[e]	4.91[e]	1.22	G^{***}	R+
Measurements at 112 days of gestation						
Litter weight, kg	4.65[d]	8.04[e]	5.63[d]	1.36	G^{***}	R^{*}
Mean piglet weight, kg	1.56[d]	1.43[d]	1.17[e]	0.25	G^{*}	
Mean FPU weight, kg	2.66[d]	2.28[d, e]	1.96[e]	0.49	G^{*}	
Total placental weight, kg	0.95[d]	1.85[e]	1.14[d]	0.40	G^{**}	R^{**}
Placental weight·kg foetus^{-1}, kg	0.20	0.23	0.20	0.04		R^{*}
Uterine horn weight, kg	2.19[d]	2.94[e]	2.49[d, e]	0.49	G+	R^{***}
Uterine horn length, m	1.66[d]	1.92[d, e]	2.08[e]	0.39	G^{*}	R^{*}
Uterus weight·foetus^{-1}, kg	0.77[d]	0.52[e]	0.55[e]	0.20	G^{*}	
Uterus length·foetus^{-1}, m	0.58	0.36	0.47	0.20		

[a] LIG: left oviduct ligated and severed; CTR: control; HHO: right hysteroovariectomy.
[b] Root mean square error.
[c] Statistical significance: G, group effect; R, replicate effect. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$.
[d, e, f] Means within a row with a different superscript letter differ significantly.

and lower in the LIG group. Litter size did not vary between 35 and 112 days in the LIG group, but decreased in the other groups, particularly in the HHO gilts. Indeed, the difference between the CTR and HHO groups was no longer significant before term, and the difference found earlier with the LIG group was reduced. The weights of litter and placentas in the uterine horn were higher in the CTR group, and did not differ between the HHO and LIG groups. Piglets in the HHO gilts were significantly lighter than in the CTR and LIG gilts (minus 18 and minus 25%, respectively). The hierarchy between the three groups was the same for the FPU weight. Placental weight per kg foetus was similar in the three groups. Uterine horns in the LIG gilts were lighter than in the CTR gilts and shorter than in the HHO gilts, but uterine weight per foetus was higher than in the other groups. The length of the uterus per foetus did not differ significantly between the groups.

3.2. Uterine blood flow

Flow probes remained functional in the gilts until 111 days of gestation. In a few cases, the file or part of the file in which the 24-h blood flow measurements were registered was cancelled due to occasional problems of connection between the probes and the flow meter. The dissection of genital tracts at slaughter showed that for all the gilts except one, flow probes were correctly implanted on the artery supplying one uterine horn. In one gilt, due to an arterial branch upstream of the flow probe, the arterial blood flow corresponding to one foetus was not taken into account. Total blood flow was then corrected by adding the mean blood flow per foetus. In all gilts, a connective tissue developed within the space between arteries and probes, and the wall of the arteries looked normal. The probes did not constrict the vessels, which developed normally during pregnancy. The development of the piglets in the horns bearing a flow probe and in the opposite horns was compared in the CTR and LIG groups. Their mean weight was similar (1.43 and 1.45 kg in the CTR gilts and 1.56 and 1.57 kg in the LIG gilts in the horns with and without a flow probe, respectively). Similarly, the mean placental weight did not differ between the horns.

3.2.1. Effect of group

Irrespective of the experimental group, UBF varied during the daily measurements. An example of registration is given in Figure 1. The intra sow × stage coefficient of variation was about 10%. Uterine blood flow increased steadily in the three groups during gestation (Fig. 2, Tab. II). At 111 days of

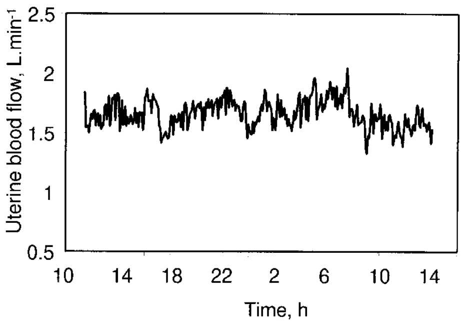

Figure 1. Example of a 24-h registration of uterine blood flow.

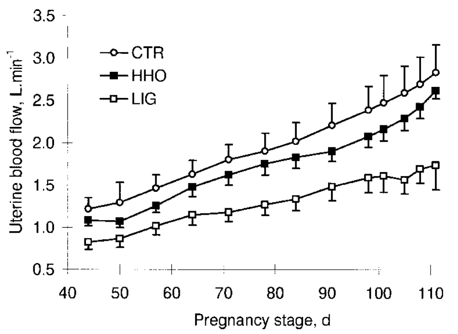

Figure 2. Effect of group on variation of blood flow in one uterine horn during pregnancy.

Table II. Effect of treatment on uterine blood flow in gilts during pregnancy (L·min^{-1}).

Item	Group[a]			RMSE[b]	Statistical significance[c]	
	LIG	CTR	HHO			
Uterine blood flow at						
44 days	0.82[d]	1.21[e]	1.09[d, e]	0.27	G*	R+
78 days	1.27[d]	1.91[e]	1.76[e]	0.41	G**	R+
111 days	1.74[d]	2.84[e]	2.63[e]	0.54	G**	
Uterine blood flow at 111 days						
·foetus^{-1}	0.60	0.56	0.53	0.18		
·kg foetus^{-1}	0.41	0.38	0.44	0.09		
·kg FPU^{-1}	0.30	0.30	0.29	0.04		R+

[a] LIG: left oviduct ligated and severed; CTR: control; HHO: right hysteroovariectomy.
[b] Root mean square error.
[c] Statistical significance: G, group effect; R, replicate effect. ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$.
[d, e] Means within a row with a different superscript letter differ significantly.

pregnancy, blood flow was 2.1, 2.3 and 2.4-fold of that measured at 44 days in the LIG, CTR and HHO groups, respectively. Blood flow per uterine horn increased by 12.4 ± 1.3, 23.7 ± 1.4 and 20.9 ± 0.9 mL·min^{-1}·day^{-1} from 44 to 111 days in these groups, respectively ($R^2 = 0.92$). The rate of increase in the LIG group was lower ($P < 0.001$) than in the other groups, and it tended to be higher in the CTR than in the HHO group ($P = 0.07$). Irrespective of the stage, UBF was significantly affected by the group ($P < 0.05$ to $P < 0.01$). It did not differ between the CTR and HHO groups ($P > 0.10$), and was higher in the CTR than in the LIG group. Blood flow differed significantly between the HHO and LIG groups during the last trimester of pregnancy.

Uterine blood flow per foetus also increased continuously during pregnancy (Fig. 3, Tab. II). The rate of increase (4.2 ± 0.3 mL·min^{-1}·day^{-1}·foetus^{-1}) did not differ between the three groups ($R^2 = 0.89$). Blood flow per foetus did not differ significantly between the three groups whatever the pregnancy stage. At 111 days, UBF per kg foetus or per kg FPU was similar in the three groups (Tab. II).

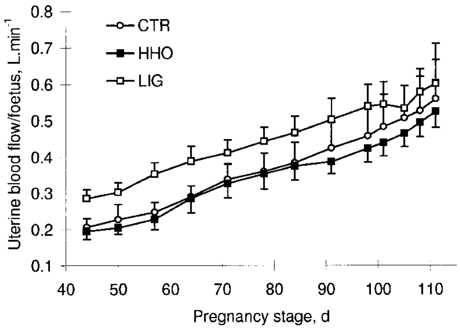

Figure 3. Effect of group on variation of blood flow per foetus in one uterine horn during pregnancy.

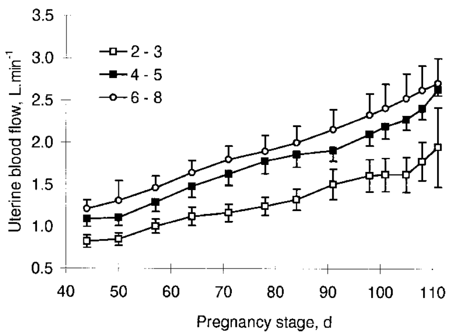

Figure 4. Effect of litter size on variation of blood flow in one uterine horn during pregnancy.

Table III. Effect of litter size on uterine blood flow in gilts during pregnancy (L·min^{-1}).

Litter size in the uterine horn	Class[a] 2–3	4–5	6–8	RMSE[b]	Statistical significance[c]
Uterine blood flow at					
44 days	0.83[d]	1.09[e]	1.23[e]	0.26	G**
78 days	1.25[d]	1.78[e]	1.90[e]	0.44	G*
111 days	1.95[d]	2.63[e]	2.71[e]	0.72	G+
Uterine blood flow at 111 days					
·foetus^{-1}	0.72	0.60	0.43	0.20	
·kg foetus^{-1}	0.47	0.45	0.35	0.10	
·kg FPU^{-1}	0.35	0.33	0.25	0.09	

[a] Class: class of litter size in the instrumented uterine horn (2 or 3, 4 or 5, and more than 5 foetuses).
[b] Root mean square error.
[c] Statistical significance: G, group effect; R, replicate effect. ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$.
[d, e] Means within a row with a different superscript letter differ significantly.

3.2.2. Effect of number of foetuses in the uterine horn

The mean numbers of foetuses in the instrumented uterine horns at 112 days of pregnancy were 2.7 ± 0.5 (n = 11), 4.4 ± 0.5 (n = 8) and 6.3 ± 0.7 (n = 9) in the 2–3, 4–5 and 6–8 classes of litter size, respectively. Uterine blood flow increased continuously between 44 and 111 days of pregnancy for the three classes. The rate of increase of UBF between 44 and 111 days amounted to 14.1 ± 1.5, 20.9 ± 1.4 and 21.7 ± 1.0 mL·min^{-1}·day^{-1} in the 2–3, 4–5 and 6–8 classes, respectively (R^2 = 0.90). It was similar in the 4–5 and 6–8 classes, and lower ($P < 0.001$) in the 2–3 class. From 44 to 84 d, UBF increased significantly with litter size (Fig. 4, Tab. III). It was significantly lower in the 2–3 class than in larger litters. From 84 to 108 days, UBF tended to differ between the three classes, and blood flow in the 4–5 class was intermediate between the two others.

Irrespective of the litter size class, UBF per foetus in the uterine horn increased continuously during pregnancy. The rate of increase was 5.1 ± 0.4, 4.7 ± 0.4 and 3.5 ± 0.3 mL·min^{-1}·day^{-1}·foetus^{-1} in the 2–3, 4–5 and 6–8 classes, respectively (R^2 = 0.89). It did not differ between the two upper classes (P = 0.4) and was higher in the 2–3 class ($P < 0.001$). Uterine blood flow per foetus was affected by litter size between 44 and 108 days of pregnancy ($P < 0.01$ to $P < 0.05$). It was significantly higher in the 2–3 class than in the 6–8 class, and intermediate in the 4–5 class (Fig. 5, Tab. III). There was no difference between the classes

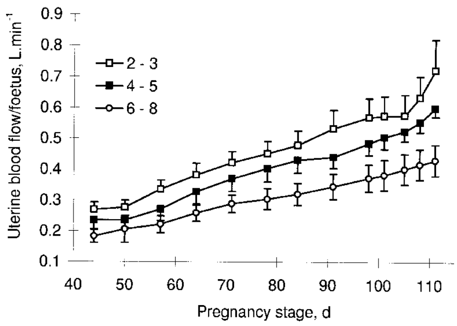

Figure 5. Effect of litter size on variation of blood flow per foetus in one uterine horn during pregnancy

for UBF at 111 days of pregnancy when expressed per kg foetus or per kg FPU (Tab. III).

3.2.3. Relationships with blood flow

Uterine blood flow at 111 days of pregnancy was correlated with litter weight (r = 0.79, P < 0.001), litter size (r = 0.56, P < 0.05), weight of placentas (r = 0.76, P < 0.001), length (r = 0.52, P < 0.05) and weight of the uterine horn (r = 0.48, P < 0.05). At this stage, an important part of the variation of UBF (mL·min^{-1}) was explained by the weight of the litter in the uterine horn (LW, g) and the length of the uterus (UL, cm), which can be expressed by the following equation:

$$\text{UBF} = 92\ (\pm 487) + 0.24\ (\pm 0.05)\ \text{LW} + 4.78\ (\pm 2.28)\ \text{UL}\ (R^2 = 0.72).$$

4. DISCUSSION

4.1. Variation of UBF with pregnancy stage

Irrespective of the group or class, UBF increased gradually between 44 days of pregnancy and term. When considering the individual sows, it increased until 98 days of gestation in all the sows, and continued to increase until term in 20 of them, without differences between classes. Total conjugated oestrogens in the plasma of gilts increase between 70 and 110 days of gestation [5, 26], and it is well established that through dilatation of the uterine blood vessels, oestrogens have a positive effect on UBF [6, 14, 23]. Increase of UBF at day 12–13 and before day 30 of gestation was described in relation to the presence of embryos in sows [24] and cows [25]. An increase of UBF during later stages has been described in many species. In rabbits, a 2.5-fold increase was reported between day 24 and 30 of gestation [28]. In sheep, UBF increases linearly over time during the second half of pregnancy [8, 35]. Christenson and Prior [10] reported that UBF increased from 958 to 1 358 mL·min^{-1} between 105 and 123 days of pregnancy in ewes. Huckabee et al. [30] with goats and Peeters et al. [39] with guinea pigs found that UBF·kg^{-1} FPU does not change during the second half of pregnancy, which means that absolute UBF increases. In cows, Reynolds et al. [49] and Reynolds and Ferrell [45] reported that UBF increases exponentially during gestation (4.5-fold greater at 250 than at 137 days of gestation). All these results show that uterine blood flow increases during a period of intense development and growth of the foetuses. This change is partly explained by a faster heart rate of the mother, as shown in ewes [11, 50], and by a redirection of blood flow to the uterus at the expense of other tissues and organs, like skin and carcass (ewe: Rosenfeld et al. [50]; cow: Ferrell and Ford [20]; guinea pig: Peeters et al. [39]). The increase in blood volume during pregnancy shown in pigs [2, 36, 51], and the greater uterine artery endothelial production of the potent vasodilatators, prostacyclin and nitric oxide, demonstrated in pregnant sheep [34], may also be involved.

In contrast, a constant UBF during the last part of gestation was found in some experiments. Ferrell and Ford [20] measured UBF in cows from 30 to 240 days of pregnancy and reported that it was constant from 178 days onward. In sows, a constant UBF was recorded between day 50 and day 90 of pregnancy [29], during the last 22 days of pregnancy [26], or between 70 and 110 days of pregnancy [48]. Methodological differences with earlier experiments on sows may partly explain this discrepancy. Reynolds et al. [48] measured UBF in anaesthetised sows which differed between the three stages of measurement. In these experiments, UBF was determined using electromagnetic blood flow transducers which may be less adapted to blood flow measurements on developing vessels as is the case for the middle uterine arteries during pregnancy.

For instance, Reynolds et al. [49] showed that UBF increased until 250 days of gestation in cows by using the steady-state diffusion procedure with deuterium oxide, whereas Ferrell and Ford [20] did not find any increase of UBF after day 178 when measured with electromagnetic transducers. Moreover, UBF varies with time during the same day, with sow position, and between night and day (Père, unpublished data), and it was measured during 10 min per day by Ford et al. [26] and Reynolds et al. [48] instead of 24 h as in the present study.

The rise of UBF during gestation appears as an adaptation allowing to increase the nutrient supply to the foetuses. However, UBF increased linearly between 44 and 111 days of pregnancy in the present experiment whereas foetal growth was exponential. When combining UBF measured in the CTR group of this experiment with the variation of foetus weight during pregnancy calculated according to Salmon-Legagneur [51], the UBF per litter weight ratio decreased from 1.77 to 0.91 and to 0.39 $L \cdot min^{-1} \cdot kg^{-1}$ at 44, 78 and 111 days of gestation, respectively. Variation of UBF may then be possible within limited values only. Reynolds et al. [48] also suggested that there may be a limit to maximal UBF in the sow. As in other species, complementary maternal and foetal adaptations like insulin resistance [18], variation of the blood content in substrates and hormones [40], and increased uptake of substrates by foetuses likely appear during the last part of gestation of the sow in order to ensure a harmonic development of the litter until term.

4.2. Variation of UBF with litter size

In the present experiment, UBF amounted to 2.40 to 2.84 $L \cdot min^{-1}$ in the CTR gilts between 98 and 111 days of gestation, and 0.56 $L \cdot min^{-1} \cdot foetus^{-1}$ at 111 days. These values were higher than those obtained in sows during the last part of gestation by Ford et al. [26] (1.57 $L \cdot min^{-1}$) and by Reynolds et al. [48] (1.51 $L \cdot min^{-1}$). When related to the number of foetuses measured at term, UBF amounted to 0.24 and 0.31 $L \cdot min^{-1} \cdot foetus^{-1}$ in these experiments, respectively. Reynolds et al. [46] reported that electromagnetic transducers gave estimates of bovine UBF that were 32 to 42% of those obtained by steady-state diffusion of D_2O or antipyrine. Similarly, bovine UBF estimated by using antipyrine [22] was considerably higher than when measured with electromagnetic transducers [20]. Values of UBF measured with antipyrine in ewes are in the same range as those estimated in the present experiment: 0.33 to 0.42 $L \cdot min^{-1} \cdot foetus^{-1}$ [10], 0.32 $L \cdot min^{-1} \cdot foetus^{-1}$ (calculated from the data obtained by Makowski et al. [35]), 0.49 $L \cdot min^{-1} \cdot foetus^{-1}$ (calculated from the data obtained by Meschia et al. [37] for sheep and goats).

In the present experiment, UBF increased with litter size at all the pregnancy stages considered. The rate of increase during pregnancy was lower in the 2–3 class than in the two others, and was similar in the 4–5 and 6–8 classes. Reynolds et al. [48] reported that UBF was correlated with the number of foetuses per uterine horn. Christenson and Prior [10] found that UBF at 105 days was greater in ewes with triplets than in ewes with twins and tended to be lower in ewes with a single foetus. Ferrell and Reynolds [21] measured a lower UBF in cows with a single foetus than with twin foetuses. In our study, UBF per foetus decreased when litter size increased. Its rate of increase during pregnancy was higher in the 2–3 class and did not differ for the two other classes. A negative relationship between UBF per foetus and the number of foetuses per uterine horn was found in sows by Reynolds et al. [48]. Present data suggest that UBF adapts to litter size, but within limits, and that there may be a maximal UBF in the sow. Indeed, UBF increased at a lower extent than the number of foetuses in the uterine horn, and then to the nutrient demand of the pregnant uterus. The limitation seemed more evident when the uterine horn contained more than 5 foetuses, which corresponded to a total litter size of 10 piglets. This explains why piglets from large litters generally have lower birth weights. In the present study, foetus mean weight at 112 days of gestation decreased from 1.52 to 1.24 kg when litter size in the uterine horn increased from 2–3 to 6–8. These results agreed with those cited by Alno [1] indicating that the number of littermates weighing more than 1.3 kg at birth is constant and equal to about seven, the additional piglets are lighter and their number increases with litter size.

4.3. Relationship between UBF and litter weight

Some results suggest that foetal growth is highly dependant on UBF. The estimates of UBF at 111 days did not differ statistically between the groups or the classes when expressed per foetus, and were even closer when expressed per kg foetus or per kg FPU. The correlation between UBF and total weight of the foetuses in the uterine horn was high, in agreement with other results in sows [29], guinea pigs [7] and rabbits [28]. A UBF per kg foetus (0.32 to 0.36 $L \cdot min^{-1} \cdot kg^{-1}$) similar to the present results (0.38 $L \cdot min^{-1} \cdot kg^{-1}$ at 111 d in the CTR group) and constant for several pregnancy stages was found in sows by Dickson et al. [13] when using antipyrine. The UBF per kg FPU near term found in the present experiment (0.30 $L \cdot min^{-1} \cdot kg^{-1}$) compares with that measured in cows (0.34 $L \cdot min^{-1} \cdot kg^{-1}$) by Ferrell et al. [22], and is higher than that in guinea pigs (0.15 to 0.17 $L \cdot min^{-1} \cdot kg^{-1}$ according to Bjellin et al. [7], and Peeters et al. [39]) or in rabbits (0.10 $L \cdot min^{-1} \cdot kg^{-1}$, Gilbert et al. [28]). This comparison agrees with the differences of placenta structure between species; the maternal-foetal exchanges being more efficient in the hemochorial placenta of rodents than in the syndesmochorial placenta of ruminants or the epitheliochorial placenta of pigs. Ferrell and Reynolds [21] suggested that reduced birth weight in twin compared to single foetuses in cows is related to reduced UBF and reduced nutrient delivery per foetus. According to Reynolds et al. [47], heat stress in cows has adverse effects on foetal growth due to reduced foetal and utero-placental nutrient uptake which probably results from decreased UBF and umbilical blood flow. In agreement with experiments involving other species, the present results suggest that variation of UBF in sows is more closely related to differences in litter weight or growth rate of the foetuses than to litter size. This relationship is most likely mediated through maternal-foetal transfer of nutrients, which partly depends on UBF.

4.4. Effect of treatments on reproductive performance and UBF

Reproductive performance in this study are in the same range as those measured with a larger number of gilts [40]. They are in agreement with the concept of uterine capacity demonstrated through superovulation with a PMSG treatment of sows [12, 33, 41] or superinduction, adding embryos to recipient pregnant sows [3, 4, 19, 42]: despite a much higher ovulation rate per uterine horn in the HHO than in the CTR group, litter size at term is not different. According to Webel and Dziuk [54], Knight et al. [31] or Chen and Dziuk [9], foetal death related to uterine capacity occurs after day 25 of gestation. Legault et al. [32] and Père et al. [40] found that death of the supernumerary foetuses occurs partly before 35 days of gestation and continues thereafter. In the present experiment, 35% of the potential foetuses of the HHO gilts died after 35 days of pregnancy compared to 11% in the CTR gilts. At 35 d, the number of live foetuses in the remaining uterine horn of the HHO gilts was still much greater than in the CTR gilts (10.8 vs. 6.6). This agrees with results indicating that foetal mortality after 35 days is high when litter size at that stage exceeds uterine capacity [17, 40].

The foetuses in the HHO group were lighter than in the other groups at the end of pregnancy, as in the studies of Legault et al. [32] and Père et al. [40]. However, the difference was greater in the present gilts. The greater litter size at 35 days of the HHO gilts may have increased intrauterine competition. The lower placental weight in the HHO group supports this hypothesis, and suggests that maternal-foetal exchanges of nutrients and hormones were more limited in these gilts.

There was no difference in absolute UBF or UBF per foetus between the CTR and HHO groups from 44 days of gestation onward. This contrasts with the difference in litter size which was 39% smaller in the CTR than in the HHO gilts at 35 days of pregnancy. Part of this discrepancy can be related to a lower mean weight of the foetuses in the HHO group due to uterus crowding in the early stages of gestation, as is the case near term. Another explanation could be that supernumerary foetuses of the HHO gilts died before the first measurement of UBF, i.e. before 44 days. Rankin et al. [43] showed that UBF was reduced 24 h after clamping a branch of the umbilical arterial tree in sheep or killing a foetus in the middle of one uterine horn in rabbits. Unilateral or bilateral occlusion of the middle uterine artery of gilts during mid-gestation reduced foetal survival and development [38]. These results show that UBF decreases when foetuses die. In the present experiment, there was no dramatic variation of UBF in any gilt after 44 days of gestation. Foetal mortality in the HHO group may then have occurred between 35 and 44 days of pregnancy. A few mummified foetuses were found at slaughter. This also suggested that most foetal death occurred in early stages. This study did not allow to determine the extent of the involvement of limited UBF in uterine capacity in sows, as suggested in mice [53]. The greater mortality rate after day 35 of pregnancy in the HHO gilts is most likely not related to the implantation of the probes because in the other groups, foetal mortality in instrumented sows was very low and foetal growth rate was not affected.

In conclusion, this study describes the evolution of UBF during the last two thirds of pregnancy of three groups of sows that differed by their embryo potential. Foetal growth was closely related to UBF, which increased steadily during pregnancy until term. It also increased with litter size. Uterine blood flow is a major adaptation of the dam to pregnancy and allows to respond to the increasing nutrient and hormone demand of the uterus. However, this adaptation is limited because when litter size exceeds five foetuses per uterine horn, UBF increases less than the number of foetuses. Their growth rate is then reduced. The limitation of litter size by uterine capacity seems to occur during the first third of pregnancy. In order to determine the role of UBF in this phenomenon, it would be necessary to measure it from the beginning of pregnancy. The variation of UBF throughout gestation and the important differences between gilts already suggest that UBF is a critical element for the survival and growth of the foetuses.

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