Effects of folic acid and vitamin B\textsubscript{12} supplements on folate and homocysteine metabolism in pigs during early pregnancy

Frédéric Guay\textsuperscript{1}, J. Jacques Matte\textsuperscript{2*}, Christiane L. Girard\textsuperscript{2}, Marie-France Palin\textsuperscript{2}, Alain Giguère\textsuperscript{2} and Jean-Paul Laforest\textsuperscript{1}

\textsuperscript{1}Department of Animal Sciences and Centre de Recherche en Biologie de la Reproduction (CRBR), Laval University, Ste-Foy, Québec, Canada G1K 7P4
\textsuperscript{2}Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Lennoxville, Québec, Canada J1M 1Z3

(Received 14 August 2001 – Revised 11 March 2002 – Accepted 15 April 2002)

The present experiment aimed to determine the effects of supplements of folic acid (FA) alone or in combination with vitamin B\textsubscript{12} on folate and homocysteine metabolism in gestating nulliparous Yorkshire–Landrace (YL) and multiparous Landrace (LD) occidental sows and multiparous Chinese Meishan–Landrace (ML) sows. LD sows were randomly assigned to two treatments: 0 or 15 mg FA/kg diet while YL and ML sows were assigned to three treatments: 0 mg FA/kg diet, 15 mg FA/kg or 15 mg FA + 160 μg vitamin B\textsubscript{12}/kg diet. Supplements were given from the oestrus preceding insemination up to slaughter on day 15 of gestation. At slaughter, a uterine flush was collected to determine uterine contents of homocysteine, methionine, tetrahydrofolate (THF), 5-methyl-THF, pyridoxal 5-phosphate (P5P) and vitamin B\textsubscript{12}. Blood samples were taken at first oestrus, at insemination and on days 5, 10 and 15 of gestation to determine plasma concentrations of homocysteine, methionine, THF, 5-methyl-THF, P5P, vitamin B\textsubscript{12} and relative total folate-binding capacity. In occidental sows (YL and LD), the FA supplement tended to decrease uterine flush content of homocysteine (P=0.06) and concentrations of plasma homocysteine (P=0.09). Nulliparous YL sows had lower concentrations of plasma homocysteine, methionine, THF and 5-methyl-THF (P<0.05) than multiparous LD sows. Multiparous ML and LD sows had similar concentrations of plasma THF, 5-methyl-THF, methionine and vitamin B\textsubscript{12}, but ML sows had lower concentrations of plasma homocysteine (P<0.05). The vitamin B\textsubscript{12} supplement increased concentrations of plasma vitamin B\textsubscript{12} (P<0.05) both in multiparous ML and nulliparous YL sows, but had no effect on the composition of either uterine flush or plasma. The present results showed also that sows had a low vitamin B\textsubscript{12} status (<200 pg/ml) and high circulating homocysteine levels (>15 μM) during the first 15 d of gestation. Furthermore, the vitamin B\textsubscript{12} content in uterine secretions represented between 180 and 300% of the total content in plasma. The low plasma concentrations of homocysteine in multiparous ML sows suggest a more efficient remethylation pathway which may not be dependent upon dietary supply of FA or vitamin B\textsubscript{12}. In nulliparous YL sows, low concentrations of both homocysteine and methionine suggest that the methionine requirement for protein deposition might have reduced the amount of methionine available for the methylation pathway. The results of the present experiment suggest that the reduction of uterine homocysteine may be an important aspect of the role of FA supplement on the uterine environment in occidental sows. The presence of high levels of vitamin B\textsubscript{12} in uterine secretions merits further investigation in relation to embryonic development.

Folates: Homocysteine: Gestation: Sow

Abbreviations: FA, folic acid; FBC, folate-binding-capacity; LD, Landrace sow; ML, Meishan–Landrace sow; P5P, pyridoxal 5-phosphate; THF, tetrahydrofolate; YL, Yorkshire–Landrace sow.

* Corresponding author: Dr J. Jacques Matte, present address, Agriculture and Agri-Food Canada, 2000 route 108, CP 90 Lennoxville, Québec, Canada J1M 1Z3, fax +819 564 5307, email mattej@em.agr.ca
The death of initially viable embryos is one of the major limitations to improving litter size in pigs. From several studies it has been accepted that during the first 25–30 d of gestation, embryonic mortality is approximately 30% and occurs especially during embryonic attachment, day 12–15 of gestation (Pope & First, 1985; Wilmut et al. 1985). Recent advances in the understanding of embryonic development and uterine physiology have shown that embryonic mortality is controlled by several factors, such as nutritional status and genotype of sows (Haley & Lee, 1993; Ashworth, 1994).

A dietary supplement of folic acid (FA) during pregnancy increases litter size by 10% in multiparous sows (Matte et al. 1984). This increased litter size is associated with modifications in endometrial secretions and decreased embryonic mortality in early pregnancy (Tremblay et al. 1989; Matte et al. 1996). However, effects of FA on litter size, embryonic mortality and endometrial secretions are less marked in nulliparous sows (Lindemann & Kornegay, 1989; Matte et al. 1993; Duquette et al. 1997). Chinese sows of the Meishan genotype are recognized for their higher litter size and lower embryonic mortality rate than occidental sows (Haley & Lee, 1993). In a recent study, it has been shown that there was no effect of FA supplementation on litter size and embryo survival in Meishan crossbred sows during the first 25 d of gestation (Guay et al. 2002).

In human subjects, FA supplementation is known as a reliable therapeutic tool to reduce the concentration of plasma homocysteine and the incidence of neural-tube defects (Czeizel & Dudas, 1992; Jacques et al. 1999). Maternal accumulation of homocysteine, a metabolite of the methionine cycle, has been linked to neural-tube defects in the child and associated with defective chorionic villous vascularization in women with recurrent early pregnancy loss (Mills et al. 1995; Nelen et al. 2000). In ovo, treatment of avian embryos with various concentrations of exogenous homocysteine during the processes of gastrulation, neurulation and crano–truncal separation showed homocysteine induced oro–facial, coro–truncal and neural-tube defects and reduced embryo survival (Rosenquist et al. 1996, 1999), but the physiological basis of effects of homocysteine on embryo–fetal development remains to be determined. However, a recent study has shown that homocysteine could inhibit conversion of retinal to retinoic acid in avian embryos (Limpach et al. 2000). Retinoic acid is important in the regulation of homocysteine by the methionine synthase pathway requires another B-complex vitamin, vitamin B12, for the demethylation of 5-methyl-tetrahydrofolate (THF) (Bässler, 1997). A deficiency in vitamin B12 induces an accumulation of homocysteine and 5-methyl-THF in plasma. This reduced utilization of folates is called ‘methyl trapping’ (Scott & Weir, 1981). Furthermore, in human subjects, neural-tube defects was associated with lower concentrations of vitamin B12 in amniotic fluid (Steen et al. 1998). Although such an association has not been established in the pig, it is known that vitamin B12 influences swine reproduction and neonatal survival (Frederick & Brisson, 1961). In a recent study, we reported low plasma concentrations of vitamin B12 (<200 pg/ml) in gestating multiparous Meishan–Landrace (ML) and nulliparous Yorkshire–Landrace (YL) as compared with multiparous YL sows. These results suggest a possible suboptimal status of vitamin B12 in these gestating sows (Guay et al. 2002).

The objectives of the present study were to evaluate, during early pregnancy, the effects of supplements of FA alone, or in combination with vitamin B12, on folate and homocysteine metabolism in nulliparous and multiparous sows (nulliparous effect) and multiparous ML sows (Meishan genotype effect) during the first 15 d of gestation.

Materials and methods

Animals and treatments

Ten multiparous ML (three to four parities, body weight 197·5 (SE 6·7 kg)) and twelve multiparous Landrace (LD, three to four parities, body weight 255·6 (SE 7·5 kg)) sows (Génétiporc Inc., St-Bernard, Qué., Canada), were compared, to evaluate impacts of the Meishan genotype. Eleven nulliparous YL sows (body weight 125·7 (SE 3·5 kg)) (Génétiporc Inc.) were also compared with multiparous ML and LD groups to evaluate effects of ‘nulliparous status’ on responses of homocysteine and folate metabolism to FA supplementation. After weaning, multiparous sows in ML and LD groups were fed with the gestation diet and transported a few days after weaning to the Research Centre. Before their arrival, nulliparous YL sows were fed with breed–gestation diet and were not cycling upon arrival at Research Centre. Beginning on arrival and for at least 2 weeks, all groups of sows received 2·5 kg experimental diet/d (Table 1) without FA supplement. After these 2 weeks, heat detection was performed twice per d, between 08.00 and 09.00 hours, and 16.00 and 17.00 hours, by introducing a boar into the pen. On the first observed oestrus (day –21) (monitored from at least 2 weeks after their arrival), the sows were allocated (Table 2) to one of the following two treatments: basal diet (0 mg FA/kg) or basal diet with 15 mg FA/kg. As a results of findings in a previous study (Guay et al. 2002), a third treatment, basal diet supplemented with 15 mg FA and 160 µg vitamin B12/kg, was also used in multiparous ML (n 5 sows) and nulliparous YL (n 6 sows) to evaluate the impact of FA plus vitamin B12 on folate utilization and homocysteine metabolism. At second observed oestrus, the
Folates, homocysteine and gestating sows

Table 1. Composition of the basal experimental diet†

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>500</td>
</tr>
<tr>
<td>Barley</td>
<td>200</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>200</td>
</tr>
<tr>
<td>Soyabean meal (480 g crude protein (N x 6-25)/kg)</td>
<td>50</td>
</tr>
<tr>
<td>Limestone</td>
<td>25</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>14</td>
</tr>
<tr>
<td>Salt</td>
<td>5</td>
</tr>
<tr>
<td>Mineral premix§</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin premix‡</td>
<td>5</td>
</tr>
</tbody>
</table>

*The analysed dietary concentrations of folates in the 0 mg/kg, 15 mg/kg and 15 mg/kg + FA diets were 1.02 (SD 0.12), 16.6 (SD 1.4) and 16.3 (SD 2.3) mg/kg respectively. The added folinic acid was pteroylglutamic acid, C19H19N7O6 (molecular mass 441.4; ICN Canada Ltd, Montreal, Que., Canada).

† The analysed dietary concentrations of vitamin B12 in the 0 mg/kg, 15 mg/kg and 15 mg folic acid/kg + B12 diets were 15.7 (SD 2.2), 17.4 (SD 1.9) and 192.3 (SD 17.1) µg/kg respectively. The added vitamin B12 was cyanocobalamin, C63H88N14O14PCO (molecular mass 1355.4; ICN Canada Ltd, Montreal, Que., Canada).

‡ Provided (kg diet): Mn as manganous oxide 30 mg, Zn as zinc oxide 100 mg, Fe as ferrous sulfate 100 mg; Cu as copper sulfate 15 mg; I as calcium iodate 30 µg, Se as selenite 300 µg.

§ Provide (kg diet): retinol acetate 35 mg, menadione 2.2 mg, thiamin 2 mg, riboflavin 5 mg, niacin 25 mg, pantothenic acid 16 mg, pyridoxine 2 mg, biotin 250 µg, choline 350 mg.

sows were inseminated twice with commercial semen (pooled semen from three Duroc boars of proven fertility: CIPQ Inc., St-Lambert, Que., Canada), 12 h and 24 h after initial oestrus detection. The first day of the second oestrus was considered to be day 0. Dietary treatments were given to day 15 of pregnancy.

Blood samples and tissues collected

At first oestrus (day - 21, baseline value), second oestrus (insemination, day 0), and days 5, 10 and 15 of gestation, blood samples were collected in morning prior to feeding (after a 20–24 h fast) with whole-blood collection tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing EDTA as anticoagulant and centrifuged for 10 min (1800 g). Plasma was frozen at −25°C, and later analysed for vitamin B12, pyridoxal 5-phosphate (PSP), total folate-binding capacity (THF), 5-methyl-THF, homocysteine and methionine. For THF and 5-methyl-THF determinations, 0.5 ml ascorbic acid (100 g/l) was added to 10 ml blood to avoid oxidation. On day 15 of gestation, sows were killed according to the recommended code of practices (Canadian Council on Animal Care, 1993). The reproductive tract was taken and transported to the laboratory. The uterine horns were separated from the mesosomium. The ovaries, oviducts and cervix were also removed. One horn, chosen at random, was flushed with 20 ml PBS according to a method described by Laforest et al. (1992). The fluid collected was designated as ‘uterine flush’ and the total volume was recorded. After assessing the presence of filamentous blastocysts, pregnant uteri were kept for further analysis. The fluid was centrifuged at 86 g for 5 min to precipitate the blastocysts and uterine flush was collected and stored at −25°C.

Table 2. Composition of the uterine flush of sows slaughtered on day 15 of pregnancy as affected by nulliparous status, Meishan genotype and folic acid supplement¶†

<table>
<thead>
<tr>
<th>Dietary folate (mg/kg)</th>
<th>Multiparous ML</th>
<th>Multiparous LD</th>
<th>Nulliparous YL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS</td>
<td>SEM</td>
<td>LS</td>
</tr>
<tr>
<td>Sows (n)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Flush volume (ml)</td>
<td>18-2 8-3</td>
<td>0-5</td>
<td>17-7  0-3</td>
</tr>
<tr>
<td>Methionine (nmol)</td>
<td>786 130</td>
<td>202</td>
<td>544  89</td>
</tr>
<tr>
<td>Homocysteine (nmol)</td>
<td>117  31</td>
<td>102 31</td>
<td>115 16</td>
</tr>
<tr>
<td>5-methyl-THF (ng)</td>
<td>432‡ 103</td>
<td>138</td>
<td>314 47</td>
</tr>
<tr>
<td>PSP (nmol)</td>
<td>56-5 7-0</td>
<td>11-3</td>
<td>26-6* 3-2</td>
</tr>
<tr>
<td>Vitamin B12 (ng)</td>
<td>1339 254</td>
<td>339</td>
<td>1207 167</td>
</tr>
</tbody>
</table>

ML, Meishian–Landrace sows; LD, Landrace sows; YL, Yorkshire–Landrace sows; THF, tetrahydrofolate; PSP, Pyridoxal 5 Phosphate. For PSP, values in LD sows were lower than in ML and YL sows whatever the dietary folic acid supplement: *P<0.05. For homocysteine, values in occidental sows (YL and LD) tended to be lower than in those fed 15 v. 0 mg FA/kg diet: †P=0.06. Values for ML sows were higher than for LD sows: ‡P=0.09. Values for YL sows were lower than for LD sows: §P=0.09.

¶ For details of diets and procedures, see Table 1 and p. 254.

† Total content = concentration x volume of uterine flush.
with 350 μl pure water. Folates were eluted twice from the column by 1 ml acetic acid pH 2·3 and washed with 2 ml ultra-pre-treated with 1 ml methanol and 1 ml water followed (Varian Analytical Instruments, Walnut Creek, CA, USA) strong anion-exchange column (Bondesil, Sax 40 μm).

Thereafter, the sample was loaded in a disposable assay tube to allow the separation of folates from binding proteins. Thereafter, the sample was loaded in a disposable strong anion-exchange column (Bondesil, Sax 40 μm: Varian Analytical Instruments, Walnut Creek, CA, USA) pre-treated with 1 ml methanol and 1 ml water followed by 1 ml acetic acid pH 2·3 and washed with 2 ml ultra-pure water. Folates were eluted twice from the column with 350 μl sodium chloride solution (100 g/l) pH 2·0 (adjusted with citric acid) containing sodium ascorbate (10 g/l) and β-mercaptoethanol (2 ml/l). Plasma THF and 5-methyl-THF were separated by HPLC coupled to a Beckman solvent delivery system (model 126; Beckman Instrument (Canada) Inc.). Validation tests showed satisfactory parallelism among dilutions (for 300–500 μl CV was 1·7 %) and recovery tests yielded 98·7 %. The inter- and intra-assay CV were 11·1 and 7·7 %, respectively.

**Statistical analysis**

The effects of Meishan genotype and nulliparous status were analysed using the Mixed procedure of the SAS (SAS Institute, Inc., Cary, NC, USA), according to a 3 × 2 factorial arrangement with the comparison between multiparous ML and LD, and nulliparous YL and dietary supplements (0 or 15 mg FA/kg) as the main factors. The following model was used: \( Y_{ij} = \mu + B_i + F_j + (B_i \times F_j) + e_{ij} \), where \( \mu \) is the mean, \( B_i \) is the dependent variable, \( B_i \) is the type of sow, \( F_j \) is the FA supplement and \( e_{ij} \) is the residual error. Preplanned single df contrasts were used to compare (1) Meishan genotype effects between multiparous ML and LD sows; (2) ‘nulliparous effects’ between multiparous LD and nulliparous YL sows; (3) specific effects of FA supplementation within occidental sows. To evaluate the effect of vitamin B\(_{12}\) on folate metabolism, an analysis using the Mixed procedure (SAS Institute Inc.) was also done within multiparous ML and nulliparous YL groups using the dietary supplement of vitamin B\(_{12}\) as the main variable; comparisons were performed between 15 mg FA/kg and 15 mg FA + 160 μg B\(_{12}\)/kg using the following model: \( Y_{ij} = \mu + F_i + e_{ij} \), where \( \mu \) is the mean, \( F_i \) is the vitamin B\(_{12}\) supplement and \( e_{ij} \) is the residual error. For plasma FBC, THF, 5-methyl-THF, homocysteine, methionine, vitamin B\(_{12}\) and P5P, days of pregnancy (days 0, 5, 10, 15) were added to the model as a third factor and were analysed using the repeat option of the Mixed procedure (SAS Institute Inc.) with the autoregressive option and then sow was considered as a random effect and included in statistical analysis. To determine effects of FA alone or FA + vitamin B\(_{12}\) on concentrations of plasma total FBC, THF, 5-methyl-THF, homocysteine, methionine and P5P, values measured at treatment allocation were used as co-variables. For plasma concentrations of total FBC, THF and 5-methyl-THF, a logarithm transformation was performed to normalize for experimental errors.

**Results**

**Meishan genotype and nulliparous effects**

During early gestation, multiparous LD and ML sows had similar concentrations of plasma THF, 5-methyl-THF and total FBC (Figs 1 and 2; \( P > 0·10 \)), whereas multiparous LD had higher plasma concentrations of THF, 5-methyl-THF and FBC than nulliparous YL sows (\( P < 0·05 \); Figs 1 and 2).

Multiparous LD sows also had higher concentrations of homocysteine than multiparous ML and nulliparous YL
sows (Fig. 3; $P<0.05$). However, the reduction of homocysteine concentrations during the first 15 days of gestation was more marked in multiparous LD sows than in multiparous ML and nulliparous YL sows (Fig. 3; Genotype $\times$ Time and Nulliparous $\times$ Time, $P<0.05$).

Multiparous LD and ML sows had similar concentrations of plasma P5P (0.27 (SE 0.012) vs. 0.29 (SE 0.015) $\mu$M respectively) and methionine (39.9 (SE 2.1) vs. 35.4 (SE 2.2) $\mu$M respectively) while multiparous LD sows had higher plasma concentrations of methionine.
Concentrations of plasma P5P were higher (0.40 (SE 0.016) v. 0.27 (SE 0.012) μM respectively, P<0.01) in nulliparous YL than multiparous LD sows. Finally, multiparous ML and LD, and nulliparous YL sows had similar concentrations of vitamin B12 (227.1 (SE 51.0), 158.5 (SE 27.6) v. 147.5 (SE 5.7) pg/ml respectively, P>0.10).

In the uterine flush, nulliparous YL and multiparous LD and ML sows had similar total contents of methionine, homocysteine and vitamin B12 (Table 2; P>0.10). Multiparous ML sows tended to have higher uterine contents of 5-methyl-THF than multiparous LD sows whereas contents of 5-methyl-THF tended to be lower in nulliparous YL compared with multiparous LD sows (Table 2; P=0.09). The content of P5P was higher in multiparous ML and nulliparous YL than in multiparous LD sows (Table 2; P<0.05).

**Folic acid effects**

The FA supplement did not increase plasma concentrations of THF and 5-methyl-THF (Fig. 1; P>0.10). However, the FA supplement increased concentrations of plasma total FBC in multiparous ML and LD, and nulliparous YL sows during the first 15 d of gestation (Fig. 2; P<0.05).

The concentrations of plasma vitamin B12, P5P, methionine (results not shown) and homocysteine (Fig. 3) were not altered by the FA supplement during the first 15 d of gestation (P>0.10). However, if analyses were done within occidental sows only, FA supplement tended to decrease concentrations of plasma homocysteine by 10% in multiparous LD and nulliparous YL sows (Fig. 3; P=0.09).

The FA supplement had no significant effect on the composition of the uterine flush in nulliparous YL, and multiparous LD and ML sows (Table 2; P>0.10). However, there was a strong tendency for the FA supplement to decrease the total content of homocysteine in occidental sows (Table 2; P=0.06).

**Vitamin B12 effects**

In multiparous ML and nulliparous YL sows, vitamin B12 had no effect on the concentration of plasma homocysteine, methionine, THF, 5-methyl-THF and P5P (results not shown). Nevertheless, the addition of vitamin B12 to the
FA supplement increased the plasma concentrations of vitamin B_{12} compared with FA supplement alone (Fig. 4; \( P < 0.05 \)). The addition of vitamin B_{12} had no significant effect on the composition of uterine flush either in nulliparous YL or ML sows (results not shown).

![Figure 3](https://www.cambridge.org/core/journals/...)

**Fig. 3.** Effects of folic acid (FA) supplement, nulliparous status and Meishan genotype on plasma concentrations of homocysteine from the oestrus preceding insemination to day 15 of gestation for (a) multiparous Meishan–Landrace, (b) multiparous Landrace sows, (c) nulliparous Yorkshire–Landrace sows. (●), 0 mg FA/kg diet; (○), 15 mg FA/kg diet. For details of diets and procedures, see Table 1 and p. 254. Values are least squares means with standard errors shown by vertical bars. Mean values for Meishan–Landrace sows were significantly different from those for Landrace sows: \( P < 0.05 \). Mean values for Yorkshire–Landrace sows were significantly different from those for Landrace sows: \( P < 0.05 \). Statistical significance of (Meishan–Landrace v. Landrace) \( \times \) Time, \( P < 0.05 \); (Yorkshire–Landrace v. Landrace) \( \times \) Time, \( P < 0.05 \). Mean values for occidental sows (Landrace and Yorkshire–Landrace) fed 15 mg FA/kg diet were significantly different from those fed 0 mg FA/kg diet.

![Figure 4](https://www.cambridge.org/core/journals/...)

**Fig. 4.** Effects of addition of vitamin B_{12} to folic acid (FA) supplement on plasma concentrations of vitamin B_{12} from the oestrus preceding insemination to day 15 of gestation for (a) multiparous Meishan–Landrace and (b) nulliparous Yorkshire–Landrace sows. (●), 15 mg FA/kg diet; (○), 15 mg FA and 160 \( \mu \)g vitamin B_{12}/kg diet. For details of diets and procedures, see Table 1 and p. 254. Values are least squares means with standard errors shown by vertical bars. Mean values for Meishan–Landrace and Yorkshire–Landrace sows given FA and vitamin B_{12} were significantly different from those given FA only: \( P < 0.05 \). (Statistical analyses were performed on logarithm values.)
Discussion

To the best of our knowledge, the present experiment was the first to evaluate the effects of FA alone and the effects of FA with vitamin B$_{12}$ on folate and homocysteine metabolism of different types of sows during early pregnancy. In growing pigs and adult human subjects, supplementation with FA reduces the concentration of plasma homocysteine (Ambrosi et al. 1999; Jacques et al. 1999). In the present experiment, the FA supplement reduced the homocysteine content in uterine flush by approximately 30 % and by 10 % in plasma in occidental sows (multiparous LD and nulliparous YL sows). In the first 15 d of gestation, large amounts of folates are taken up by the uterine tissues and transferred into the uterine lumen (Matte et al. 1996; Vallet et al. 1999a). Those folates can be readily used by the conceptus in order to respond to the intense metabolic activity and cellular division rates at this stage of development (Pusateri et al. 1990). This high rate of cellular division is likely to increase FA requirements and induces an increased uterine uptake of FA to allow an efficient methylation of homocysteine within the uterine environment. In culture of post-implantation rat embryos, homocysteine is recognized to be teratogenic for 10-d-old rat embryos (VanAerts et al. 1994). The teratogenic effect of homocysteine may be associated with the specific ability of homocysteine to inhibit conversion of retinal to retinoic acid which could regulate gene expression prior to and during rapid trophoblastic elongation in the porcine conceptus and also be involved in the development and growth of the porcine placenta (Yelich et al. 1997; Limpach et al. 2000; Johansson et al. 2001). According to Menezo et al. (1989), during early embryonic development of the mouse, homocysteine would act also as an inhibitor of methylation, would decrease the uptake of methionine and the S-adenosyl methionine pool, and would partially impair blastocyst formation.

Several aspects of folate and homocysteine metabolism in early pregnancy appear to be influenced by the Meishan genotype. In the present study, multiparous ML sows had lower concentrations of plasma homocysteine although multiparous LD and ML sows had similar concentrations of plasma THF, 5-methyl-THF, vitamin B$_{12}$, P5P and methionine. Such result suggests that the remethylation pathway is more efficient in multiparous ML than in LD sows (Scott, 1999). In human subjects, polymorphisms of methionine synthase and methylene tetrahydrofolate reductase have been identified. Mutations in human methionine synthase and methylene tetrahydrofolate reductase genes (A2756G and C677T mutations respectively) are a fairly common polymorphism and have been significantly associated with modified homocysteine concentrations (Goyette et al. 1994; Jacques et al. 1996; Chen et al. 1997; Schwartz et al. 1997; Harmon et al. 1999). Although polymorphisms of methionine synthase and methylene tetrahydrofolate reductase have not yet been identified in pigs, it is possible that different DNA polymorphisms for methionine synthase and methylene tetrahydrofolate reductase exist between Chinese Meishan and occidental sows that could explain the homocysteine metabolism discrepancy observed between multiparous ML and LD sows (Signer et al. 2000).

Although the vitamin B$_{12}$ supplement had no effect on folate and homocysteine metabolism, the vitamin B$_{12}$ supplement did increase concentrations of plasma vitamin B$_{12}$, suggesting a relative efficiency of absorption of the dietary supplement. Moreover, according to the present results, the transfer of vitamin B$_{12}$ towards the uterus is an important process in early pregnancy of pigs. Indeed, the total content of vitamin B$_{12}$ in uterine horns represents 180–300 % of the total content in plasma, assuming that the total volume of plasma represents 4 % of pig body weight (Matte & Girard, 1996). In human subjects, the receptor of transcobalamin II–vitamin B$_{12}$ complex is present in placental tissues (Friedman et al. 1977), allowing active transport of vitamin B$_{12}$. In pigs, the receptor of transcobalamin II has not yet been characterized in endometrial and placental tissues, but transcobalamin I was identified in endometrial tissues (Pearson et al. 1998). Although there was no effect of vitamin B$_{12}$ supplement on homocysteine metabolism when sows were fed a dietary supplement of FA, such a large additional uterine transfer of vitamin B$_{12}$ suggests an important requirement for embryo development and for uterine metabolism during first 15 d of gestation.

At the endometrial level, multiparous ML and LD sows had similar uterine flush content of vitamin B$_{12}$, homocysteine and methionine. For 5-methyl-THF, multiparous ML sows had a higher uterine flush content than multiparous LD sows in spite of similar plasma concentrations. Such results suggest a more efficient uterine transfer of 5-methyl-THF in ML sows. One component of the mechanism controlling folate delivery to tissues are folate-binding proteins (Antony, 1996) that have been isolated in porcine endometrium during first 15 d of gestation (Vallet et al. 1999b). However, such an explanation is unlikely to apply in the present case since Vallet et al. (1999a) have shown that the amount of folate-binding protein in uterine secretions are similar between pure-bred Meishan and Large White sows from day 10–15 of pregnancy. Alternatively, it is possible that accumulation of 5-methyl-THF in the uterine flush of multiparous ML sows was due to a reduced utilization of 5-methyl-THF for conceptus cellular division in early pregnancy. It is recognized that Meishan conceptuses are smaller and have fewer cells compared with Yorkshire conceptuses on day 12–15 of gestation (Rivera et al. 1996; Wilson & Ford, 1997).

In the present experiment, multiparous LD and nulliparous YL sows differed not only by their nulliparous status, but also by a heterosis effect (multiparous LD v. nulliparous YL). Nevertheless, it was assumed that, in such a situation, the nulliparous status was the dominant factor in the difference between multiparous LD and nulliparous YL sows (Johnson, 1981; Yen et al. 1987). Moreover, in nulliparous YL, lower concentrations of THF and 5-methyl-THF agree with previous results reported by Guay et al. (2002), where concentrations of total folates were lower in nulliparous YL compared with multiparous YL sows. The lower plasma concentration of folates in nulliparous YL as compared with multiparous LD sows may be due to a greater utilization of folates required for both gestation and growth (Matte et al. 1993). An age effect was also reported by Natsuhori et al. (1996), who
showed that plasma THF and 5-methyl-THF were higher in adult pigs than in 158-d-old pigs. This low concentration of plasma folates during the growing period could also be linked to an increase of folate catabolism, as observed in rats (McNulty et al. 1995).

Plasma homocysteine was lower in nulliparous YL than in multiparous LD sows. In nulliparous YL sows, the increased utilization of methionine to sustain protein synthesis and rapid growth (Whittemore et al. 1988) is likely to reduce the amount of circulating methionine and methionine available for methyl transfer. Moreover, it is known that the activity of methionine synthase and betaine methyltransferase decreases with ageing, whereas the activity of PSP-dependent cystathionine β synthase (Finkelstein, 1990), an alternative pathway to catabolism of homocysteine to cysteine, increases (Nakagawa & Kimura, 1968). It is possible that in nulliparous YL sows, the reduced level of free methionine combined with intense activity of methionine synthase would bring about a decrease in concentrations of homocysteine, whereas in multiparous LD sows, the lower activity of methionine synthase, along with a lower concentration of PSP, an essential cofactor for the activity of cystathionine β synthase (Smolin et al. 1983), increases concentrations of homocysteine.

### Conclusion

The reduction of systemic and uterine homocysteine induced by the FA supplement might be a mode of action of this vitamin in the uterine environment and on embryonic development in occidental sows. In multiparous ML sows, low circulating homocysteine suggests an intrinsically more efficient remethylation pathway of homocysteine which did not seem to be dependent on folate or vitamin B12 status. In nulliparous sows, the high requirement for both gestation and growth would decrease the amount of methionine available for methyl transfer, decreasing the formation of homocysteine. Finally, the important uterine transfer of vitamin B12 during pregnancy merits further investigations with regard to its metabolic role in embryonic development.

### Acknowledgements

The authors are grateful to M. Guillette for technical assistance and D. Morissette, J. Boudreau, E. Bérubé, M. Turcotte, C. Mayrand and F. Phaneuf for animal care. F.G. is supported by a Fellowship from NSERC and FCAR. This work is partially subsidized by Génétiporc Inc. (St-Bernard, Québec, Canada), Hoffmann-LaRoche (Basel, Switzerland and Mississauga, Ont., Canada) and Agriculture and Agri-Food Canada.

### References


Johansson S, Dencker L & Danzner V (2001) Immunohistochemical localization of retinoid binding proteins at the maternal–


