Effects of dietary supplements of folic acid on reproductive performance in ewes

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Méthot, H., Girard, C. L., Matte, J. J. and Castonguay, F. W. 2008. **Effects of dietary supplements of folic acid on reproductive performance in ewes.** Can. J. Anim. Sci. **88**: 489–497. The objective of this project was to assess the impact of periconceptional folic acid supplementation on the reproductive performance of prolific and non-prolific ewes, in the estrous and anestrous seasons. Two initial trials took place during the estrous season at two experimental sites where 38 Dorset and 39 half-Finn half-Dorset ewes (site A) as well as 80 Dorset ewes (site C) were divided into two groups receiving either 0 or 210 mg ewe^{-1} d^{-1} of folic acid over a period which extended from 21 d premating to 30 d postmating. Three other trials were conducted in the anestrous season where 80 Dorset ewes (site A), 56 half-Romanov ewes (site B) and 78 Dorset ewes (site C) were subjected to the same protocol as the one used in the breeding season. In all the trials, the folic acid supplement increased plasma and red cell folates, but had no effect on fertility, embryonic mortality, or the size or weight of the litter at birth. Folic acid supplementation did not improve the reproductive performance of prolific and non-prolific ewes, either in the estrous season or in the anestrous period.

Key words: Ewes, reproduction, vitamins, folic acid, fertility, prolificacy

Méthot, H., Girard, C. L., Matte, J. J. et Castonguay, F. W. 2008. **Effet de la supplémentation en acide folique sur les performances de reproduction des brebis.** Can. J. Anim. Sci. **88**: 489–497. L'objectif de ce projet était d'évaluer l'impact d'une supplémentation périconceptionnelle en acide folique sur les performances de reproduction de brebis prolifiques et non prolifiques, en saison et en contre-saison sexuelle. Deux premiers essais ont eu lieu en saison sexuelle sur deux sites expérimentaux où 38 brebis Dorset et 39 demi-Finnois demi-Dorset (site A) ainsi que 80 Dorset (site C) ont été séparées en deux groupes recevant soit 0 ou 210 mg tête^{-1} jour^{-1} d'acide folique sur une période qui s'est étendue de 21 j précédant la saillie jusqu'à 30 j après la saillie. Trois autres essais ont été réalisés en contre-saison sexuelle où 80 brebis Dorset (site A), 56 demi-Romanov (site B) et 78 Dorset (site C) ont été soumises au même protocole que celui réalisé en saison sexuelle. Dans tous les essais, le supplément d'acide folique a augmenté les folates plasmatiques et érythrocytaires, mais n'a eu aucun effet sur la fertilité, la mortalité embryonnaire, la taille et le poids de la portée à la naissance. Les performances reproductives des brebis prolifiques et non prolifiques n'ont pas été améliorées par la supplémentation en acide folique, autant en saison qu'en contre-saison sexuelle.

Mots clés: Brebis, reproduction, vitamines, acide folique, fertilité, prolificité

The sheep industry is an emerging agricultural sector in North America. Unfortunately, sheep farm profitability is often low owing to the low productivity of livestock operations. Of the various means available to producers to increase their income, increasing the fertility and prolificacy of breeding ewes are certainly at the top of the list of foreseeable short-term solutions.

In the estrous season, ewe productivity is limited primarily by the low number of lambs born per ewe lambing (prolificacy). Widespread use of non-prolific breeds, which often give birth to only one lamb per lambing, remains the main reason for this low productivity. However, in sheep, 20 to 40% of fertilized ova are lost during gestation (Edey 1969; Ashworth 1995), primarily during the first month of gestation (Quinlivan et al. 1966). While, in the estrous season, this loss of embryos reduced the number of lambs born per lambing, in the anestrous season, the embryonic mortality could not only affect prolificacy, but also fertility rate. Indeed, in this period of the year, the number of ovulations is reduced and the loss of an embryo could mean the loss of the one and only embryo present in the uterus, leading to the end of pregnancy (Torrès et al. 1987; Webb et al. 1992). Hence, ewe productivity could be considerably improved if embryo survival at the start of gestation could be more effectively controlled and increased.

Some studies have shown that embryo mortality could be modulated by nutrition, particularly vitamin

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Abbreviations: DP, Dorset; **FLDP,** half-Finn half-Dorset; **THF,** 5,10-methylene tetrahydrofolic acid

supplementation. In fact, it has been demonstrated that folic acid, a B complex vitamin, is critical for embryo survival and fetal development in polytocous species such as rats (Tagbo and Hill 1977), hamsters (Moiij et al. 1993) and guinea pigs (Habibzadeh et al. 1986). In livestock animals, studies show that sows could benefit from folic acid supplementation, which would increase litter size (Matte et al. 1984; Thaler et al. 1989; Lindemann 1993) by decreasing embryonic mortality (Tremblay et al. 1989).

To date, there are only indirect observations to suggest that ewe reproduction could benefit from dietary supplements of folic acid. For instance, it has been demonstrated that serum folate concentrations fall sharply in ewes in the first half of gestation, and that this drop is more pronounced in prolific breeds (Girard et al. 1996). These changes are strikingly similar to those observed in sows (Matte et al. 1984; Harper et al. 1994), where beneficial effects of folic acid supplementations are observed on litter size (Matte et al. 1984; Thaler et al. 1989; Lindemann 1993). It has also been demonstrated that ewes from prolific breeds have a higher serum folate concentration than ewes from non-prolific breeds (Girard et al. 1996). In ewes, dietary supplements of folic acid increase serum folate concentrations (Girard et al. 1999). However, no studies have ever been conducted in sheep to measure the impact of long-term periconceptional folic acid supplementation on reproductive performance according to breed prolificacy and time of the year.

The objective of the present project was to verify the effect of periconceptional folic acid supplementation on folate-related blood parameters and on reproductive performance (fertility, ovulation rate, prolificacy, embryonic mortality) of prolific and non-prolific ewes, in the estrous and anoestrous seasons.

MATERIALS AND METHODS

The care and handling of the sheep used in this study were approved by the Laval University Animal Care Committee and were in accordance with the guidelines established by the Canadian Council on Animal Care (1993).

Preliminary Experiment

A preliminary experiment was conducted to determine the amount of dietary folic acid needed to substantially increase plasma concentrations of folates in ewes.

In a commercial flock (site A), 32 Dorset (DP) ewes were divided into four groups of eight animals each, based on body condition [range from 1 (very thin) to 5 (very fat)] and live weight. "Headgate" type feeders were installed inside each pen to ensure individual feeding. Forage was available ad libitum. Each group was then assigned to one of the four levels of folic acid supplementation. The day of the experiment, the ewes received 500 g ewe^{-1} d^{-1} of a commercial concentrate (15% crude protein) distributed in two meals (0700 and 1800). Folic acid supplement was served as a top dressing on the concentrate at doses of 0, 70, 140 or 210 mg ewe^{-1} d^{-1}, divided equally between the two meals. Supplementary folic acid (Rovimix, 80% pteroylmonoglutamic acid, Hoffman-LaRoche, Cambridge, ON) was mixed in ground corn (2.63% dilution). In order to monitor changes in plasma folates, jugular blood samples were collected by venipuncture using Vacutainer® EDTA tubes (Becton Dickinson and Co., Franklin Lakes, NJ), before the first supplemented meal, at 4, 7 and 10 h after the first meal, as well as 4 h and 13 h after the second meal of the day. Immediately after collection, the samples were centrifuged at $1800 \times g$ for 12 min. The plasma was then transferred into polypropylene microtubes and frozen at $-20°C$ until analysis.

Experiment 1 – Estrous Season

Two similar trials were conducted in the estrous season (matings in October and November) in commercial flocks (sites A and C; Table 1).

At site A, 38 non-prolific DP ewes and 39 prolific half-Finn half-Dorset (FLDP) hybrid females were allocated to two equal groups of DP and FLDP ewes with uniform characteristics within each genotype in terms of body condition and live weight, as well as average lifetime prolificacy. The two groups were subjected to the same protocol, but 21 d apart, in order to distribute the lambings over time. Within each group, the ewes of each genotype were allocated to two sub-groups, balanced for live weight, body condition and average lifetime prolificacy. The two sub-groups of each genotype were then assigned to one of the two levels of folic acid supplementation described below.

At site C, 80 DP ewes were allocated to two groups also balanced for live weight, body condition and average lifetime prolificacy. Each of these groups was then assigned to one of the two levels of folic acid supplementation. In this case, the experiment was carried out at the same time for all the ewes.

The animals were housed in pens with approximately 10 animals per pen. Feeders similar to those used during the preliminary experiment were installed. Forage was distributed ad libitum. The same protocol was repeated at both sites. Based on the results of the preliminary experiment, it was decided that the dose of 210 mg

Table 1. Number of ewes in exps. 1 and 2, for each site, genotype and folic acid treatment

				Folic acid treatment (n)	
Exp.	Season	Site	Breed	0 mg d^{-1}	210 mg d^{-1}
1	Estrous	A	Prolific	19	20
			Non-prolific	19	19
		C	Non-prolific	40	40
2	Anoestrous	A	Non-prolific	40	40
		B	Prolific	28	28
		C	Non-prolific	38	40

ewe^{-1} d^{-1} was the most appropriate for use in subsequent experiments (see the Results Section). The ewes were therefore assigned to one of the two levels of folic acid supplementation, either 0 mg ewe^{-1} d^{-1} (control) or 210 mg ewe^{-1} d^{-1} of folic acid. The dietary treatments began 21 d (day −21) before ram introduction (day 0) and ended on day 32 postmating (day 32) at site A and on day 30 postmating (day 30) at site C. Each ewe received two daily meals (at 10- to 12-h interval) of 250 g of commercial concentrate (16% crude protein). The supplemented ewes received their daily dose of folic acid divided into two meals and distributed as a top dressing on the feed. The control ewes received only the concentrate.

Five days after the start of the dietary treatments, the ewes were treated for 14 d with progestagen-impregnated vaginal sponges (Veramix, Upjohn, Orangeville, ON), in order to synchronize estrus and mating. At sponge removal, 500 IU of eCG (Folligon, Intervet, Whitby, ON) were administered by intramuscular injection to each ewe to improve estrus synchronization. Forty-eight hours later, rams were introduced to ewes for a period of 7 d with a ram:ewe ratio of 1:10. The rams were then removed for 7 d, and subsequently re-introduced for another 7-d period in order to cover the return to heat of non-pregnant ewes following mating at synchronized estrus. The rams were equipped with marking harnesses, which made it possible to record the exact mating date of each female. Seven days after the first introduction of the rams, laparoscopies were performed on the ewes to count the number of corpora lutea, corresponding to the number of ovulations.

In order to measure plasma folates, blood samples were collected from all ewes at site A and from half of the ewes at site C using Vacutainer EDTA tubes. All blood collections were taken before the first meal of the morning. At site A, blood was collected before the first supplemented meal (day −21), on the day of mating (day 0) as well as on days 10 and 32 of gestation. At site C, blood samples were taken on days −21, 0, 12 and 30. In addition, in order to monitor postprandial changes in plasma folates, blood samples were taken from 39 ewes at site A and 40 ewes at site C (approximately 20 ewes per treatment per site) just before the morning meal on day 0 as well as at 4 and 8 h following the meal. Immediately after collection, the samples were centrifuged at $1800 \times g$ for 12 min, and the plasma was collected and frozen at −20°C until analysis.

At site A, total folates and packed cell volume were measured in all the samples in order to calculate the red cell folate concentration. For this purpose, blood was collected using Vacutainer EDTA tubes, and 0.5 mL was then transferred into microtubes and mixed with 0.5 mL of an ascorbic acid (Sigma Chemical Corp., St-Louis, MO) solution (0.4 g of ascorbic acid in 100 mL of distilled water). The mixture was then frozen at −20°C.

The plasma concentration of vitamin B_{12} at the start of the experiment was determined from a plasma sample (Vacutainer EDTA tubes) taken from all the ewes on day −21.

The ewes' weight and body condition were recorded at the start of the experiment and at mating. The fertility rate at synchronized estrus (induced by vaginal sponge) and the overall fertility rate (which includes the ewes that conceived at synchronized estrus and those that conceived at return to heat) were calculated. The embryonic mortality, the number of lambs born per lambing (prolificacy), the birth weight of the lambs as well as the total weight of the litter at birth were recorded for ewes that conceived at synchronized heat. The embryonic mortality rate (%) was calculated as follows: [1 − (number of lambs born/number of corpora lutea)] × 100.

Experiment 2 – Anestrous Season

Three trials were conducted in the anestrous period (matings in June) in three commercial flocks, two of which were the same as in exp. 1 (sites A and C; Table 1).

At site A, 80 non-prolific DP ewes were separated into two uniform groups balanced for live weight, body condition and average lifetime prolificacy. The mating of the two groups was spaced 22 d apart. Each group of ewes was divided into two uniform sub-groups and one of the two dietary treatments was assigned to each sub-group (protocol similar to exp. 1 at site A). At site B, 56 prolific half-Romanov (half-RV) ewes were divided into two uniform groups balanced for live weight, body condition and genetic composition. Matings were synchronized with an interval of 9 d between the groups. Each group was then divided into two uniform sub-groups and one of the two dietary treatments was assigned to each sub-group (protocol similar to exp. 1 at site A). At site C, 78 DP ewes were divided into two uniform groups balanced for live weight, body condition and average lifetime prolificacy. Each of these groups was assigned to one of the two levels of folic acid supplements. The experiment was conducted at the same time for all the ewes.

At sites A and C, the animals were housed in pens with approximately 10 animals per pen. At site B, all the ewes receiving the same treatment were housed in a single pen. Individual feeders were installed to ensure uniform distribution of the concentrates. Forage was available ad libitum.

For this second series of experiments, folic acid was incorporated directly into the concentrate served to the ewes. The concentrates (16% CP), with or without added folic acid, were prepared in a commercial mill. The feeding regimen, the quantity of concentrate served (500 g ewe^{-1} d^{-1}), the dose of folic acid (210 mg ewe^{-1} d^{-1}) as well as the mating procedures were identical to exp. 1. A 600 IU dose of eCG (Folligon, Intervet, Whitby, ON) was administered at sponge withdrawal, since these trials took place during the seasonal

anestrous period. The rams were left with the ewes for a minimum mating period of 35 d. Laparoscopies were performed on the ewes at site C in order to determine the ovulation rate.

At sites A (40 ewes), B (27 ewes) and C (40 ewes), blood samples were collected before the first supplemented meal (day −21), on the day of mating (day 0) and on days 12 and 30 of gestation to measure the plasma concentrations of folates. On day 0, blood samples were taken 4 h following the morning meal in half of the ewes from the three sites to measure plasma folates. On day −21, a plasma sample from all the subjects was taken to measure vitamin B_{12} concentration at the beginning of the experiment. Blood samples were treated as described in exp. 1. The same production variables as those listed in exp. 1 were measured.

Blood Assays

Red cell folates, plasma folates and plasma vitamin B_{12} concentrations were measured in duplicate by radioimmunoassay with commercial assay kits used for human blood (Quantaphase Folate and Quantaphase B_{12}, Bio-Rad Laboratories Ltd., Mississauga, ON) as described by Lévesque et al. (1993), Girard et al. (1999) and Girard and Matte (1988) and validated for sheep (Girard et al. 1996). The interassay coefficients of variation were 3.0% ($n=1246$), 3.6% ($n=426$) and 2.8% ($n=184$) for plasma folates, red cell folates and plasma vitamin B_{12}, respectively. Packed cell volume was measured by microcentrifugation, in duplicate, in fresh blood.

Statistical Analyses

In preliminary study, concentration of plasma folates was analyzed in repeated measures using the MIXED procedure of the SAS Institute, Inc. (2001) with dose of folic acid supplementation, time of the day and dose × time interaction as sources of variation. The spatial power law was used as the covariance structure. The overall daily means of plasma folate levels were compared using orthogonal polynomial contrasts for linear, quadratic and cubic effects of the supplementation dose.

Red cell folate level was assayed only at site A in the estrous season (exp.1). These repeated measures were analyzed using PROC MIXED of SAS software (covariance structure = spatial power) with sources of variation including folic acid treatment (control and supplemented), genotype (DP and FLDP), day of collection and their interactions. For plasma folate concentration in exps. 1 and 2, analyses were performed using the same procedure of SAS software with folic acid supplementation, site (A and C), day of collection, season (estrous and anestrous seasons) and their interactions in the statistical model. The spatial power law was used as the covariance structure for these repeated measures. When significant treatment × day or season × day interactions were detected, simple effects of treatment or season were compared using the SLICE option of the LSMEANS statement of SAS software. In order to compare sites and seasons of reproduction, the blood samples taken on days 10 and 32 postmating at site A in the estrous season were considered as being days 12 and 30, respectively.

The categorical parameters (ovulation rate, fertility, number of lambs born, number of fetuses lost) were analyzed using the LOGISTIC procedure of SAS software.

The other production variables (ewes' weight and body condition, birth weight of the lambs and total weight of the litter at birth) and concentration of vitamin B_{12} were analyzed with the MIXED procedure. The dependent variables were studied within each season of reproduction (estrous and anestrous seasons) according to a model with main effects of folic acid treatment, site and their interaction. Comparison between genotypes (DP and FLDP) for the effect of folic acid supplementation on production traits (exp. 1 at site A) was performed using the MIXED procedure. Folic acid treatment, genotype and genotype × treatment interaction were factors included in the model. In order to ensure that the ewes included in the analyses on reproductive performance had received the treatment during a similar length of time (21 d before conception to 30 d after conception), only the data from ewes that lambed following conception at synchronized estrus were used for these analyses.

RESULTS

Preliminary Experiment

The postprandial changes in plasma folates varied according to the dose of folic acid (dose × time interaction, $P<0.001$; Fig. 1). The dose of folic acid of 210 mg ewe^{-1} d^{-1} resulted in a greater increase ($P<0.001$) in the average plasma concentration of folates than the other doses administered (1.26 ± 0.44, 1.49 ± 0.44, 3.13 ± 0.44 and 5.57 ± 0.44 ng mL^{-1} for 0, 70, 140 and 210 mg ewe^{-1}

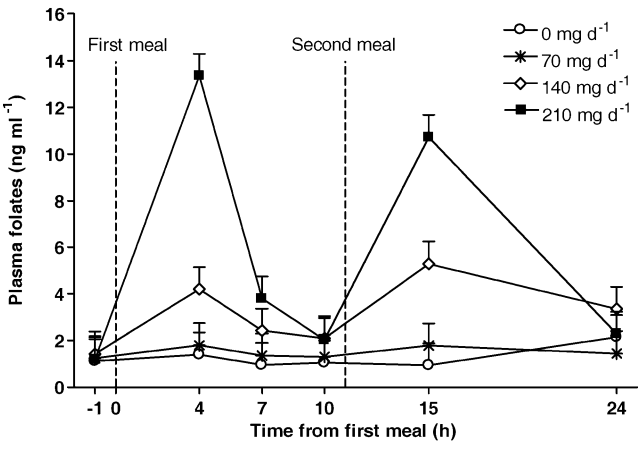

Fig. 1. Plasma folate concentrations in ewes supplemented with different daily doses of folic acid. Two daily meals of concentrate were given, at 0 h and 11 h later.

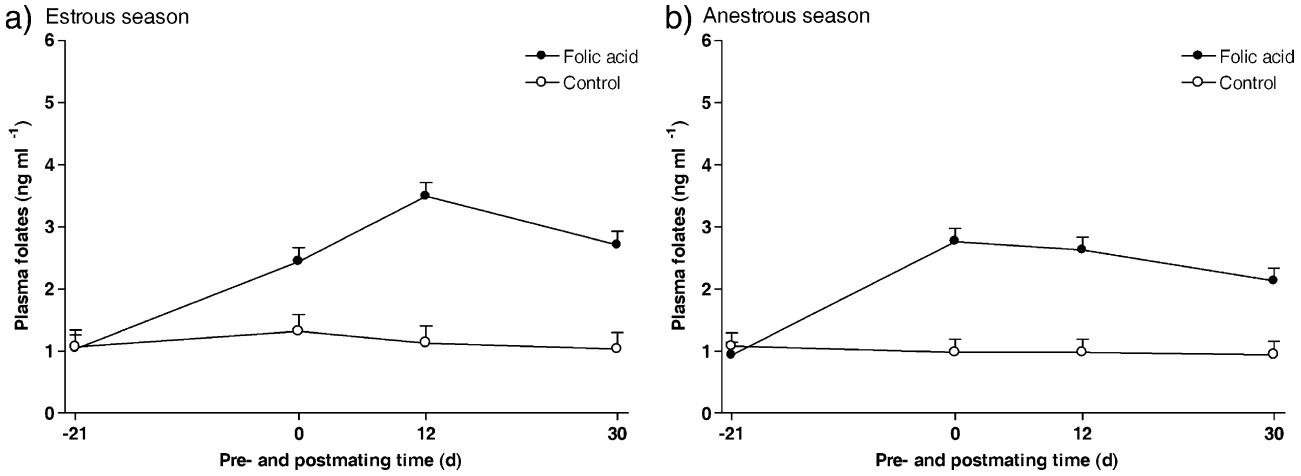

Fig. 2. Plasma folate concentrations in ewes according to the dietary supplement of folic acid (0 or 210 mg ewe^{-1} d^{-1}) given 21 d before to 30 d after mating (day 0) in (a) estrous season and (b) anestrous season (sites A and C).

d^{-1}, respectively). This dose was therefore used in the subsequent experiments.

Experiments 1 and 2

Plasma Folates

In all trials, changes in plasma concentrations of folates over time differed between the two treatments (treatment × day interaction, $P<0.001$) regardless of the season (Fig. 2) or genotype (Fig. 3). Ewes fed supplementary folic acid had higher concentrations than the control ewes. Plasma concentration of folates, 4 h after the meal was higher in ewes fed supplementary folic acid than 1 h before the meal (treatment × time interaction, $P<0.001$; data not shown), whereas, in the controls, there was no difference between the pre- and postprandial concentrations. The effect of the treatment differed according to the season of reproduction only at site A (treatment × season × site interaction, $P<0.01$), due to a greater plasma folate concentration at day 12 in the estrous than in the anestrous season. At site A, in the estrous season, plasma concentrations of folates, before the first folic acid meal, did not differ between non-prolific (DP) and prolific (FLDP) breeds (Fig. 3). Moreover, the response of the two genotypes to supplementation was similar ($P=0.9782$).

Red Cell Folates

At site A during the estrous season, the red cell concentration of folates (Fig. 3) varied according to the day of sample collection ($P<0.001$), the ewe's genotype (3.72 ± 0.16 and 4.19 ± 0.17 ng mL^{-1} for FLDP and DP, respectively; $P<0.05$) and the treatment (3.70 ± 0.16 and 4.21 ± 0.16 ng mL^{-1} for 0 and 210 mg ewe^{-1} d^{-1} of folic acid, respectively; $P<0.05$). Red cell

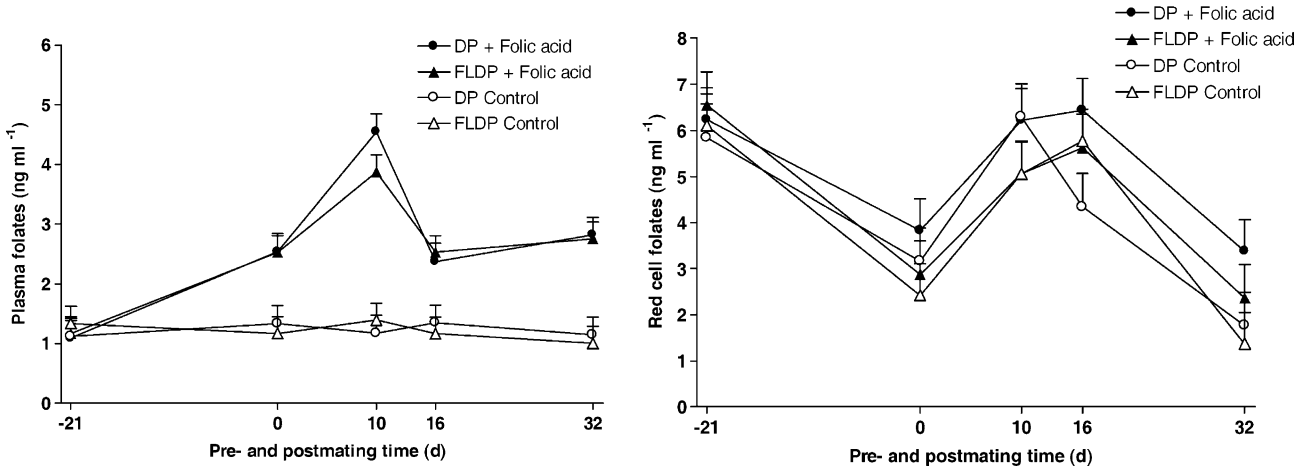

Fig. 3. Plasma and red cell folate concentrations in ewes according to the dietary supplement of folic acid (0 or 210 mg ewe^{-1} d^{-1}) given 21 d before to 32 d after mating (day 0) in non-prolific Dorset (DP) and prolific half-Finn half-Dorset (FLDP) ewes, treated in estrous season (site A).

concentration of folates dropped between day −21 and day 0 (mating) in all groups. Concentrations of red cell folates increased at day 10 and day 16 postmating, but decreased thereafter at day 32.

Vitamin B_{12}

Average plasma concentration of vitamin B_{12} at the beginning of the experiment (day −21) ranged from $1\,623 \pm 170$ to $4\,207 \pm 191$ pg mL^{-1}. Plasma concentration of vitamin B_{12} was higher ($P<0.001$) during the anestrous than the estrous season (site A: $2\,984 \pm 124$ vs. $1\,792 \pm 88$ pg mL^{-1}; site C: $4\,081 \pm 153$ vs. $3\,167 \pm 136$ pg mL^{-1}). At site A, plasma concentrations of vitamin B_{12} were similar between genotypes ($1\,757 \pm 116$ and $1\,813 \pm 115$ pg mL^{-1} for DP and FLDP, respectively).

Production Performance

There was no significant site × treatment interaction in either the estrous or anestrous season trials for production performance (Tables 2 and 3). Therefore, data from the experimental sites were analyzed together for each breeding season. In the estrous season, site effects ($P<0.05$) were observed for the ewes' body condition at mating, ovulation rate and the birth weight of the lambs and litter (data not shown). In the anestrous period, site effects were observed for the ewes' body condition at mating and the birth weight of the lambs (data not shown). In all trials, regardless of breeding season or ewe genotype, dietary supplements of folic acid had no effect on reproduction variables such as ovulation rate, fertility at synchronized estrus or overall fertility, number of lambs born or embryonic mortality rate (Tables 2 to 4). The comparison of prolific and non-prolific genotypes at site A (Table 4) showed that the ovulation rate of FLDP ewes receiving the folic acid treatment was higher than in the other three groups (genotype × treatment interaction, $P<0.05$). As anticipated, the total weight of the litter at birth was higher ($P<0.05$) in the prolific FLDP ewes (7.2 ± 2.2 vs. 8.2 ± 1.6 kg for DP and FLDP, respectively), a result mainly explained by a near significant ($P=0.076$) increase in the number of lambs born in FLDP ewes (1.47 ± 0.51 vs. 1.79 ± 0.55 for DP and FLDP, respectively).

DISCUSSION

Effect of Dietary Supplements of Folic Acid on Concentrations of Plasma and Red Cell Folates and Plasma Vitamin B_{12}

One of the objectives of this study was to verify the effects of long-term folic acid supplementation on plasma folate concentrations. Regardless of the breeding season, the periconceptional supplementation of 210 mg ewe^{-1} d^{-1} of folic acid administered for a period of approximately 50 d increases plasma and red cell concentrations of folates for the entire duration of supplementation as compared with control. These observations confirm previous findings in women (Scholl et al. 1996), pigs (Tremblay et al. 1986; Matte et al. 1996; Matte and Girard 1999), calves (Girard et al. 1992) and cows (Girard and Matte 1999). In sheep, experiments have been conducted only with one-time supplementation during gestation (Girard et al. 1999).

Moreover, absorption of dietary supplements of folic acid is confirmed by a significant increase in plasma folate concentrations measured 4 h after a supplemented meal (Fig. 1) as previously observed by Girard et al. (1999). Plasma concentrations of folate fell 7 h after the meal, confirming the direct and rapid impact of the addition of the vitamin to the ewes' ration.

In a previous study, Girard et al. (1996) showed that, in the absence of folic acid supplementation, ewes of prolific breeds had higher plasma concentrations of folates than less prolific ewes (Romanov > Finn > Suffolk) at the time of mating and during gestation. In the present study, there were no differences in plasma folate concentrations between prolific and non-prolific ewes in the control group. Moreover, genotypes of different prolificacy did not influence the response of plasma folates to supplementation. However, the prolific crossbred ewes used in this study had smaller litters than the prolific pure breeds studied by Girard et al. (1996).

Table 2. Reproductive performance in ewes receiving (210 mg ewe^{-1} d^{-1}) or not (0 mg ewe^{-1} d^{-1}) a supplementation of folic acid in estrous season (sites A and C)

Item	Folic acid supplementation		SEM	*P* value
	0 mg d^{-1}	210 mg d^{-1}		
No. of synchronized ewes	78	79		–
Body condition at mating	3.1	3.0	0.1	NS
Ovulation rate at synchronized estrus	2.0	2.2	0.1	NS
Fertility rate at synchronized estrus (%)	85.3 (66/77)	88.5 (70/79)		NS
Overall fertility rate (%)	92.2 (71/77)	96.2 (76/79)		NS
Prolificacy[z]	1.75	1.74	0.07	NS
Embryonic mortality[z] (%)	14.1	16.5	2.9	NS
Lamb weight at birth[z] (kg)	5.2	5.3	0.1	NS
Litter weight at birth[z] (kg)	8.7	8.7	0.3	NS

[z]Calculated only with ewes that conceived at the synchronized estrus.

Table 3. Reproductive performance in ewes receiving (210 mg ewe^{-1} d^{-1}) or not (0 mg ewe^{-1} d^{-1}) a supplementation of folic acid in anestrous season (sites A, B and C)

Item	Folic acid supplementation		SEM	*P* value
	0 mg d^{-1}	210 mg d^{-1}		
No. of synchronized ewes	106	108		–
Body condition at mating	3.2	3.4	0.1	NS
Ovulation rate at synchronized estrus[z]	3.0	2.6	0.3	NS
Fertility rate at synchronized estrus (%)	78.3 (83/106)	73.1 (79/108)		NS
Overall fertility rate (%)	82.1 (87/106)	79.6 (86/108)		NS
Prolificacy[y]	2.07	2.09	0.11	NS
Embryonic mortality[zy] (%)	26.6	19.3	4.9	NS
Lamb weight at birth[y] (kg)	4.2	4.2	0.1	NS
Litter weight at birth[y] (kg)	8.1	8.0	0.3	NS

[z]Only evaluated on site C ($n = 78$).
[y]Calculated only with ewes that conceived at the synchronized estrus.

Folic acid supplementation produced an overall increase in red cell folate concentration (Fig. 3), which is an indicator of the body's folate reserves. The cause of the decline observed in red cell concentration of folates between day −21 and mating in all groups is unknown, and such decline has never been reported previously. Concentrations of red cell folates increased at day 10 and day 16 postmating, but decreased thereafter as gestation progressed, even in supplemented animals. This decline in red cell folates during gestation was previously observed in ewes (Girard et al. 1999), and could be explained by increased fetal uptake of folates at the expense of maternal reserves. Higgins et al. (2000) reported, in humans, a higher demand for folic acid during gestation owing to the high requirements for cell synthesis. It, therefore, appears that the level of folic acid supplementation used in this study, i.e., 210 mg ewe^{-1} d^{-1}, was not successful in preventing the drop in red cell folates and then, possibly, failed to maintain constant the pregnant female's body reserves of folates throughout the critical period for embryo survival. It may, therefore, be relevant to study the impact of folic acid supplementation beginning earlier than 21 d before mating in order to prevent this drop in red cell folates observed during gestation despite supplementation. In addition, since no plateau in the increase in plasma folates in response to the different doses of folic acid was observed in the preliminary experiment of this study (Fig. 1), trials with doses higher than 210 mg ewe^{-1} d^{-1} could also be attempted.

In the comparison of genotypes of different prolificacy, red cell folate concentrations were lower in the FLDP ewes. This could be explained by a greater weight of the litter at birth in these ewes compared with the DP ewes, which would increase fetal uptake of folates in these more prolific females. The fact that the FLDP ewes had lower red cell folate values than the DP ewes, but equivalent plasma folate concentrations, suggests that, in prolific ewes, plasma folates were preferentially redirected towards fetuses rather than maternal tissues.

One of the known factors that can interfere with the action of folic acid is the presence of vitamin B_{12} (Bässler 1997). The sole biochemical function of folic acid in mammals is to mediate the transfer of one-

Table 4. Reproductive performance in non-prolific Dorset (DP) and prolific half-Finn half-Dorset (FLDP) ewes receiving (210 mg ewe^{-1} d^{-1}) or not (0 mg ewe^{-1} d^{-1}) a supplementation of folic acid in estrous season (site A)

Item	DP		FLDP		SEM	*P* value[y]		
	0 mg d^{-1}	210 mg d^{-1}	0 mg d^{-1}	210 mg d^{-1}		G	S	G × S
No. of synchronized ewes	19	19	19	20				
Body condition at mating	3.7	3.7	3.3	3.1	0.2	<0.01	NS	NS
Ovulation rate at synchronized estrus	1.8	1.6	1.9	2.3	0.1	NS	NS	<0.05
Fertility rate at synchronized estrus (%)	84.2 (16/19)	84.2 (16/19)	78.9 (15/19)	90.0 (18/20)		NS	NS	NS
Overall fertility rate (%)	94.7 (18/19)	100.0 (19/19)	84.2 (16/19)	95.0 (19/20)		NS	NS	NS
Prolificacy[z]	1.44	1.5	1.81	1.78	0.15	<0.1	NS	NS
Embryonic mortality[z] (%)	14.6	6.3	11.6	20.4	6.1	NS	NS	NS
Lamb weight at birth[z] (kg)	5.2	5	4.9	4.8	0.2	NS	NS	NS
Litter weight at birth[z] (kg)	7.3	7.2	8.6	7.9	0.5	<0.05	NS	NS

[z]Calculated only with ewes that conceived at the synchronized estrus.
[y]G, genotype; S, supplementation of folic acid.

carbon units (Choi and Mason 2000). Folic acid, as 5,10-methylene tetrahydrofolic acid (THF) and 10-formyl-THF, gives one-carbon units for pyrimidine and purine synthesis, essential for DNA formation. Moreover, 5,10-methylene-THF can be irreversibly reduced to 5-methyl-THF, which under the action of the vitamin B_{12}-dependent enzyme, methionine synthase, will transfer its methyl group to homocysteine for regeneration of methionine and THF (Bässler 1997). A lack of vitamin B_{12} would block 5-methyl-THF demethylation and reduce folate utilization at the cell level in spite of the accumulation of 5-methyl-THF in plasma (Scott 1999). To verify that the ewes were not B_{12}-deficient, plasma concentration of B_{12} was measured at the start of the experiment. According to Clark et al. (1989), the acceptable lower limit for sheep is approximately 500 pmol L^{-1}, or approximately 675 pg mL^{-1}, whereas the mean values of the ewes in this study ranged from 1623 ± 170 to 4207 ± 191 pg mL^{-1}. Therefore, these animals likely had an adequate vitamin B_{12} status to ensure normal physiological functions and normal metabolic activity of folates.

Effect of Dietary Supplements of Folic Acid on Reproductive Performance

This study is the first to examine the effects of folic acid supplementation on the reproductive performance of ewes. The present periconceptional supplementation did not improve ewe fertility either in the estrous or in the anestrous season. Nor did it increase litter size, as has been demonstrated in some studies on pigs (Matte et al. 1984; Thaler et al. 1989; Lindemann 1993). However, it should be pointed out that, even in pigs, the effects of folic acid supplementation can vary. For instance, no significant effect of folic acid supplementation on litter size was observed during a number of other studies conducted on primiparous sows (Matte et al. 1992, 1993; Harper et al. 1996). In pigs, the effect of folic acid supplementation on litter size, embryo survival and uterine secretions would be dependent upon the parity (Matte et al. 2006).

An increased ovulation rate generally leads to an increase in embryonic mortality (Restall et al. 1976; Ricordeau et al. 1986; Scaramuzzi and Downing 1997). Therefore, it is possible that the ovulation rate in the present study (between 1.6 and 3.0 ovulations) may have been too small to allow the full expression of embryonic mortality potential and, then, to demonstrate the impact of folic acid supplementation. It would be of interest to conduct a similar protocol with breeds recognized for their high ovulation rate, such as purebred Romanov or Finnish Landrace breeds, to evaluate the effects of folic acid supplements on embryo survival, given the known positive correlation between embryo losses and increased ovulation rate. In the present study, although folic acid supplementation did not change the ovulation rate of the non-prolific ewes, the value increased in FLDP ewes receiving folic acid supplementation as compared with control ewes. This observation is in contradiction with studies on pigs in which folic acid supplementation has little (Harper et al. 1996) or no effect on the ovulation rate of females (Tremblay et al. 1989; Matte et al. 1996). Such an observation might be an indication of another mode of action of folic acid in sheep, and deserves to be confirmed and further evaluated using breeds recognized for their high ovulation rate, as mentioned above.

CONCLUSION AND IMPACT

Although a dietary folic acid supplement of 210 mg ewe^{-1} d^{-1}, given between 21 d premating and 30 d postmating, increased plasma and red cell folate concentrations, it had no effect on embryonic mortality, fertility, the number of lambs born or the weight of the litter at birth. Season of reproduction and ewe prolificacy had no observable impact on these results. However, some of the results suggest that it may be relevant, from a research perspective, to repeat the experiment with breeds of ewes that have a much higher ovulation rate. Nevertheless, from a practical standpoint, it seems premature to recommend any folic acid supplementation in the ewe's ration during the periconceptional period. Additional trials should be attempted with higher doses and/or a longer period of supplementation before mating.

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