

# Serum and Milk Folates During the First Two Gestations and Lactations in Romanov, Finnsheep, and Suffolk Ewes<sup>1</sup>

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**ABSTRACT:** Concentrations of folates in serum and milk were determined during the first two parities in 7 Suffolk, 12 Finnsheep, and 15 Romanov ewes at 0 (mating), 60, 110, and 140 d of gestation and 1, 7, 14, 21, and 50 d of lactation. The number of lambs born at first and second parturitions was  $1.38 \pm .13$  and  $1.71 \pm .13$  for Suffolk,  $2.00 \pm .32$  and  $2.33 \pm .57$  for Finnsheep, and  $2.67 \pm .24$  and  $3.20 \pm .22$  for Romanov, respectively, and the concentration of folates at d 0 was  $1.17 \pm .21$ ,  $1.87 \pm .19$ , and  $2.14 \pm .17$  ng/mL, respectively. During gestation, serum concentrations of folates declined in the three breeds, more quickly in Romanov and Finnsheep (from d 0 to 60) than in Suffolk (from d 0 to 110). This decline was followed by an increase in serum concentrations of folates from d 110 to 140 of gestation in the three breeds; the largest increase was observed in Romanov (breed  $\times$  quadratic effect of time,  $P = .001$ ). During lactation, Romanov had higher

serum concentrations of folates than Finnsheep, which in turn had a higher concentration than Suffolk ( $P = .001$ ). Colostrum of Romanov had the highest concentration of folates ( $164.9 \pm 11.9$  ng/mL), followed by that of Finnsheep ( $125.0 \pm 9.5$  ng/mL) and Suffolk ( $98.2 \pm 13.3$  ng/mL). On d 7 of lactation, concentration of folates was similar in milk of Romanov ( $97.1 \pm 5.6$  ng/mL) and Finnsheep ( $94.9 \pm 4.0$  ng/mL) and was higher than in Suffolk ( $72.4 \pm 3.7$  ng/mL). From d 14, the concentration of milk folates was similar among breeds and decreased slowly to approximately 40 ng/mL at d 50 (breed  $\times$  quadratic effect of time,  $P = .005$ ). The changes in serum concentrations of folates during gestation of ewes were similar to those observed in sows, suggesting that exogenous supply of folates may not be optimal in ewes during gestation and lactation.

Key Words: Folic Acid, Sheep, Pregnancy, Lactation, Serum, Milk

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## Introduction

It is generally considered that synthesis of folates by ruminal microorganisms makes the inclusion of folic acid in the diet unnecessary (McDowell, 1989). However, in dairy cows, supply of folates by diet and ruminal microorganisms is not sufficient to optimize folate status and lactational performance (Girard et al., 1989, 1995). In many species, the demand for folic acid increases during gestation and lactation. Fetal storage of folates increases during the last part of gestation in humans (Loria et al., 1977) and rats (Potier de Courcy and Terroine, 1979). In polytocous

species, folic acid is considered essential for embryo survival (Habibzadeh et al., 1986; Tagbo and Hill, 1977; Tremblay et al., 1989; Moijj et al., 1993). It is possible that in sheep, especially in prolific breeds, the requirements for folic acid increase during gestation and cannot be completely fulfilled by diet and microbial synthesis. Serum folates are accurate indicators of folate status when feed ingredients and feeding times are kept constant throughout the experimental period (Gee et al., 1989; Clifford et al., 1990). Consequently, the present experiment was undertaken to observe variations of folate concentrations in serum and milk, as indicators of folate status, during the first two gestations and lactations of ewes of three breeds with different levels of prolificacy.

## Materials and Methods

**Animals.** Blood and milk samples were taken from 7 Suffolk, 12 Finnsheep, and 15 Romanov ewes that had completed their first two parities in the herd of the

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Sheep Research Farm of Agriculture and Agri-Food Canada at La Pocatière. The number of lambs born was  $1.38 \pm .13$  and  $1.71 \pm .13$  for Suffolk,  $2.00 \pm .32$  and  $2.33 \pm .57$  for Finnsheep, and  $2.67 \pm .24$  and  $3.20 \pm .22$  for Romanov at the first and second parturitions, respectively. At approximately 8 mo of age, the ewes were synchronized for estrus using vaginal sponges (Véramix, Upjohn, Orangeville, ON). At sponge removal, 14 d later, each ewe received 350 IU of pregnant mare serum gonadotropin (Équinex, Laboratories Ayerst, St-Laurent, QC) to stimulate follicular development and was mated to a sire of her respective breed.

The ewes were housed continuously indoors in large pens of about 10 animals each. One week before the expected date of lambing, ewes were transferred to individual lambing pens of 1.8 m  $\times$  2.9 m each. The ewes and their lambs were returned to the large pens 24 h after lambing and kept there until weaning at 50 d. A maximum of two lambs were left to suckle the ewe; when necessary, the extra lambs were raised artificially on milk replacer. The same procedures were repeated for the second parity.

During the whole experimental period, ewes had free access to a high-moisture grass silage (approximately 25% DM and 16% CP), fresh water, and minerals. Six weeks before parturition, the ewes were fed 200 g/d of a commercial concentrate (15% CP); starting at 200 g/d, the quantity of concentrate was increased gradually to reach 700 g/d at lambing. During lactation, ewes nursing single lambs received 500 g/d of concentrate, whereas those nursing twins received 700 g/d. The concentrate was given to meet the NRC (1985) recommendations. Fresh feed was served every day at 0900.

**Blood and Milk Sampling.** For the two parities, blood samples were taken at 1000 with a Vacutainer<sup>®</sup> system by jugular venipuncture at mating (d 0), at 60, 110, and 140 d of gestation, and at 1, 7, 14, 21, and 50 d of lactation. Blood was allowed to clot in the dark at 4°C for 24 h. The serum was separated by centrifugation at  $1,854 \times g$  for 10 min, transferred in polypropylene tubes, and stored at -20°C until assayed.

Milk was collected at 1, 7, 14, 21, and 50 d after lambing. One hour before manual milking, lambs were removed from the dams. Milk samples were immediately stored at -20°C until assayed.

**Folate Determination.** Procedures for preparation of samples and folate determination were described by Girard et al. (1989). The only modification was the addition of 100  $\mu$ L of bovine serum albumin (7%) to 100  $\mu$ L of hydrolysate before running the assay to determine milk folates to restore a viscosity similar to serum. Two hydrolysates were made per milk sample. Serum and milk folates were measured in duplicate in two different assays by radioassay with a commercial kit used for human serum (Quantaphase Folate, Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON)

and validated for sheep serum and milk. The validation tests for both assays showed satisfactory parallelism (CV among dilutions < 3.8% for serum and 2.5% for milk); for values smaller than 1 ng/mL, the quantity of serum used in the assay was doubled (400  $\mu$ L). In this case, however, the value obtained was overestimated by 27%, as observed from recovery tests, and the appropriate correction was made. With 200  $\mu$ L, the interassay coefficient of variation and recovery tests were 3.55% (SE = .17, n = 400) and 100.2% (SE = 1.4, n = 26), respectively, for serum and 3.75% (SE = .18, n = 400) and 97.7% (SE = 1.1, n = 24) for milk.

**Statistical Analysis.** The concentrations of serum folates during gestation and lactation and milk folates during lactation were analyzed using the General Linear Model procedure of SAS (1985) according to the following model:  $Y_{ijkl} = \mu + B_i + P_j + BP_{ij} + E_k(BP_{ij}) + T_l + BT_{il} + PT_{jl} + BPT_{ijl} + \epsilon_{ijkl}$ , where  $Y_{ijkl}$  indicates the dependent variables, serum or milk folates. The overall mean is  $\mu$ ,  $B_i$  is the breed effect,  $P_j$  is the effect of parity, and  $T_l$  is the effect of time of gestation or lactation. The error  $\epsilon_{ijkl}$  is ewe within breed and parity,  $E_k(BP_{ij})$ , and  $\epsilon_{ijkl}$  is the residual error. A Bonferroni *t*-test was used to test for significant differences among means when the effects reached a level of significance of 95%. The analysis of repeated measurements (time and its interactions) was made according to Rowell and Walters (1976).

## Results

The effect of breed on serum concentrations of folates was similar during the two gestations (breed  $\times$  number of gestation,  $P = .93$ ). The mean concentration of folates during gestation was higher in Romanov ( $1.71 \pm .11$  ng/mL) than in Finnsheep ( $1.52 \pm .12$  ng/mL), with Suffolk showing the lowest concentration ( $1.35 \pm .12$  ng/mL;  $P = .001$ ). However, serum concentrations of folates changed differently during gestation according to the breed (breed  $\times$  quadratic effect of time,  $P = .001$ ; Figure 1). In Finnsheep and Romanov, serum concentrations of folates declined markedly from mating to 60 d of gestation, whereas in Suffolk, they decreased at a slower rate from mating to 110 d of gestation. Serum concentrations of folates increased from 110 to 140 d of gestation in the three breeds; the greatest increase was observed in Romanov. Concentrations at 140 d of gestation were similar to those observed at the previous mating.

The effect of breed on serum concentrations of folates was similar during the two lactations (breed  $\times$  number of lactation,  $P = .49$ ). The serum concentrations of folates during lactation varied according to the breed ( $P = .001$ ). Romanov had higher serum folate concentrations ( $1.81 \pm .12$  ng/mL) than Finnsheep ( $1.41 \pm .10$  ng/mL), and the lowest concentration was also observed in Suffolk ( $1.05 \pm .10$  ng/mL). However,

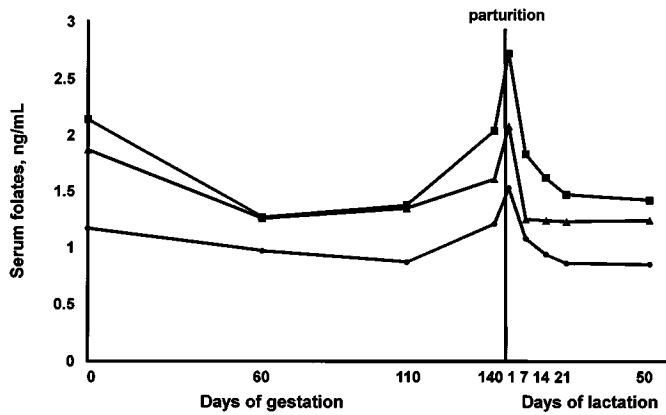


Figure 1. Serum concentrations of folates during gestation and lactation of Suffolk (—●—), Finnsheep (—▲—), and Romanov (—■—). At 0, 60, 110, and 140 d of gestation, SE were, respectively, .21, .10, .07, and .13 for Suffolk; .19, .11, .09, and .10 for Finnsheep; and .17, .08, .05, and .13 for Romanov. At 1, 7, 14, 21, and 50 d of lactation, SE were, respectively, .14, .13, .08, .05, and .08 for Suffolk; .22, .07, .07, .07, and .07 for Finnsheep; and .21, .15, .12, .08, and .06 for Romanov.

the pattern of serum concentrations of folates during lactation was similar for the three breeds (breed × time,  $P = .37$ ; Figure 1).

The parity modified the pattern of serum folates during gestation (parity × quadratic effect of time,  $P = .001$ ) and lactation (parity × quadratic effect of time,  $P = .01$ ; Figure 2). During the first gestation, serum folates were higher at mating and 60 d gestation than in the second gestation. However, as the gestation

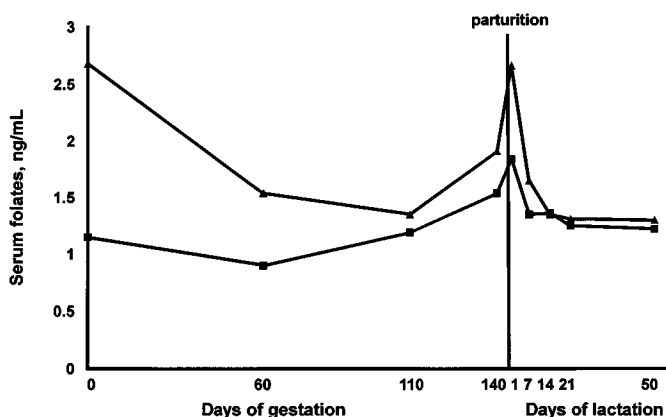


Figure 2. Serum concentrations of folates during the first (—▲—) and the second (—■—) parity of ewes. At 0, 60, 110, and 140 d of gestation, SE were, respectively, .12, .07, .07, and .14 in first gestation and .07, .04, .06, and .09 in second gestation. At 1, 7, 14, 21, and 50 d of lactation, SE were, respectively, .21, .14, .09, .07, and .07 in first lactation and .14, .10, .11, .08, and .06 in second lactation.

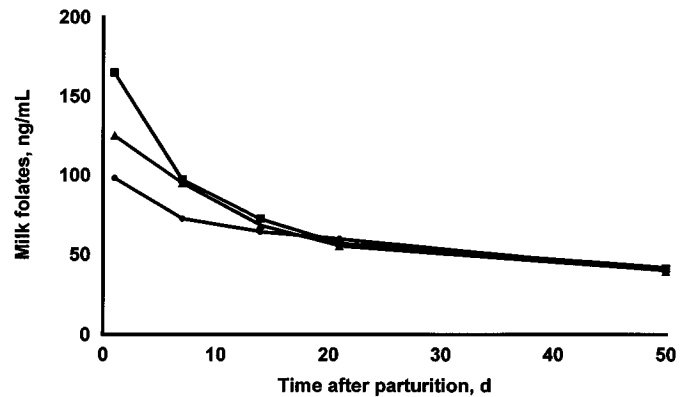


Figure 3. Milk concentrations of folates during lactation of Suffolk (—●—), Finnsheep (—▲—), and Romanov (—■—). At 1, 7, 14, 21, and 50 d of lactation, SE were, respectively, 13.33, 3.71, 3.50, 3.34, and 2.10 for Suffolk; 9.47, 3.97, 3.37, 2.17, and 1.41 for Finnsheep; and 11.87, 5.61, 3.49, 2.52, and 2.42 for Romanov.

progressed, at 110 and 140 d, the effect of parity became negligible. The first day after parturition, serum concentration of folates was higher in first than in second lactation. No further differences between the first and second lactations were observed with advance in lactation.

Milk folates were not affected by the lactation number ( $P = .21$ ) or the interaction of lactation number × breed ( $P = .64$ ), but the pattern of milk concentrations of folates during lactation differed according to the breed (breed × quadratic effect of time,  $P = .005$ ; Figure 3). Concentration of folates was higher in colostrum of Romanov than in that of Finnsheep ewes, whereas that in Suffolk ewes ranked lowest. On d 7 of lactation, concentration of folates in milk was similar for Romanov and Finnsheep and higher than that of Suffolk. Milk concentrations of folates were similar in the three breeds and declined slowly from 14 to 50 d of lactation.

### Discussion

Serum concentrations of folates observed in the present experiment were in the same range as those determined by radioassay (Wang and Masters, 1990) and microbiological (*Lactobacillus casei* ssp.) assay (Wang et al., 1991) in growing sheep.

Whatever the breed studied, serum concentrations of folates decreased between mating and 60 d of gestation. This decrease was greater in prolific breeds; they were of 17%, 32%, and 41% for Suffolk, Finnsheep, and Romanov, respectively. The pattern observed in ewes in the present experiment is similar to that observed in sows, in which serum concentration of folates decreases by 30 to 40% from conception to midgestation (Anzhi and Cooper, 1989; Harper et al.,

1994; Matte et al., 1984b, 1992; O'Connor et al., 1989; Thaler et al., 1989). The decrease of serum concentrations of folates during the first part of the gestation could reflect an increased demand for folic acid by developing embryos and their membranes that go through rapid and intensive cell division during this period. Serum concentrations of folates increased near lambing time (140 d of gestation), as frequently observed near farrowing in sows (Matte et al., 1984b, 1992; Thaler et al., 1989). O'Connor et al. (1989) and Harper et al. (1994) reported, however, that serum folates of sows remain low until the end of gestation. In the present study, serum folates of ewes were higher at the beginning of the first gestation than at the second one. Thaler et al. (1989) observed a similar phenomenon in sows fed a diet unsupplemented with folic acid. This could be related to a depletion of maternal reserves due to the demand of repeated gestations and lactations.

During lactation, serum folates decreased drastically during the first days of lactation and remained low until weaning, at 50 d. This pattern is opposite to that observed in sows, in which serum folates increase from the end of gestation or at parturition to weaning (Harper et al., 1994; Matte and Girard, 1989; Matte et al., 1992; O'Connor et al., 1989; Thaler et al., 1989). The decrease of serum folates and, moreover, the low concentration maintained throughout the lactation could reflect a high demand of folates for milk synthesis and transfer of folates to milk.

It is unlikely that changes of serum concentrations of folates observed during repeated gestations and lactations of ewes were related to changes in diet (i.e., addition of concentrates to the ration from 6 wk prepartum to weaning). Work with steers demonstrated that the effect of diet unsupplemented with folic acid on serum folates is small; serum concentrations of folates were similar for steers fed a diet based on forages (30% barley - 70% hay) or grain (70% barley - 30% hay) (Girard et al., 1994).

Regardless of the breed, folate concentration in milk decreased drastically between colostrum and milk and that was more marked in prolific breeds such as Romanov and Finnsheep than in Suffolk. Differences among breeds had vanished after 14 d of lactation. This absence of breed effect could be attributable to the herd management used in which no more than two lambs were left to suckle the ewe.

In litter-bearing species, dietary folic acid is critical for normal reproduction and fetal development. In sows, research has shown an improvement in the number of pigs born, total and alive, when sows were given a supplement of folic acid during gestation (Lindemann, 1993). In rats (Tagbo and Hill, 1977; Potier de Courcy and Terroine, 1979), in hamsters (Moijj et al., 1993), and in guinea-pigs (Habibzadeh et al., 1986), folic acid is essential to embryo survival. In swine, the increase in the number of pigs born is

believed to be caused by an increased rate of embryo survival during the first 30 d of gestation. Moreover, the favorable effect of folic acid in reducing embryonic mortality is especially noticeable when ovulation rate is high (Matte et al., 1984a; Lindemann and Kornegay, 1989; Tremblay et al., 1989). The effects of supplementary folic acid seem to be less marked in primiparous than in multiparous sows (Lindemann and Kornegay, 1989), in which ovulation rate is higher and folate stores depleted by repeated gestations and lactations.

Supplementary folic acid increases the concentration of protein in the embryo (Tremblay et al., 1989), which may explain the increase in litter and pig weights (Matte et al., 1984a, 1992). In humans (Goldenberg et al., 1992; Tamura et al., 1992) and in hamsters (Moijj et al., 1993), supplementary folic acid reduces the incidence of fetal growth retardation and increases birth weight.

In dairy cows, total serum folates decrease from 2 mo postpartum to calving (Girard et al., 1989). The demand of tissue for folic acid is higher during late gestation than during early lactation (Girard and Matte, 1995). A supplement of folic acid increases milk production and content of milk protein during the last half of the lactation but increases only milk protein content during the first weeks after parturition in multiparous dairy cows (Girard et al., 1995).

Observations in sheep in the present experiment are similar to those made on other species and could give an indication that the demand for folic acid increases during gestation and lactation. In sheep and swine, embryonic mortality is responsible for the majority of losses during gestation (Pope, 1994; Nancarrow, 1994). Consequently, in sheep as in other polytocous species, a supplement of folic acid provided during gestation might have a beneficial effect on survival rate of embryos and subsequently on the number of lambs born if the observed fall of serum folates was representative of a lack of folic acid at the tissue level. Similarly, supplementary folic acid given to ewes during lactation could also improve milk production, as previously observed in dairy cows (Girard et al., 1995). In the present study, differences among breeds of varying degrees of prolificacy might provide an indication of the role of folic acid on embryo survival, and the more prolific breeds might have developed an ability to retain more folates through evolution. Literature references were not found in which serum folates were compared in prolific and non-prolific breeds in sheep or in other species to corroborate this theory.

### Implications

In ewes, supply of folates by the diet and the synthesis by ruminal microorganisms is not sufficient

to maintain the serum concentrations of folates throughout gestation and lactation at a constant level. Similarity between ewes and sows in the variations of serum folates during gestation indicate that, as in sows, the demand for folic acid increases during gestation and could not be completely fulfilled in ewes during this period. Consequently, the effect of supplementary folic acid on prolificacy in ewes needs to be investigated, taking into account the beneficial effect on survival rate of embryos and litter size in other polytocous species.

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