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FETAL SURVIVAL AND NEONATAL GROWTH WITH INTRAMUSCULAR INJECTIONS OF FOLATE DURING GESTATION IN THE RAT

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ABSTRACT

Folic acid (2.0 mg/ml) was injected subcutaneously to rat dams beginning on day 2 after mating (day 0 = day of sperm in vaginal smear). At parturition litter size per dam and the weight of fetuses were determined. Pups were thereafter equalized among the two groups (n=16 each) and assigned to four dams in each group (n=4 pups per dam). They were weighed every 5 days until day 20. On day 20 pups were killed, dried, and the crude protein levels of carcasses assessed using the Kjeldhal's method. Litter size (7) and weight (6.21) of pups from treated dams were not significantly different from those of control (6.4 and 5.9 respectively, $P<0.05$). Regression analysis and t-test indicated a faster growth rate and significantly higher crude protein level among pups from treated dams than those of controls. It was concluded that folic acid treatment of dams during gestation and subsequent lactational performance of treated dams may have ameliorated the protein-synthesizing function of cells of neonates from treated dams but further experiments are needed to confirm this conclusion.

Key words: Folic acid, rat, pregnant, fetus.
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INTRODUCTION

Folic acid (pteroglutamic acid) is a vitamin of the B complex which is essential in animal metabolism. It is necessary for the synthesis of nucleic acids because of its requirement for the synthesis of purines and pyrimidine which are components of nucleic acids (Valencia, 1974, Huennekens, 1968, EB, 2004). It is therefore required for the production of DNA and RNA (Herbert and Das, 1976; Davis and Nicol, 1988) and proteins (Chang and Kaiser, 1972). Tissues that require intense cellular hyperplasia and hyperthrophy (such as the mammalian fetus) require protein accretion (Whittemore *et al.* 1988; McDowell, 1989) and hence folic acid supplementation. For this reason, pregnant women with insufficient intake of folic acid are more likely to give birth prematurely or to deliver babies with low birth weight (EB, 2004).

In ruminants, folic acid is synthesized by the gut microflora (Girald, *et al.* 1989) so that exogenous supply of the vitamin to ruminants, in most feeding regimen, is unnecessary. Non-ruminants (such as the rat) and humans, however, cannot synthesize folic acid (Blakley, 1969) and requirements must be met, for the most part, through exogenous supply, especially in the diet.

The following experiment was to test the effect of exogenous administration of folic acid on fetal survival and development and neonatal growth performance in the rat.

MATERIALS AND METHODS

Ten female albino rats at second parity were randomly selected from the animal house of the Department of Animal and Environmental Biology, University of Port Harcourt. They were housed in flat-bottomed cages (33cm x 21cm x 18cm) equipped with feeding trough and water nozzle in groups of 3 or 4 at laboratory temperature and fed with commercial chow *ad libitum*. Following mating (day 0) the females were separated into two groups of five and housed in individual cages. From day 2 of mating, 5 animals received 2mg/ml folic acid in saline subcutaneously every two days until day 20. At parturition, litter size was recorded and pups were weighed. The number of fetuses were then equalized among the two experimental groups (n=16 each) by the random removal of pups from both groups. The pups were then weighed individually and thereafter, every 5 days until day 20 post-partum. They were killed under anaesthesia on day 20 and percentage protein content of the carcass were analyzed using the Kjeldahl's method in the Department of Biochemistry, University of Port Harcourt.

RESULTS

Litter size (Fig. 1) and weight (Table 1) were not significantly affected by folic acid treatment, ($P>0.05$). Average litter size for treated group was 7.0 while that for control group was 6.4 per dam.

Average litter weight for treatment group was 6.2 ± 0.02 and that for control was 6.0 ± 0 .12. Regression analysis (Fig. 2) indicated that average growth rate of pups of treated dams was significantly higher than that for controls.

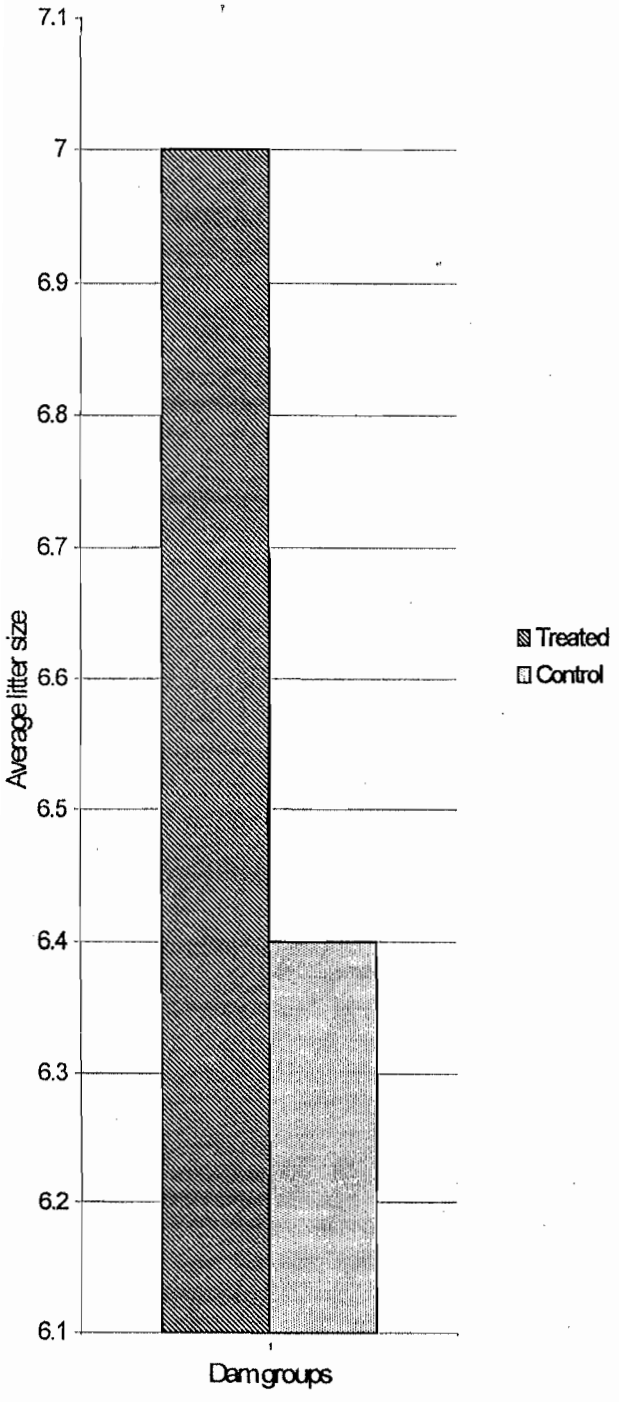

FIG. 1: LITTER SIZE OF FEMALE RATTUS NORVEGICUS TREATED WITH FOLIC ACID AND CONTROLS

TABLE 1: FETAL BIRTH WEIGHTS FROM DAMS TREATED WITH FOLIC ACID OR PLACEBO DURING GESTATION

S/No	Treated	Control
1	6.4	5.8
2	5.9	5.6
3	6	5.4
4	7.2	5.6
5	6.2	5.4
6	6.8	6.2
7	6.2	5.4
8	6.2	5.6
9	7	6.1
10	6.4	6.2
11	8	5.8
12	5.9	5.9
13	5.3	6
14	5	6
15	5.5	6.4
16	5.4	7
Total	99.4	94.4
mean	6.21	5.90
Stdev	0.77	0.43

Average crude protein levels (Table 2) on day 20 post-partum was significantly higher in pups from treated dams (55.02±0.5) than those of control animals (38.77±0.12; $P<0.05$).

TABLE 2: PROTEIN LEVELS (% CRUDE PROTEIN) IN THE CARCASS OF RAT FETUSES ON DAY 20 POST-PARTUM FROM DAMS TREATED WITH FOLIC ACID OR PLACEBO DURING GESTATION

S/No.	Treated	Control
1	68.5	40.1
2	69.08	45.36
3	67.81	37.25
4	48.13	40.92
5	50.4	37.52
6	53.3	38.9
7	47.72	38.45
8	50.02	37.14
9	50.75	36.74
10	52.43	40.68
11	49.02	43.06
12	50.8	38.75
13	53.34	37.42
14	50.15	35.82
15	49.46	35.01
16	69.43	37.25
Total	880.34	620.37
Mean+SEM	55.02±0.5	38.77±0.12

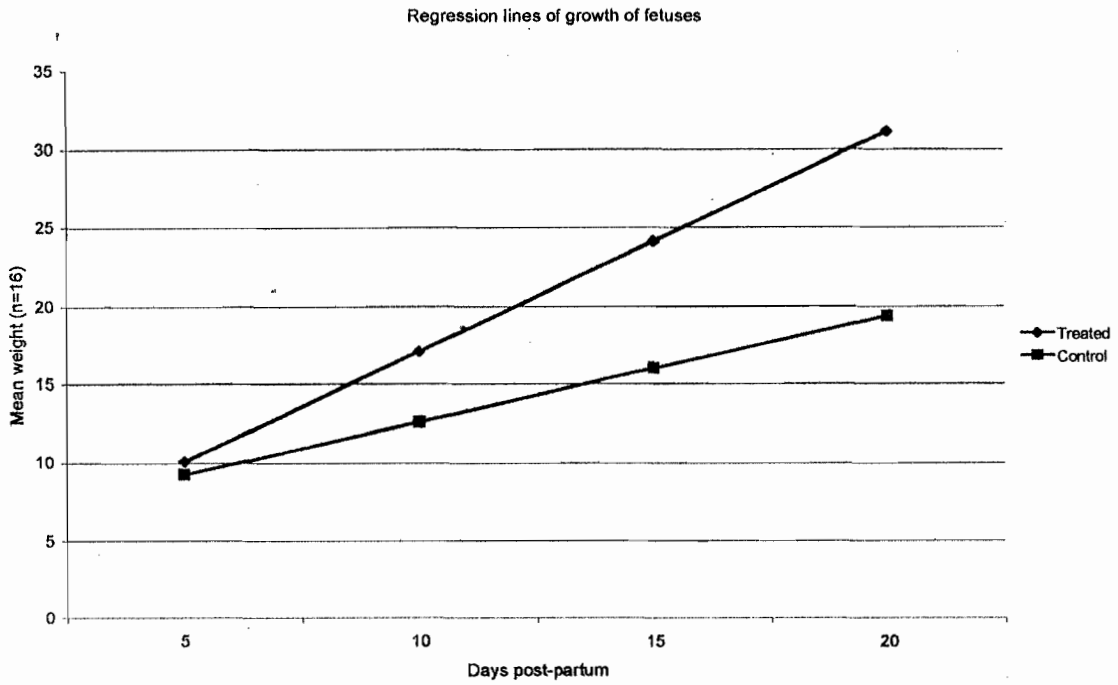

FIG. 2. REGRESSION OF WEIGHT OVER DAYS OF NEONATES FROM DAMS TREATED WITH EITHER FOLIC ACID OR PLACEBO DURING GESTATION. (TREATED: Y= 3.05 + 1.4X, CONTROL: Y=5.85 + 0.68X).

DISCUSSION

The present experiment shows that folic acid treatment of female rats during gestation improves the growth rate and protein accretion of the neonates within the first twenty days post-partum. Initial birth weight and litter size were, however, not affected. Earlier work in the pig (Lee *et al* 1994) found no effect of folic acid on litter size and Guay, *et al*, 2002 showed that dietary supplement of folic acid and glycine (which markedly increased serum folic acid levels in the embryo) had no effect on litter size and embryo survival but "tended to increase" embryo DNA. This, they suggested, was probably because the levels of dietary folic acid was adequate for maternal metabolism but not to optimize embryo development. That report agrees with the present observation in the rat in which neither litter size nor birth weight of embryos were affected by folic acid treatment.

Others have reported that dietary supplement of the vitamin during gestation increased both litter size and embryo survival in multiparous pigs (Matte *et al*, 1984, Lindermann and Kornegay, 1989, Tremblay, *et al*, 1989) but not in gilts (Matte, *et al*, 1993) suggesting that folic acid effect may be dependent on number of parity. The observation that serum folic acid actually decreased during pregnancy in the pig (Matte *et al*, 1984a) suggests that more than basal supply of the vitamin may be required for improved litter size and weight. Whether this explanation also applies to the rat is not clear because optimal requirement of folic acid by the albino rat is not known. To raise litter birth weight, DNA and protein levels in Sprague-Dawley rats, Morgan and Winick (1978) reportedly administered 1.8mg/kg diet *ad libitum* of folic acid in addition to a daily intraperitoneal injection of 1mg to the dams in which case the level of folic acid in the present experiment may be less than adequate. Furthermore, the report of Bassler (1997) indicated that optimal response to folic acid is potentiated by the catabolism of glycine, so that deficiency of this amino acid could reduce the effects of folic acid on initial embryo parameters.

The current experiment also shows that neonates from mothers treated with folic acid grew faster and had higher crude protein levels on day 20 post-partum. Folic acid treatment therefore must have affected the embryos through a mechanism not quite clear. The post-partum manifestation of improved growth suggests a delayed effect in which fetal storage of folic acid was, perhaps, demobilized after birth. But this

explanation seems unlikely since folic acid, a water-soluble vitamin (Lee, *et al* 1994) would have a limited storage capacity in the body, as reported in the sow. Alternatively, folic administration during gestation could have altered the protein-manufacturing capacity of fetal cells, enabling more protein synthesis post-partum and hence greater growth and protein accretion. It is suggested that this mechanism, perhaps in addition to lactational factors from the treated dams may have markedly enhanced the growth rate of the neonates from treated mothers.

It is concluded that folic acid treatment of pregnant rats is important for the growth and subsequent development of neonatal albino rats.

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