DOI: 10.7860/JCDR/2018/31208.11313

Original Article

Anatomy Section

Effect of Folic Acid Supplementation and/or its Absence on Body Weight of Wistar Rats in Pregnancy and in their Offspring

LOKADOLALU CHANDRACHARYA PRASANNA[1], ASHOK KUMAR[2], ROHINI PUNJA[3], HOSAPATNA MAMATHA[4], SNEHA GURUPRASAD KALTHUR[5], DINESH UPADHYA[6]

ABSTRACT

Introduction: Foetal birth weight is the best predictor of pregnancy outcome as the growth restriction has been associated with high morbidity and mortality. The need of Folic Acid (FA) supplementation is essential during the period of pregnancy to prevent untoward effects on pregnancy related complication in mother and to prevent birth defects in foetus.

Aim: To compare the effects of FA supplementation and FA absent diets during the pregnancy on maternal and their offspring body weight.

Materials and Methods: This was a prospective study comprising of 18 Wistar albino rats procured from the Animal house, Manipal Academy of Higher Education, Karnataka, India (January 2017 to June 2017), after the Institutional Ethical Committee clearance. Rats were randomly divided into three groups and given different diets: Control group with normal diet, group with FA supplementation diet, and group with FA absent diet (with added succinyl sulfathiazole) for five weeks. Later the female adult Wistar rats could mate with male rats of their own strain and allowed the female to complete their pregnancy. After the delivery, delivered pups were counted for each rat and gross malformation in each pup were noted. Immediately after the delivery, all the pups born to each rat were weighed individually in digital weighing machine. The statistical data were analysed using SPSS version 6.0.

Results: Among the pregnant dams the body weight gain was observed more in control group with an average weight of 40.56 gm, followed by diet with absent FA (32 gm) and least in rats fed with FA supplementation diets (31 gm). Average number of pups born to pregnant dams fed with FA supplementation, FA absent diet, and in normal diet was found to be 8, 8, and 9 respectively. Regarding the birth weight of pups born to pregnant dams fed with FA supplementation the average weight was found to be 6.01-5.09 gm, with normal diet it was 5.51-5.08 gm, and least weight was observed in pups born with FA absent diet (4.74-3.31 gm).

Conclusion: The present study concluded that the FA status in pregnancy is inversely proportional to the prenatal death in pups but in offspring's born to mothers fed with FA supplementation, average body weight was more than those born to mothers fed with FA absent diet and with control groups. However, this needs further confirmation as the tests were done on limited samples.

Keywords: Folate, Folic acid supplememntation diet, Prenatal nutrition

INTRODUCTION

The current recommendation for FA supplementation is based only on the evidence that FA reduces the chances of developing neural tube defects and other birth outcomes but the consequences of long-term supplementation remain unsettled.

There is no consensus about the safe upper limit of FA intake, but is usually considered to be 400-600 mcg/day in pregnancy. Limited evidences suggested that serum concentrations of FA >45 to 59 nmol/L are often considered to be supraphysiological [1]. Though we are unaware of the harmful levels of concentrations, such high concentrations may cause epigenetic changes in many tissues [2].

It was found that high maternal folate status (4 mg/day) in pregnancy resulted in high total fat mass, greater risk of insulin resistance, chances of cancer in few organs [3].

Research has been done to elucidate the effects of periconceptional intake of folate to reduce the hazards of neural tube defects without its deleterious postnatal outcome. Folate restriction may cause defects in developing organs. The developing foetus can have teratogenic effects due to severe deficiencies or excess micronutrients (like folate, calcium, magnesium, and zinc) [4]. Moderate nutritional deficiencies or excesses may cause more subtle damage, potentially impaired organ development during critical periods of foetal development [3,4].

The present study was undertaken to compare the effects of long term FA supplementation and FA absent diets during the pregnancy on maternal and foetal birth weights.

MATERIALS AND METHODS

This experimental prospective study on Wistar rats (approximately 150 gm) were carried between January 2017 to June 2017. Eighteen Wistar female rats were approved for experimental use by the Institutional Animal Ethical Committee (Ref no: IAEC/KMC/06/2017), Manipal Academy of Higher Education, Manipal, Karnataka, India. Rats were randomly divided into three groups with six rats in each group. The control group was fed with a normal diet, second group was fed a diet containing no FA. About 1% succinylsulfathiazole was added to the diet with no FA content to reduce/inhibit the gut flora responsible for synthesis of FA in their body and to avoid coprophagy during pregnancy [3]. Third group was given diet with excess of FA (40 mg/kg body weight). All rats were maintained in separate cages in a specific-pathogen-free animal room under controlled environment (12 hours light and 12 hours of darkness, 25°C, and 35% humidity) with free access to food and water. Three types of diet (diet with normal amount of FA, diet with excess of FA, and diet with absent FA with succinylsulfathiazole) as described above were given to the experimental female rats for three weeks prior to mating and continued the same diets till the completion of their pregnancy. Also, the different diet pattern as described above

for three different groups was continued for three weeks after the delivery (till the weaning period). To check whether they mated or not, vaginal smear was taken in the early morning and stained with methylene blue and confirmed the presence of sperm plug under microscopy. On confirmation of sperm plug, the gestational day was termed as day 1 (GD1). Pups were remained with the mother until three weeks of postnatal life (weaning period). Body weights of experimental female rats were recorded every week from the time of procurement to their completion of pregnancy. Rats were remained in their cage and with their respective diets until they delivered the pups. Delivered pups were counted for each rat and gross malformation in each pup were noted. Immediately after the delivery, all the pups born to each rat were weighed individually in digital weigh machine.

STATISTICAL ANALYSIS

Data were analysed using the Statistical Package for the Social Sciences (SPSS) statistical software (version 6.0). Body weight of Wistar rats obtained at the time of experiment (from 0 week five weeks) were analysed using two-way ANOVA. Ordinal variables were assessed using non parametric tests: Kruskal-Wallis and Mann-Whitney U-test significant differences obtained in the two-way ANOVA were followed by Tukey's Honestly Significant Differences (HSD) post-hoc analysis. All significance tests were considered significant at p-value <0.05.

RESULTS

Weight gain among the three groups (control, diet with increased FA, diets with absent FA) were compared after feeding with their respective diets as prescribed in the protocol from the day of procurement of Wistar rats for experiment to fifth week. Towards the end of fifth week, the body weight gained was more in control group, followed by diet with absent FA and least in rats fed with FA supplementation diets as shown in the [Table/Fig-1].

Week	Group	Mean body weight gained (in grams)	Standard deviation	No. of samples used
Week-0	Increased folic acid	175.5385	25.69895	13
	Absence of folic acid	159.8333	6.17669	12
	Control	141.6667	9.68246	9
	Total	161.0294	21.47795	34
Week-5	Increased folic acid	206.5385	21.22740	13
	Absence of folic acid	191.8333	25.60836	12
	Control	182.2222	13.48868	9
	Total	194.9118	22.96027	34

[Table/Fig-1]: Comparison of weight gain by the mother fed with the diet with increased FA, absent FA, and normal FA content (control) groups (from 0 week to 5 week).

With multivariate test application, weight gained by pregnant Wistar rats fed with increased FA was significantly (p-value 0.001) increased when compared with other two groups [Table/Fig-2]. Tests of within-group (factor1=0.001, and factor 1*=0.58) showed no significant association between the weight gain observed in pregnant rats fed with increased FA and FA absent diets. Tukey's test among the multiple comparison showed significant differences (p-value 0.05) among the three groups. Pair-wise comparison of three groups showed statistically significant differences in weight gain among the FA supplementation (p-value 0.001) and control groups but whereas with other groups (p-value 0.130), it was non significant.

Average number of pups born to pregnant dams fed with FA supplementation, FA absent diet, and in normal diet was found to be 8, 8, and 9 respectively. Kruskal-Wallis test showed no significant p-value (0.575) statistically.

Group	Vs Group	Mean difference	Standard error	Sig.	95% Confidence interval
					Lower bound
Increased folic acid	Absence of folic acid	15.2051	6.32048	0.056	-0.3508
	Control	29.0940*	6.84637	0.001	12.2438
Absence of folic acid	Increased folic acid	-15.2051	6.32048	0.056	-30.7610
	Control	13.8889	6.96209	0.130	-3.2461
Control	Increased folic acid	-29.0940*	6.84637	0.001	-45.9442
	Absence of folic acid	-13.8889	6.96209	0.130	-31.0239

[Table/Fig-2]: Pairwise comparison of the significance of weight gain by the mother during pregnancy with the diet containing increased FA, absent FA, and normal amount of FA (Tukey's HSD test).
*statistically significant (p-value 0.001)

Regarding the birth weight of pups born to pregnant dams fed with FA supplementation the average weight was found to be 6.01-5.09 gm, with normal diet it was 5.51-5.08 gm, and least weight was observed in pups born with FA absent diet (4.74-3.31 gm) [Table/Fig-3]. ANOVA between the three groups was found to be statistically (p-value 0.001) significant than within the groups. Pairwise comparison was done to correlate the significant association between the different groups. It showed significant difference when compared with pups born to FA supplementation diet (p-value 0.002) to pups born to FA absent diet (p-value 0.008) when compared with control group. Also, significant differences were noted when compared pups born to FA absent diet mother with pups born to FA supplementation and with pups born to normal diet [Table/Fig-4].

Group	No. of pups born	Mean body weight of pups in each group (in grams)	Std. deviation	Std. error	95% Confidence interval for mean	
					Lower bound	Upper bound
Increased folic acid	42	5.4991	0.39552	0.17688	5.0080	5.9902
Absence of folic acid	34	4.2832	0.57922	0.25904	3.5640	5.0024
Control	38	5.2567	0.17246	0.07713	5.0425	5.4708
Total	114	5.0130	0.66696	0.17221	4.6436	5.3823

[Table/Fig-3]: Mean birth weight of pups born to pregnant dams fed with the diet containing increased FA, absent FA, and normal amount of FA.

Group	Vs group	Mean difference	Std. error	Sig.	95% confidence interval
					Lower bound
Increased folic acid	Decreased folic acid	1.21583*	0.26374	0.002	0.5122
	Control	0.24239	0.26374	0.639	-0.4612
Absence of folic acid	Increased folic acid	-1.21583*	0.26374	0.002	-1.9194
	Control	-0.97344*	0.26374	0.008	-1.6771
Control	Increased folic acid	-0.24239	0.26374	0.639	-0.9460
	Decreased folic acid	0.97344*	0.26374	0.008	0.2698

[Table/Fig-4]: Pairwise comparison of birth weight of pups born to pregnant dams fed with the diet containing increased FA, absent FA, and normal amount of FA (Tukey's HSD test).
*statistically significant (p-value 0.05)

DISCUSSION

The term folate is a generic name for the group of chemically related compounds based on the FA structure. Folate (Vitamin B9), an essential vitamin that must be administered either in diet (green leafy vegetables, legumes, egg yolk, liver and citrus fruit) or through

supplementation [5]. FA used in food fortification (artificially enriched foods and pharmaceutical vitamins) is synthetic and differ from naturally occurring FA in our diet because it is in the oxidized state, has only one conjugated glutamate molecule, higher bioavailability, and rapidly absorbed in the intestine [1].

The normal range of the serum folate concentration in humans is 2.7-17 ng/mL [6]. Studies reported that consumption of 400 µg/day of naturally available FA in food and FA supplementation resulted in supraphysiological levels with greatest increase in pregnant women [5]. Concern has been raised if such over FA exposure in pregnancy will have any detrimental effects if not benefited. In present study, the level of FA supplementation chosen was 40 mg FA/kg diet, i.e., 20 times the normal level considered for pregnant rats (2 mg FA/kg) [7].

National Institutes of Health (NIH) have recommended 600 µg of FA be taken daily by pregnant women throughout their pregnancy to have adequate FA stored in pregnancy, and that this supplementation be continued in lactation with reduced amount to 500 µg.

Higher doses of FA (5 mg) strategy was recommended only in patients with a history of recurrent spontaneous abortions and neural tube defects, life style issues like variable diet, inconsistent birth control, and consumption of alcohol, smoking, and recreational drugs [8].

Earlier studies recorded an impressive increase in the weight of rats and pregnant dams when fed with excess FA supplementation [9,10]. The present study showed that weight gain of the Wistar rats fed with normal diet was found to be high with an average gain of 40.56 gm, followed by rats fed with FA absent diet (32 gm), and was least in FA supplementation rats (31 gm). This is in line with Achon M et al., study as they found no significant increase in weight among the rats fed with highest possible doses (4 gm/day) of FA supplementation [1]. On the contrary, FA supplementation showed significant reduction in the weight of pups as well as their lengths at the time of birth [7]. However, several studies put forth their hypothesis that FA supplementation to maternal protein restriction diet provided significant contribution in increase in the body weight [9,10].

The present study gave statistically significant differences among the body weight of the three groups but the same significance was not found when applied within each group. Also, when compared with the estimated marginal means of body weight among the three groups, we found no association between the body weight and FA status in the body.

One of the pregnant dam from FA supplementation group delivered 11 pups, with an average weight of 6.56 gm and all of them were died immediately after the delivery including the mother. This could be due to over weight of the pups causing severe blood loss in the mother.

In the present study, the mean birth weight of pups born to pregnant dams fed with FA supplementation, FA absent diet, and control group was 5.49, 4.28, and 5.01 gm respectively. It was reported that an impressive increase in the weight of both mother and their offspring upon FA supplementation during the time of pregnancy was confirmed by Shaw GM et al., in California [10].

In India, nutrition based study on children below six years of age and during pregnancy showed greater total fat mass (birth weight) with high doses of FA supplementation but found greater risk of developing insulin resistance and altered immune function [1].

Burgoon JM et al., described a mouse model for FA deficiency in vivo and found that FA deficient (with added succinylsulfathiazole) diet fed mice for four weeks before mating showed decrease in the concentration of plasma and increase in homocysteine levels, decreased body weight, developmental delays, and 20% chances of resorption (spontaneous abortions) [11]. In the present study, we found an average weight of 4.28 gm pups compared to 5.01 gm in control and 5.49 gm pups in FA supplementation groups. FA deficiency leads to a decrease in nucleic acid synthesis and developmental delays in orofacial and cardiovascular development. Embryos after toxic insult by FA deficiency were unable to repair itself successfully or undergo catch-up growth [12].

Ly A et al., found that the mean body weight of the pups born to pregnant dams supplemented with FA was significantly higher by 13% than its counterpart fed with normal diet. Also, they noted the weight of the pups has no relation with maternal and post weaning FA supplementation [13].

It is expected that congenital anomalies do exist in pups born to pregnant dams fed with FA absent [2]. However, no gross malformation of any kind was noticed in all the three groups.

Researchers reported inconsistent report on the effect of FA supplementation during pregnancy on maternal weight and their offspring. Some reported no association between FA supplementation and weight gain in both mother as well as their offspring, few observed weight gains in both mother and their offspring and few recorded appreciable weight losses among the offspring and in mothers [1,3,7,9,10,13]. However, excess FA supplementation and unmetabolised FA in pregnancy can cause detrimental effects on the offspring with T allele of Methylenetetrahydrofolate Reductase (MTHFR) which in turn increases the risk of spontaneous abortion in pregnancy. Children born with T allele carrier state are at greater risk of developing depression, schizophrenia, bipolar disease, male infertility, neural tube defects, and rarely Down's syndrome [5,13-18]. Several meta-analysis and randomised control trials have reported that prenatal FA supplementation are protective against the childhood cancers and asthma [19]. On the other hand, FA deficiency during pregnancy has been associated with the development of neural tube, orofacial, cardiovascular defects, preterm, low-birth weight in children and anaemia in mothers.

LIMITATION

Present study limited to the correlation of the body weight of pregnant rats and their offspring with FA supplementation, FA absence and with normal dietary dose of FA in the diet. Though we found significant differences in the weight gain observed among the three different diets, this needs further confirmation as the tests were done on limited samples.

CONCLUSION

Birth weight of pups born to pregnant dams fed with FA supplementation was found to be significantly increased when compared to those with diet with normal amount of FA, and least weight was observed in pups born with FA absent diet. FA supplementation during periconcetional and through pregnancy is essential to prevent adverse effect on mother as well as in fetus to prevent birth defects.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Anatomy, Manipal Academy of Higher Education, Manipal, Karnataka, India.
2. Student, Department of Anatomy, Manipal Academy of Higher Education, Manipal, Karnataka, India.
3. Assistant Professor, Department of Anatomy, Manipal Academy of Higher Education, Manipal, Karnataka, India.
4. Associate Professor, Department of Anatomy, Manipal Academy of Higher Education, Manipal, Karnataka, India.
5. Professor, Department of Anatomy, Manipal Academy of Higher Education, Manipal, Karnataka, India.
6. Assistant Professor, Department of Anatomy, Manipal Academy of Higher Education, Manipal, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Lokadolalu Chandracharya Prasanna,
Associate Professor, Department of Anatomy, Kasturba Medical College, Manipal-576104, Karnataka, India.
E-mail: anatomylcp@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jul 14, 2017**
Date of Peer Review: **Aug 17, 2017**
Date of Acceptance: **Dec 02, 2017**
Date of Publishing: **Mar 01, 2018**