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ORIGINAL ARTICLE

Folic acid supplementation and its effects on the oestrus cycle of rats.

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ABSTRACT… Objective: To study the effects of long-term supplemental dose of Folic acid on the oestrous cycle of female Sprague Dawley rats. **Study Design:** Laboratory-based Experimental study. **Setting:** Department of Anatomy, CMH Multan Institute of Medical Sciences in Pakistan conducted this research. **Period:** September 2020 to September 2021. **Material & Methods:** In this study, 60 female Sprague Dawley rats were used and divided into two groups. The animals of control group A were given distilled water (1ml/100g) by oral gavage daily for four weeks. Folic acid was administered orally to the experimental group B once per day at a dosage of 0.2mg/kg of body weight, diluted in 100 grams of pure distilled water. A vaginal smear was taken every day between 11 am and 12 pm daily for four weeks and the slides were stained with Papanicolaou's stain. SPSS version 26 was used for data entry and analysis. **Results:** In experimental group B, the animals had an abnormal oestrus cycle. A statistically significant p-value of 0.000 was obtained after comparison between the groups. **Conclusion:** The current study showed that long-term supplemental dose of Folic Acid leads to prolongation of oestrous cycle of Sprague Dawley rats.

Key words: Endocrine Disrupting Chemicals (EDC), Folic Acid, Oestrous Cycle of Rats.

INTRODUCTION

The B vitamins, which are soluble in water, function as essential cofactors for enzymes that play a crucial role in cellular processes. This group of vitamins includes Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxine (B6), Biotin (B7), Folate (B9), and Cobalamin (B12).[1] Folic acid is a dietary essential. When a cell is actively undergoing chromosomal replication and division, the absence of sufficient folic acid leads to a disruption in DNA synthesis.[2] Recommended dietary allowance of folate is 400 microgram/day.[3] Because of its importance, this vitamin is added to food as a fortification measure. The fortification of food has led to a widespread rise in folate consumption among the population, surpassing the body's physiological requirements. Serum or plasma folate concentrations of >45 nmol/L (19.8 ng/mL) in fasting blood samples are often considered supraphysiologic.[4] The structure of folic acid, also known as pteroyl glutamic acid, consists of a pteridine ring connected by a methylene bridge to para-aminobenzoic acid and glutamate components. Folic acid itself is not biologically active; instead, it undergoes conversion into its active forms, dihydrofolic acid and tetrahydrofolic acid, primarily in the liver.[5] Tetrahydrofolate plays a role in one-carbon metabolism, contributing to the production of thymidylate, purines, and pyrimidines necessary for DNA synthesis. Folic acid finds extensive use in the treatment of megaloblastic anemia and the prevention of neural tube defects (NTDs).[6] According to a human study, folic acid interacts with homocysteine and methionine in the one-carbon metabolism pathway and disrupts the endocrine system.[7] Androgen receptors indirectly influence one-carbon metabolism, thereby influencing epigenetic processes, DNA metabolism, and the balance of redox reactions. The ovaries, uterus, vulvar epithelium, vaginal mucosa, omental tissue, and mammary glands exhibit significant androgen receptor (AR) activity.[8] Endocrine disruption occurs by the

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effect of endocrine-disrupting chemicals (EDCs). Folic acid functions as a chemical that disrupts the endocrine system.[9] These chemicals act on androgen receptors, interfering with their normal signaling and actions.[10] Disrupting the androgen receptor signaling pathway affects the hypothalamic-pituitary-gonadal axis, leading to increased secretion of sex hormones such as GnRH, LH, and FSH. Consequently, this leads to higher serum estradiol levels and prolonged oestrous cycle.[11]

The objective of this study was to investigate and provide concrete evidence regarding the potential interaction between folic acid and the endocrine system, particularly its impact on androgen receptors. This necessitated conducting further comprehensive research and relying on well-documented scientific studies.

MATERIAL & METHODS

This experimental study was conducted within the research laboratory at the Department of Anatomy, CMH Multan Institute of Medical Sciences in Pakistan from September 2020 to September 2021 after approval from ethics committee (TW/25/CIMS).

The University of Veterinary and Animal Sciences (UVAS) Lahore provided sixty healthy female Sprague Dawley rats. Rats that were pregnant or had any physical abnormalities were not allowed to participate in the study. Rats were chosen using a random sampling technique.

Thirty rats each were placed in groups A (Control) and B (Experimental) of the rats. For four weeks, Group A received 1ml/100g of pure distilled water by oral gavage each day. For four weeks, Group B received an additional dose of 0.2 mg/kg of folic acid in 1 mg/100 g of pure distilled water by oral gavage.[12,13] The optimum dose was determined through a combination of consulting a reference article and conducting a pilot procedure. A daily vaginal smear was collected within the time frame of 11 am to 12 pm for four weeks. The rat was put inside the lid, and the tail was raised to expose the genital area. A plastic pipette, containing 10 µL of distilled water, was carefully inserted into the rat's vaginal area. The pipette bulb was gently compressed two or three times, allowing for the collection of vaginal fluid. The pipette's tip had a diameter of less than 2 mm. The procedure was conducted with great precision, and the rats were handled gently to minimize stress. The entire collection process was efficiently completed in just 30 seconds.[14] After performing vaginal lavage, individual samples from each animal were evenly distributed onto separate microscope glass slides. These slides were left to air dry for a period of 3-4 hours before being stained using the Papanicolaou stain to determine the oestrous cycle. Following staining, the slides were examined under a light microscope with a 40X objective lens to analyze the different stages of the oestrous cycle. Rats were considered to have a normal reproductive cycle when they exhibited a consistent four-stage pattern, which included proestrus (lasting twelve to fourteen hours), estrus (lasting twenty-five to twenty-seven hours), metestrus (lasting six to eight hours), and diestrus (lasting fifty-five to fifty-seven hours). This four-stage cycle typically spanned approximately 4 to 5 days.[15, 16] The histological changes observed in a vaginal smear include nucleated epithelial cells during proestrus, cornified squamous epithelial cells during estrus, an equal proportion of cornified cells and leukocytes during metestrus, and predominantly leukocytes during diestrus.[17]

However, if there were any variations in the duration or sequence of these stages, it was classified as an abnormal cycle.[18] Data was expressed in percentages, and the Chi-square was applied. The p-value of 0.05 or less was considered statistically significant.

RESULTS

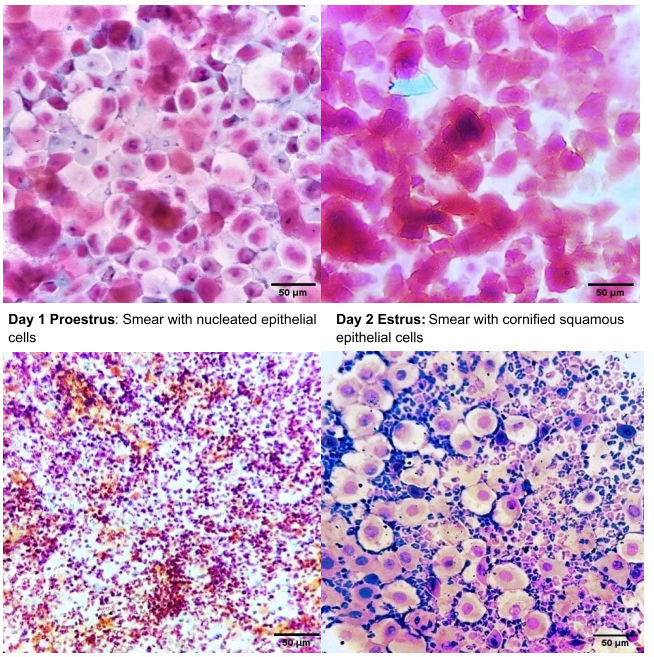
The animals of group A showed regular pattern of oestrus cycle and the animals of group B were arrested in diestrus phase as shown in Figure-1 & 2. In group A- Control, out of 30 rats, five rats (17%) showed abnormal oestrous cycle, in the B- Folic Acid group, all of the 30 rats (100 %), showed abnormal oestrous cycle. (Table-I).

Groups	Normal	Abnormal	P-Value

A (n= 30)	83%	17%	0.000*
B (n= 30)	0%	100%	

Table-I.

P-value <0.05 was considered significant & marked by Asterix (*)



Day 1 Proestrus: Smear with nucleated epithelial cells

Day 2 Estrus: Smear with cornified squamous epithelial cells

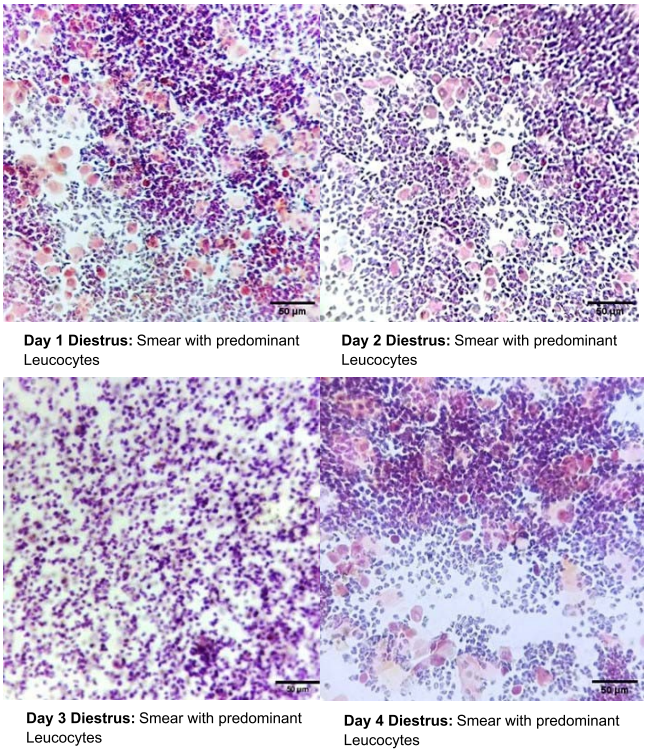
Day 3 Diestrus: Smear with predominant Leucocytes

Day 4 Diestrus: Smear with predominant Leucocytes

Figure-1. Photomicrograph of a representative animal from Group A showing the regular pattern of Oestrus Cycle

DISCUSSION

The oestrous cycle is of paramount importance in the reproductive well-being of female mammals, including rats. Several factors, including dietary elements, can impact this cycle. Folic acid is commonly employed to manage megaloblastic anemia and reduce the risk of neural tube defects.[6] Because of its significance, folic acid is added to food for fortification purposes. Food fortification has resulted in a broad increase in folate consumption among the populations. Fasting blood samples showing serum or plasma folate levels surpassing 45 nmol/L (19.8 ng/mL) are typically considered to be beyond the physiological range.[4] Elevated levels of folic acid beyond the physiological range can act as an endocrine-disrupting chemical. Endocrine disruption is known to interfere with normal hormone signaling and action.



Day 1 Diestrus: Smear with predominant Leucocytes

Day 2 Diestrus: Smear with predominant Leucocytes

Day 3 Diestrus: Smear with predominant Leucocytes

Day 4 Diestrus: Smear with predominant Leucocytes

Figure-2. Photomicrograph of a representative animal from Group B showing the regular pattern of Oestrus Cycle

The specific area of interest revolves around the consequences of elevated folic acid levels beyond the normal physiological range on reproductive health. This is primarily due to the presence of unmetabolized folic acid, which can have adverse effects. Empirical data indicates that increased concentrations of folic acid can interfere with the typical functioning of reproductive hormones, potentially impacting fertility, menstrual cycles, and hormone-related processes.[9,19] These findings raise concerns about the potential implications for both male and female reproductive systems.

Rats exhibit a four-stage estrous cycle consisting of proestrus, estrus, metestrus, and diestrus. Each stage is characterized by distinct hormonal changes and behaviors. Proestrus involves the preparation of the uterus for potential implantation, estrus marks the period of sexual receptivity, metestrus reflects a transitional phase, and diestrus signifies the luteal phase. Any disruption in the duration or sequence of these stages is considered abnormal and may have implications

for fertility and reproductive health.[18,20]

This research suggests that excessive folic acid supplementation can disrupt the normal oestrous cycle of rats. It has been observed that prolonged exposure to high folic acid levels may alter the duration and sequence of oestrous stages, leading to irregular and prolonged cycles. These changes could potentially affect fertility, hormone regulation, and the overall reproductive health of rats. However, further studies are needed to establish the precise mechanisms underlying these effects and determine the long-term consequences. The possible mechanisms through which folic acid exerts its endocrine-disrupting effects are DNA methylation patterns and gene expression. These changes in epigenetic control can result in the disruption of hormone production, release, and signaling pathways. In the experimental group, all animals displayed an irregular estrous cycle, possibly attributable to folic acid's influence on the androgen signaling pathway. Prior research has shown that modifications in the androgen receptor signaling pathway can impact the hypothalamic-pituitary-gonadal axis, leading to heightened secretion of sex hormones like GnRH, LH, and FSH. This, in turn, elevates serum estradiol levels and extends the duration of the oestrous cycle.[11]

Understanding how excess folic acid affects the rat estrous cycle requires a thorough investigation of its effects beyond what is deemed biologically required. These studies are essential for assessing the possible dangers of using too much folic acid as a supplement. The knowledge gathered from this study has potential applications for human reproductive health that go beyond the field of rat reproductive physiology. It is essential to investigate the complex molecular and cellular mechanisms underlying folic acid's influence on the estrous cycle in order to fully comprehend these effects. By revealing the intricate systems governing reproductive cycles, the investigation of these mechanisms may offer valuable insights applicable to both rats and, consequently, humans. Additionally, it is crucial to investigate the various facets of folic acid supplementation, including different dosages and durations.

CONCLUSION

The present study demonstrated that the administration of supplemental dose of Folic Acid over an extended period of time resulted in prolongation of the oestrous cycle in Sprague Dawley rats.

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