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The role of folic acid in the physiological parameters and efficiency of sperm in male rabbits

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ABSTRACT

This study s investigated the improving effects of administration of folic acid to male rabbits, using 16 adult male rabbits divided into two groups the first(control)group- administered Normal saline while the second (treatment)group administrated 5mg/kg folic acid for 30 days.

As a result, hematological parameters (RBC. Hb. PCV. and Platelets) and serum protein and its fractions (albumin, globulin) were greatly improved in the treatment animal relative to untreated animal (Normal saline). also, reductions in the lipid profile (T.C, T.G, LDL-C, VLDL-C) and significant elevation in HDL-C were found in the treatment animal.

Morphological examination of testes referred to a change in testes length, width, and diameter, significantly even epididymal sperm characteristics indicated increase in individual motility, and forward movement , decrease in abnormal movement (backward, vibration, circulation), dead and abnormal sperm percentage significantly in folic acid-treated group compared with the control group.

Keywords: folic acid, hematology, lipid profile, fertility, rabbits

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INTRODUCTION

Focusing on causes or abnormalities affecting normal sperm functions is essential. Focusing on causes or abnormalities affecting normal sperm functions is necessary. Several circumstances environmentally and physiologically have been connected with decreased sperm production and infertility (Gul Baykalir *et a*l., 2016). Vitamin B9 or folic acid deficiency causes general health problems such as weakness, fatigue, and premature birth (Campbell, 1996; scientific committee, 2000).

The documented decline in semen quality can outcome from easily manipulated interactions between genetic and environmental factors (Kuroki et al., 1999).

vitamin B9 which also referred to Folic acid identified as a member of co-factors implicated in Metabolism and cellular processes of single carbon such as biosynthesizes of purine, thymidylate, and methionine (Kamel, 2012). It is a body coenzyme that acts specifically on, exhibits antioxidant impact, erythropoiesis and Synthesis of DNA (Stanger, 2002), Metabolism of DNA, amino Acid, (Tolba *et al*.,2015), sperm formation (Kamen,1997), and male fertility in General (Hussein *et al.,* 2012).

Folate depletion is connected with male fertility reduction indicators. Reduction of its level in seminal plasma, for example, it's Associated with reduced sperm quality and elevated disruption human's sperm DNA (Wallock *et al*.,2001, Boxmeer, *et al*., 2009). An inverse relation between the total administrated in day of folate was also observed with production of aneuploid sperm in humans (Young et al. 2008). For DNA, RNA conversion, and protein synthesis, folate generally found in vegetables with green leaf, is essential. Since the synthesis of DNA is a primary part of spermatogenesis (Ciereszko and Dabrowski, 1995).

It is necessary to have sperm with good movement and high activity. just active sperm may pass through the genital tract, and movement is compromised with metabolism, dietary, and climate variables (Al-Qarawi, 2005). Reproductive efficiency is also impaired by oxidative stress. (Rui *et al*.,2017).

Amis of study:

Identify the influences of folic acid administration on hematological, biochemical, morphological examination, and sperm characteristics in rabbits.

Material and methods

Sixteen male rabbits of (1000-1250) g weight and 12 months age were used in this experiment placed in metal cages then after 15 days of acclimatization, they were separated into two groups. The first one was administrated Normal saline and the second group, folic acid (5 mg/kg) orally for the 12 weeks.

Blood collection

finally, blood samples (10 ml) of each animal were collected by direct cardiac puncture and divided into two parts, first one, with anticoagulant (EDTA) for RBC count, Hb concentration, hematocrit percentage, platelet count. second part without anticoagulant left at room temperature for one hour then serum samples were separated by centrifugation and stored in Eppendorf tubes at -20°C for biochemical tests.

Spermatozoa characteristics

Morphology, Motility and Sperm live / dead percentage and was measured using solution consisted of 1% Eosin stain and 5 % Nigrosin stain in the 3 % Tri-sodium citrate di-hydrate as mentioned by (Melissa 2002).

Statistical Analysis

All reported data were analyzed for One direction ANOVA table and t-test was used to analyze data using a computer bundled software (SPSS) (Statistical Research Packages) (V19). The findings were described as Mean ± SD with significant P-value at 0.05, while the Sperm analysis was closely observed and analyzed to determine some difference between the groups.

RESULTS

Hematological parameters

Animals treatment by folic acid substantially (P<0.05) improved hemoglobin (Hb), hematocrit percentage (PCV), RBC, platelet, and WBC count relative to the untreated animals (Table 1).

Table (1). Effect of folic acid on some blood parameters (mean ± SD)

Groups	RBC $*10^6/mm^3$	Hb mg/dl	PCV %	PLATELETS $*10^3/mm^3$	WBC $*10^3/mm$
Normal saline	4.28±0.55b	8.89 ±0.34b	40.00 ±1.41b	202.50 ±2.07b	5.73 ±0.37b
5mg/kg	5.85±0.34a	11.17 ±0.33a	44.88 ±1.48a	242.33 ±5.60a	6.96 ±0.13 a

*different letters denote significant P<0.05

Folic acid acts as an antioxidant compound which prevents the damaging effect of free radicals (El-Barody,2002); also, it prevents the occurrence of pernicious anemia by increasing the absorption of B_{12} (Change *et al.* .1997), besides that the folic acid increasing cell division (Matte *et al.* .1990).

The observed change in hematological parameters following treatment with folic acid is due to its significant antioxidant effect on hematopoietic stem cells. and this cells tend to be especially susceptible in the presence of unregulated accumulation of ROS. reduction in many scavengers ROS. caused anemia, which is extreme or even fatal in certain cases, and hematopoietic tissue cancer (Kong et al., 2004).

Increasing the PCV percentage and Hb concentration showed that folic acid causing increasing in RBC counting ((Matte *et al.* .1990).), which led to increasing the packed cell volume percentage and Hb concentration in treated groups compared with control. The vitamin was causing increasing the absorption of ascorbic acid, which stimulating erythrocytosis (Church and Pound,1988).

The total WBC count in the adult male rabbit was significant increase in the treatment group with folic acid as compared with normal saline; this may be due to the antioxidant effect of this vitamin, which leads to an increase in the phagocyte process (El-Barody,2002).

Folic acid therapy was found to be substantially improved in certain bio-chemical parameters in terms of serum protein , albumin, globulin. (Table 2).

Table (2). Effect of folic acid on serum protein, Albumin, and Globulin g/dl (mean ± SD).

Groups	TOTAL PROTEIN	ALBUMIN	GLOBULIN
Normal saline	5.80 ±0.29b	3.40 ±0.27b	2.37 ±0.16b
5mg/kg	6.30 ±0.30a	3.84 a±0.21a	3.30 ±0.29a

*different letters denote significant P<0.05

These changes may occur because the folic acid has stimulated effects on the synthesis of methionine, which interacted in protein synthesis (Hoffbrand and Jackson, 1993). These findings indicated the beneficial effects of treatment with antioxidants on protein metabolism, concerning folic acid's positive effect.

Kamel (2012) confirmed that the administration of folic acid elevated the total protein and albumin concentration of rabbit bucks seminal plasma. Also, it increases the level of immunoglobins, which leads to improving the immune capacity (Kolb and Sechawer, 1999) (Grieshop and Stahly, 2000) and (Suhdoon et al. .2009).and increases the liver activity, which it represents as the resource of protein synthesis in blood. While (Komatsu and Tsukamoto,1998) mentioned that folic acid elevated protein level through protection versus breakdown of proteins.

table (3) illustrated a significant reduction in concentrations of total cholesterol (T.C), triglycerides (T.G.), and LDL and a significant elevation (P<0.05) in serum HDL concentrations of the treatment group relative to the control group.

Table (3). Effect of folic acid on serum lipid profile mg/dl (mean ± SD)

Groups	T.C	T.G	LDL-C	HDL-C	VLDL-

Normal saline	**102.14 ±1.48 a**	**80.58 ±1.57 a**	**89.65 ±1.06 a**	**65.45 ±1.85 b**	**16.11 ±0.31a**
5mg/kg	**90.77 ±0.73 b**	**70.75 ±5.00 b**	**75.21 ±0.88 b**	**72.13 ±2.40 a**	**14.15 ±1.00b**

*different letters denote significant P<0.05

Folic acid intake effects on serum lipids have been analyzed in this experiment since it influences tissue metabolic process and may involve in bio-chemical interaction or mechanisms that also evaluate the health or disease profile of serum lipids (Robert et al., 2002).

reduction in total cholesterol reported following folic acid intake can be due to decline in the level of acetyl CoA related to reduced fatty acids ß-oxidation, because acetyl CoA is a crucial component in cholesterol production (Rang *et al*., 1995). hypocholesterolaemia is advantageous can help to minimize arteriosclerosis and the occurrence of hypertension since all diseased cases are linked with elevated LDL cholesterol (Enas, 1999).

LDL-cholesterol serum content significant decrease is understandable, as a decline in total cholesterol usually results in a decrease in LDL-cholesterol concentration, leading to a shift in the degradation of VLDL-cholesterol, according to existing LDL. in last step of degradation (Mayes, 1996).

Following folic acid consumption, the decrease of LDL has been advantageous, several epidemiological trials have demonstrated that increased LDL-cholesterol levels are associated with elevation coronary heart disease incidence . (Nelson and Cox, 2000; Woo et al. , 2002).

Increases in HDL-cholesterol, defined as healthy cholesterol after folic acid administration, could also be advantageous clinically. This finding was agreement with the reported finding that an improvement in HDL-cholesterol is inversely associated with coronary heart disease (Philip, 1995). The bio-chemical relevance of HDL-C is that it extracts cellular cholesterol and excreted it by the (Mayes, 1996).

A substantial elevation in tri-glycerides (primary store fatty-acids) after 5 mg of folic acid intake perhaps involve in increased lipo-lysis. However, folic acid intake at a 10 mg dosage induced opposing effects by inhibiting lipolysis, decreasing plasma homocysteine levels, and eventually reducing cardiovascular disease (Woo et al., 2002.-2002).

Result in Table (4) showed a substantial improvement (P<0.05) in morphological results on rabbit testes following folic acid administration of 5mg / kg. in testes dimensions by increase in length, width and diameter in compare with control group. And the result illustrated improve in the sperm quality after folic intake as significant (P<0.05) Increased sperm motility, forward movement, and a considerable decrease in abnormal movements like backward, vibration, and circulation also substantial (P<0.05) improved in sperm quality as a decrease in the dead and deformity sperms percentage in comparison with animals administrated normal saline was also seen (Table-5).

Table (4). Effect of folic acid on male rabbit testes dimension.cm (mean ± SD)

Groups	Length	Width	Diameter
Normal saline	**2.01 ±0.11b**	**0.98 ±0.07b**	**1.03±0.05b**
5mg/kg	**2.56 ±0.12a**	**1.31 ±0.04a**	**1.20 ±0.06a**

*different letters denote significant-P<0.05

Table (5). Effect of folic acid on sperm Motility and viability percentage (mean ± SD)

Groups	Sperms characteristics %						
	Individual Motility	Forward	Backward	Vibration	Circulation	Dead.	Deformity
Normal saline	**71.50 ±5.31b**	**60.83 ±8.95b**	**5.00 ±0.59b**	**3.50 ±0.87b**	**3.00 ±0.78**	**28.83 ±5.41b**	**8.16 ±1.72b**

5mg/kg	**89.16 ±2.63a**	**85.00 ±3.68a**	**1.32 ±0.19a**	**1.21 ±0.30a**	**1.43 ±0.21a**	**11.83 ±3.31a**	**3.00 ±1.26a**

*different letters denote significant P<0.05

Kamel (2012) and (Yousef *et al*., 2006) on rabbits , (Tolba *et al*., 2015).in Japanese quail males, were documented this increase in physical semen aspects of bucks administered with folic acid on rabbit bucks,

Audet *et al*. (2004) documented a positive connection in this line littel concentration of folic acid in seminal fluid and the formation of semen in young pigs. possible change in the physical features of male semen administrate folic acid, likely due to folic acid, may be essential for correct spermatozoa production due to its role in DNA production (Wallock *et al*., 2001).

And the discovery of a result agreement with (El-Ratel, 2017) that demonstrated a substantial improvement in the semen production and fertilizing potential of rabbit buck spermatozoa. Following treatment of rabbit bucks with folic acid in drinking water. However, Landau et al. (1974) recorded that a 30-day daily treated with of 10mg folic acid had no positive affect on sperm quantities in normosperm and oligozoosperm males.

(wong *et al*.,2002) showed improvement overall normal sperm numbers of (74 percent) after adjustment for an associated rise in the amount of defective sperm (4 percent) in subfertility males administrated zinc sulfate and folic acid daily for proximally 6 months . generally, physiological amounts of micronutrients have a greater influence on assimilation, transportation , metabolic processes as long as there are un significant abnormalities . he was believed that a much greater beneficial effect could be obtained if reduce folic acid or zinc sulfate dosage intake. This is in line with our result as an increase in the physical properties of the sperm has been identified.

Ethics

All animal experiments are subjected to established stander ethics in the Veterinary Medicine College of Basrah University.

CONCLUSION

According to results illustrated in this study we found that administration of vitamin 9 or folic acid to rabbits bucks have improvements effects on hematological aspects and proteins parameters which lead to improve immune capacity and decrease in lipid profile and improve in the sperm quality and viability .

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