The Role of Folic Acid on Some Physiological Parameters and Efficiency of Sperm in Male Rabbits

Rashad Fadhil Gadhban, Nawras A. Alwan

Physiology, Pharmacology and Chemistry Department Basrah University / Veterinary Medicine College Corresponding Email: rashad.gadhban@uobasrah.edu.iq

ABSTRACT
This study is 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 epididymis 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, hematolgy, lipid profile, fertility, rabbits

Correspondence:
Rashad Fadhil Gadhban
Physiology, pharmacology, and chemistry department Basrah University, Veterinary Medicine College.
Email: rashad.gadhban@uobasrah.edu.iq
ORCID ID: 0000-0003-3518-6653

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 al., 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 biosyntheses 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).

AIMS 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 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).
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 B12 (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 these cells tend to be especially susceptible in the presence of unregulated accumulation of ROS. reduction in many scavenger’s 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 erythropoiesis (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, and globulin. (Table 2).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC *10^6/mm³</th>
<th>Hb mg/dl</th>
<th>PCV %</th>
<th>PLATELETS *10^3/mm³</th>
<th>WBC *10^3/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>4.28±0.55b</td>
<td>8.89 ±0.34b</td>
<td>40.00 ±1.14b</td>
<td>202.50 ±2.07b</td>
<td>5.73 ±0.37b</td>
</tr>
<tr>
<td>5mg/kg</td>
<td>5.85±0.34a</td>
<td>11.17 ±0.33a</td>
<td>44.88 ±1.48a</td>
<td>242.33 ±5.60a</td>
<td>6.96 ±0.13 a</td>
</tr>
</tbody>
</table>

*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 buck’s seminal plasma. Also, it increases the level of immunoglobulins, 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 2. Effect of folic acid on serum protein, Albumin, and Globulin. g/dl (mean ± SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>TOTAL PROTEIN</th>
<th>ALBUMIN</th>
<th>GLOBULIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>5.80 ±0.29b</td>
<td>3.40 ±0.27b</td>
<td>2.37 ±0.16b</td>
</tr>
<tr>
<td>5mg/kg</td>
<td>6.30 ±0.30a</td>
<td>3.84 ±0.21a</td>
<td>3.30 ±0.29a</td>
</tr>
</tbody>
</table>

*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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>T.C</th>
<th>T. G</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>VLDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>102.14 ±1.48 a</td>
<td>80.58 ±1.57 a</td>
<td>89.65 ±1.06 a</td>
<td>65.45 ±1.85 b</td>
<td>16.11 ±0.31 a</td>
</tr>
<tr>
<td>5mg/kg</td>
<td>90.77 ±0.73 b</td>
<td>70.75 ±5.00 b</td>
<td>75.21 ±0.88 b</td>
<td>72.13 ±2.40 a</td>
<td>14.15 ±1.00 b</td>
</tr>
</tbody>
</table>

*different letters denote significant P<0.05
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 diseases are linked with elevated LDL cholesterol (Enas, 1999). LDL-cholesterol serum content higher increase 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 triglycerides (primary store fatty-acids) after 5 mg of folic acid intake perhaps involve in increased lipolysis. 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)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Length</th>
<th>Width</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>2.01 ±1.11b</td>
<td>0.98 ±0.07b</td>
<td>1.03±0.05b</td>
</tr>
<tr>
<td>5mg/kg</td>
<td>2.56 ±0.12a</td>
<td>1.31 ±0.04a</td>
<td>1.20 ±0.06a</td>
</tr>
</tbody>
</table>

*different letters denote significant-P<0.05

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Individual Motility</th>
<th>Forward</th>
<th>Backward</th>
<th>Vibration</th>
<th>Circulation</th>
<th>Dead.</th>
<th>Deformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>71.50 ±5.31b</td>
<td>60.83 ±8.95b</td>
<td>5.00 ±0.59a</td>
<td>3.50 ±0.87a</td>
<td>3.00 ±0.78a</td>
<td>28.83 ±5.41a</td>
<td>8.16 ±1.72a</td>
</tr>
<tr>
<td>5mg/kg</td>
<td>89.16 ±2.63a</td>
<td>85.00 ±3.68a</td>
<td>1.32 ±0.19b</td>
<td>1.21 ±0.30b</td>
<td>1.43 ±0.21b</td>
<td>11.83 ±3.31b</td>
<td>3.00 ±1.26b</td>
</tr>
</tbody>
</table>

*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 little 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-Rael, 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 effect 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 sub fertility 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 unsignificant 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.

REFERENCES

1007 Systematic Reviews in Pharmacy Vol 11, Issue 9, Sep-Oct 2020


