Egypt Poult. Sci. Vol (25) (II): (373-394)

EFFECT OF FOLIC ACID ON SEMEN QUALITY AND BIOCHEMICAL PARAMETERS OF RABBITS

By

Kamel I. Kamel
Animal Production Research Institute, Dokki, Giza, Egypt.
E-mail: ki_kamel@yahoo.com

Received: 16/ 04/ 2005 **Accepted: 08/ 05 /2005**

Abstract: *The objective of this study was to determine the effects of different levels of folic acid supplementation on sperm characteristics, seminal plasma lipid peroxidation and enzyme activities of mature rabbits. Twenty-four male New Zealand White rabbits (7 months old) were divided into four equal groups. The first group was served as control, while groups 2, 3, and 4 were orally treated with low (40 µg/kg of body weight), medium (80 µg/kg of body weight) and high doses (160 µg/kg of body weight), respectively of folic acid every other day for 12 weeks. Ejaculate volume (EV), sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate, packed sperm volume, total functional sperm fraction (TFSF) compared to the control. Normal sperms and initial fructose were found to be significantly ($P < 0.05$) increased due to treatments with low or medium doses of folic acid compared to the control. However, reaction time and dead sperm were significantly ($P<0.05$) decreased. On the other hand, high dose of folic acid did not show any significant ($P < 0.05$) effect on the most of previous semen parameters compared to the control group.*

Administration of folic acid increased ($P < 0.05$) seminal plasma total protein (TP), albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (AcP). Conversely, seminal plasma urea and glucose were significantly decreased compared to the control. Concentration of thiobarbituric acid-reactive substances (TBARS) was significantly ($P < 0.05$) reduced in seminal plasma of rabbits treated with all doses of folic acid. While, the activities of glutathione S-transferase (GST) and superoxide dismutase (SOD) were significantly increased compared with control. These results showed that treatments with folic acid caused an improvement of some semen characteristics in rabbits especially with low and medium doses without any harmful or negative effects on male fertility.

INTRODUCTION

Folate and folic acid are forms of a water-soluble vitamin B. Folic acid is the synthetic form of this vitamin that is found in supplements and fortified foods. Folic acid is also known as folate, folacin and pteroylmonoglutamate (Herbert, 1999).

Folic acid provided as a supplement is well absorbed (Brouwer et al., 2004). Folate participates in several key biological processes, including the synthesis of DNA, RNA and proteins. It is necessary for DNA replication and repair, the maintenance of the integrity of the genome, and is involved in the regulation of gene expression (Jacob, 2000). Mechanisms of folate metabolism are complex, involving multiple enzymes, coenzymes, and pathways. The principal role of folate coenzymes is to transport one-carbon units at various stages of oxidation to participate in methylation reactions, for example those essential for the synthesis of purines for DNA and RNA synthesis or for the metabolism of methionine (Wagner, 1995).

Little research on the role of folate in male reproductive function has been reported. Few published reports suggested that adequate folate may be necessary for male reproductive function. Animal research data suggested that nutrition affects spermatogenesis (Ciereszko and Dabrowski, 1995). Folate, which is mainly present in green leafy vegetables, is essential for synthesis of DNA, transfer RNA, and protein. Treatment with folate antagonists for various diseases has been shown to impair reproductive functions in men (Costabile, 1993). A high-affinity folate-binding protein has been identified in human semen, this protein has characteristics similar to those of membrane-bound folate binding proteins and does not appear to be derived from sperm (Holm *et al*., 1991). Human prostate gland also contains a high-affinity folate-binding protein (Holm *et al*., 1993). These findings suggest an association between folate status and male reproductive function. Because DNA synthesis is a main part of spermatogenesis, Wallock *et al.* (2001) reported that total seminal plasma folate concentrations were on average 1.5 times higher than blood plasma folate concentrations in men. They suggested that the presence of non-methyltetrahydrofolates in seminal plasma may indicate that the seminal fluid serves as a medium for active folate metabolism, or perhaps these folate forms are required in some manner for spermatogenesis or the sperm maturation process. Also, Zhang, (2000) found that seminal plasma non-methyltetrahydrofolate levels correlated significantly with sperm density and total sperm count.

Therefore, the present study aimed at investigating the effects of folic acid on semen characteristics and biochemical parameters of male rabbits.

MATERIALS AND METHODS

In this study, the effects of folic acid on the reproductive performance and seminal plasma biochemistry of male rabbits were investigated. Twenty four male New Zealand White rabbits 7 months old and initial body weight of 2800 ± 25 g were used. Animals individually housed in stainless steel cages were provided feed and water *ad libitum*. Rabbits were fed on a commercial ration pellets with following constitution (Table 1):

Rabbits were randomly divided into four equal groups. Group 1 served as control, however, groups 2, 3 and 4 were orally supplemented with 40 (low dose), 80 (medium dose) and 160 (high dose) µg/kg body weight of folic acid every other day for 12 weeks, respectively. The doses of folic acid were calculated according to the animal's body weight on the week before dosing. Folic acid was supplied from Pharaonia Pharmaceuticals, Alexandria, Egypt.

Semen collection was carried out weekly from all animals throughout the 12 weeks of the experimental period. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (using a graduated collection tube) after removal of the gel mass.

Table 1: Proximate analysis of pelleted concentrate feed (% on a dry matter basis)

	Pelleted concentrate
Ingredients (%)	
Berseem hay	30.0
Yellow corn	25.0
Wheat bran	26.2
Soybean meal	14.0
Molasses	3.0
$CaCl_2$	1.0
NaCl	0.4
Vit.&Min. mix.*	0.3
Methionine	0.1
Chemical analysis**	
Crude protein (%)	17.5
Crude fiber (%)	14.0
Crude fat (%)	2.7
Nitrogen free extract	56.4

*The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: A-4,000,000, D3-5000,000, E-16,7 g, K-0.67 g, B1-0.67 g, B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.).
**The chemical analysis of the pellets (AOAC, 1990).

A weak eosin solution (Smith and Mayer, 1955) was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide. Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in semen was carried out immediately after collection according to Mann (1948). Assessments of live, dead, and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture. The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage (Blom, 1950). Total number of motile sperm was calculated by multiplying percentage of motile sperm and total sperm output. Reaction time was recorded from the moment of subjecting a doe to the buck and completion of erection; it was measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universal indikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional

sperm fraction (TFSF) parameter was also calculated as the product of total sperm output by motility by normal morphology (Correa and Zavos, 1996)

Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C, and was stored at -20 °C until later analysis. Seminal plasma samples were analyzed for total protein (TP) by the Biuret method according to Henry et al. (1974). Albumin (A) concentrations were determined by the method of Doumas et al. (1977). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). For assaying acid phosphatase (AcP) activity, the method of Moss, (1984) was used. Seminal plasma glutathione S-transferase (GST) activity was determined according to Habig et al., (1974) using P-nitrobenzylchloride as a substrate. Thiobarbituric acid-reactive substances (TBARS) were measured in the seminal plasma by using the method of Tappel and Zalkin (1959). Superoxide dismutase (SOD) activity was measured according to Misra and Fridovich (1972).

Data were analyzed as a completely randomized design (Steel and Torrie, 1980) using the General Linear Model procedure of SAS (1986). Means were compared by Least Significant Difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Semen Characteristics: Data of ejaculate volume (EV), initial hydrogen ion concentration (pH), reaction time (RT), sperm concentration, total sperm output (TSO), packed sperm volume (PSV), sperm motility (%), total motile sperm per ejaculate (TMS), normal and dead sperm, total functional sperm fraction (TFSF) and semen initial fructose of rabbits treated with low (40 µg/kg of body weight), medium (80 µg/kg of body weight), and high doses (160 µg/kg of body weight) of folic acid every other day for 12 weeks are presented in Table 2. Figures from 1 to 4 represent the weekly mean values of these parameters expressed as % of control. Results shows that treatment male rabbits with low, medium and high doses of folic acid increased ($P < 0.05$) libido (decreased the reaction time), sperm concentration, PSV, sperm motility, normal sperm, and initial fructose. However, dead sperm was decreased ($P < 0.05$) compared to control group. Total sperm output was significantly ($P < 0.05$) increased in rabbit treated with low dose of folic acid compared to other treatment groups. On the other hand, pH, TMS and TFSF were significantly ($P < 0.05$) increased with low and medium. While, the high dose did not show

significant ($P < 0.05$) difference in these parameters compared to control group. Ejaculate volume was significantly ($P < 0.05$) increased in rabbit treated with low and high doses of folic acid compared to control group. Whereas, change ($P < 0.05$) was observed in medium dose-treated group. It is clear from the present results that administration of folic acid improved semen characteristics and has positive effect on semen quality and quantity. These results are in agreement with the finding obtained by Audet et al., (2004) who found positive correlation between the increased seminal plasma folic acid concentrations and increased sperm production and motility in young boars. Folate concentrations in blood and seminal plasma were markedly increased after folic acid administration and decreased during the wash-out period and the bioefficiency of folate from some foods is less than 50 % that of folic acid (Brouwer et al., 2004).

Also, Comhaire and Mahmoud (2003) found that sperm quality and function improved with the intake of complementary food supplementation using a combination of zinc and folic acid. Seminal plasma non-methyltetrahydrofolate levels correlated significantly with sperm density and total sperm count (Zhang, 2000). In addition, Wong et al. (2002) showed that daily treatment with 5 mg folic acid caused significant increase (74%) in total normal sperm count of subfertile men. Wallock (2001) reported that folic acid might be vital to proper sperm development because it is required for the production of DNA.

Seminal plasma characteristics: Seminal plasma characteristics of rabbits treated with different doses of folic acid were presented in Tables 3 and 4 and Figures 5 and 6. Data showed that administration with low, medium and high doses of folic acid increased ($P < 0.05$) seminal plasma TP and Alb, while, seminal plasma urea and glucose decreased ($P < 0.05$) compared with control. Seminal plasma ALT, AST and AcP increased ($P < 0.05$) with low and medium doses of folic acid, whereas, decreased ($P < 0.05$) with high dose of folic acid compared to control (Table 3). The total protein consists of nonprotein nitrogen, amino acids, and peptides that contribute towards the amphoteric property of seminal proteins (Dabas et al. 1982). The increase in TP and the decrease in urea and glucose in animals received folic acid was coincided with the improvement in semen parameters (sperm concentration, total motile sperm, total functional sperm fraction and packed sperm volume, Table 2).

Previous studies showed that rabbits seminal plasma contained a number of enzyme activities (Yousef et al., 2003). These enzymes play a pivotal role in providing substrate energy forming essential link in the

energy generating cycles in sperm metabolism, in fertilization process and in the maintenance of constant osmotic pressure during preservation (Dhami and Kodagali, 1987). The transaminases and phosphatases in semen play an important role in transamination and phosphorylation processes in sperm metabolism (Dhami *et al.,* 1994) and thus explain the differences observed in the semen quality. The increase in the activities of AST and ALT (Table 3) may be due to the increased secretory activity of male accessory sex glands. Irrespective of its origin, AST enzyme plays an important role in sperm metabolism through its involvement in the vital cellular process (Dhami and Kodagali, 1987). The increase in the activities of these enzymes coincided with the increase of semen quality of treated rabbits with folic acid. In addition, Dhami and Kodagali (1987) suggested a positive correlation between AST activity and sperm concentration, live sperm percent, motility, seminal total protein, semen volume and fertility rate of semen. Also, they reported that static semen sample had significantly lower AST value than motile sample ejaculated from Surti-buffalo bulls. These findings may confirm the role of such enzyme on the improvement of semen quality recorded in the present study of rabbits received folic acid.

Glutathione S-transferase (GST) and superoxide dismutase (SOD) in seminal plasma of rabbits treated with low, medium and high doses of folic acid were significantly increased, while, seminal plasma Thiobarbituric acid-reactive substances (TBARS) concentration was decreased ($P < 0.05$) as compared with the control (Table 4). These results are agreement with Chen *et al*., (2001) who found a positive correlation between the levels of reactive oxygen species (ROS) production and both of sperm motility and morphological defect, and negative correlation between the levels of vitamin B_{12} and folate and ROS production ($P < 0.01$) in semen. They concluded that the increase of ROS production is one of the most important factors related to poor sperm quality and human infertility. Also, Sharma and Agarwal (1996) reported that seminal plasma confers some protection against ROS damage because it contains enzymes that scavenge ROS, such as catalase, superoxide dismutase (SOD) and glutathione S-transferase (GST). Glutathione S-transferase (GSTs) are a family of proteins that catalyze the conjugation of glutathione with various electrotrophils, many of which are toxic. Also, many studies have been reported in the literature on the role of SOD as an antioxidant in reproductive biology. Alvarez *et al.,* (1987) reported that Superoxide dismutase protects spermatozoa against spontaneous O_2 toxicity. Holland et al., (1982) suggests that SOD plays a major role in protecting rabbit sperm against damage from lipid peroxidation. Also, Plante *et al.,* (1994) reported that the SOD level in spermatozoa is positively correlated with sperm motility. Dandekar *et al.,* (2002) found that astheno-zoospermic (< 10 x 106 sperm/ml, <

20% motility) patients showed decreased SOD and glutathione peroxidase levels. This correlated positively with the degree of lipid peroxidation seen in the samples. Folate acts directly to produce antioxidant effects, interactions with enzyme endothelial nitric oxid syntheses (eNOS) and effects on cofactor bioavailability of nitric oxid (Stanger, 2002). Stanger et al. (2002) reported that folate administration was associated with increased antioxidative capacity as determined by total antioxidant status. In contrast, folate deficiency was associated with increased lipid peroxidation in rats (Verma, and Kanwar, 1999) and decreased of cellular antioxidant defense (Dabas et al., 1982).

Conclusion

The present results showed that the maximal effects on semen characteristics especially sperm production in male rabbit seemed to be highly respond to the lowest given dose of folic acid (40 µg/kg), followed by the medium dose (80 µg/kg body weight). However, greater doses of folic acid (160 µg/kg of body weight every other day, high dose) are not as effective as low or medium doses for most parameters studied.

Table 2. Effect of different doses of folic acid on semen characteristics of male rabbits (means ± SE).

Item	Folic acid doses			
	Control 0	Low 40µg/kg*	Medium 80 µg/kg	High 160 µg/kg
Ejaculate volume(ml)	0.67±0.08^{b}	0.77±0.09^{a}	0.69±0.08^{b}	0.60±0.07^{c}
PH	7.43±0.87^{c}	7.80±0.92^{b}	8.00±0.94^{a}	7.50±0.88^{c}
Reaction time (sec.)	8.40±0.99^{a}	4.05±0.48^{d}	6.19±0.73^{c}	7.48±0.88^{b}
Sperm concentrate (×10^{6}/ml)	265±31.2^{d}	330±38.9^{a}	274±32.2^{c}	298±35.1^{b}
Total sperm output (×10^{6})	176±20.7^{b}	255±30.0^{a}	187±22.1^{b}	180±21.2^{b}
Packed sperm volume	17.8±0.18^{c}	20.2±0.43^{a}	19.9±0.30^{a}	18.8±0.28^{b}
Sperm motility (%)	67.8±8.0^{d}	80.0±9.4^{a}	76.2±9.0^{b}	69.6±8.2^{c}
Total motile sperm (×10^{6})	120.0±14.1^{c}	206.3±24.3^{a}	142.8±16.8^{b}	125.3±14.8^{c}
Dead sperm (%)	29.7±3.5^{a}	23.3±2.8^{b}	22.3±2.6^{b}	22.7±2.7^{b}
Normal sperm (%)	82.7±9.70^{d}	86.3±10.17^{b}	87.1±10.26^{a}	84.5±9.96^{c}
TFSF**	99.3±11.7^{c}	178.1±21.1^{a}	124.3±14.7^{b}	105.9±12.5^{c}
Initial fructose (mg/dl)	242.5±8.49^{d}	274.8±22.05^{b}	278.4±8.80^{a}	254.6±2.25^{c}

^{abcd} Within row, means with different superscript letters differ significantly ($p < 0.05$).
*Kg of body weight every other day.
**TFSF: Total functional sperm fraction.

Table 3. Effect of different doses of folic acid on seminal plasma total protein (TP), albumin (Alb), urea, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (AcP), of male rabbits (means ± SE).

Item	Folic acid doses			
	Control 0	Low (40µg/kg)*	Medium (80 µg/kg)	High (160 µg/kg)
TP (gm/dl)	5.65 ± 0.092 [d]	6.21 ± 0.301 [a]	6.12 ± 0.221 [b]	5.98 ± 0.178 [c]
Alb (gm/dl)	3.34 ± 0.098 [d]	3.72 ± 0.063 [a]	3.65 ± 0.044 [b]	3.62 ± 0.054 [c]
Urea (mg/dl)	42.38 ± 1.354 [a]	37.18 ± 3.600 [d]	37.77 ± 4.082 [c]	39.20 ± 3.512 [b]
Glucose (gm/dl)	39.58 ± 1.491 [a]	31.79 ± 2.593 [d]	33.68 ± 1.040 [c]	37.72 ± 2.335 [b]
ALT (IU)	26.96 ± 0.723 [b]	30.62 ± 4.022 [a]	30.69 ± 2.249 [a]	25.33 ± 1.972 [c]
AST (IU)	47.76 ± 1.692 [c]	53.01 ± 7.187 [a]	51.40 ± 4.811 [b]	45.86 ± 2.168 [d]
AcP (U/L)	23.55 ± 1.022 [c]	28.00 ± 1.836 [a]	27.85 ± 1.087 [a]	25.63 ± 0.342 [b]

[abcd] Within row, means with different superscript letters differ significantly ($p < 0.05$).
*Kg of body weight every other day.

Table 4. Effect of different doses of folic acid on seminal plasma TBARS concentration, glutathione s-transferase (GST), and super oxide dismutase (SOD) of male rabbits (means ± SE).

Item	Folic acid doses			
	Control 0	Low (40µg/kg)*	Medium (80 µg/kg)	High (160 µg/kg)
TBARS (nmol/ml)	1.17 ± 0.054 [a]	0.95 ± 0.099 [b]	0.89 ± 0.116 [c]	0.97 ± 0.090 [b]
GST (µmol/hr)	1.33 ± 0.020 [d]	1.47 ± 0.060 [a]	1.39 ± 0.064 [c]	1.41 ± 0.026 [b]
SOD (U/ml)	25.50 ± 1.610 [d]	36.00 ± 1.988 [a]	32.48 ± 2.640 [b]	26.68 ± 2.439 [c]

[abcd] Within row, means with different superscript letters differ significantly ($p < 0.05$).

*Kg of body weight every other day.

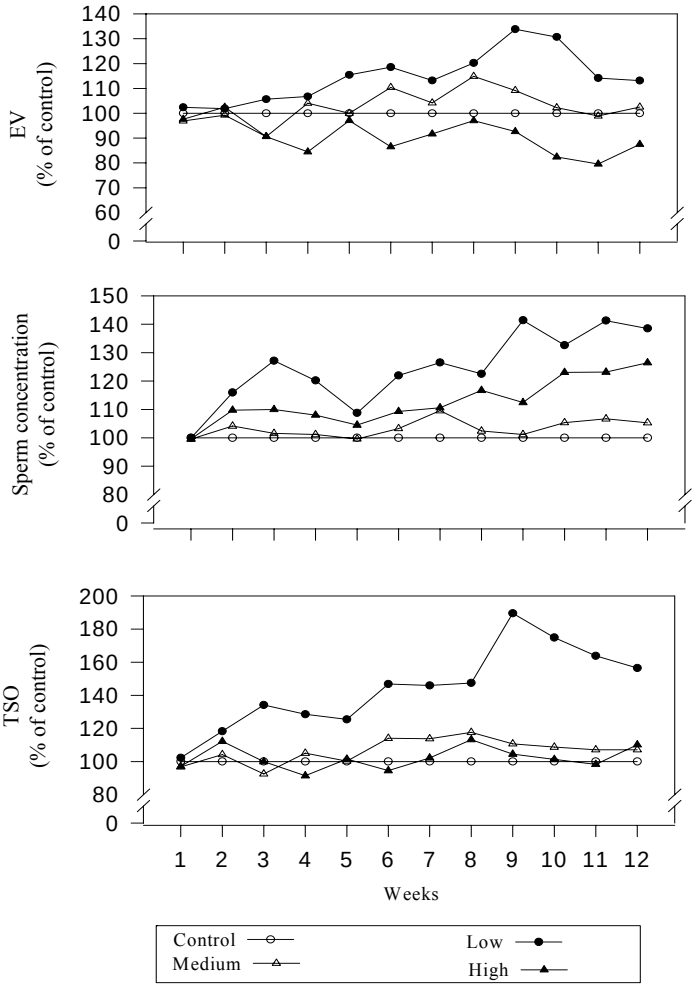

Figure 1:Effect of different doses of folic acid on ejaculate volum (EV), sperm concentration and total sperm output (TSO)as a % of control for male rabbits.

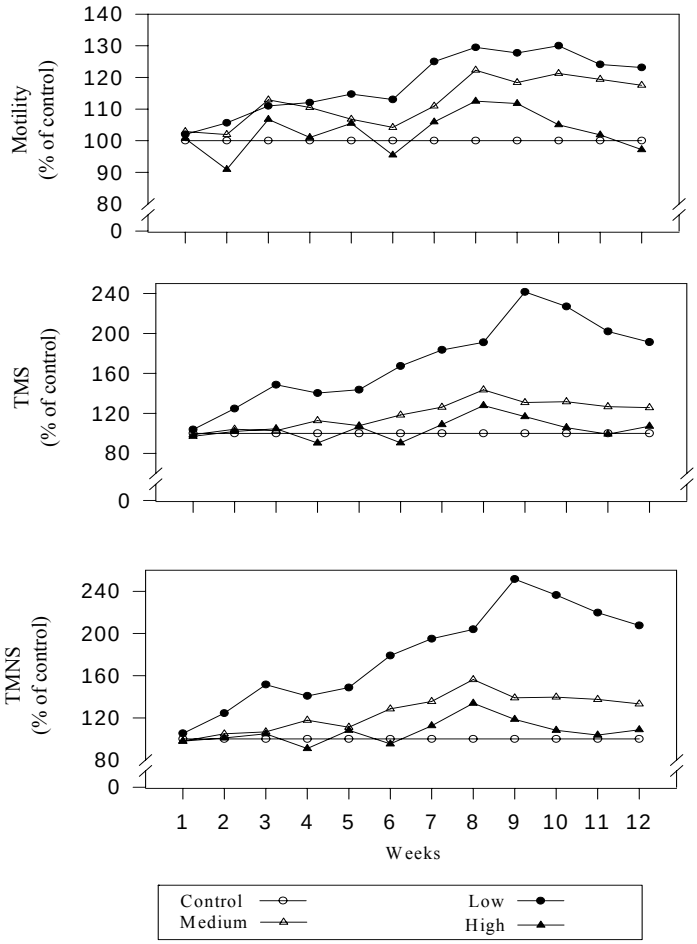

Figure 2: Effect of different doses of folic acid on motility, total motile sperm(TMS) and total motile normal sperm (TMNS) as % of control for male rabbits.

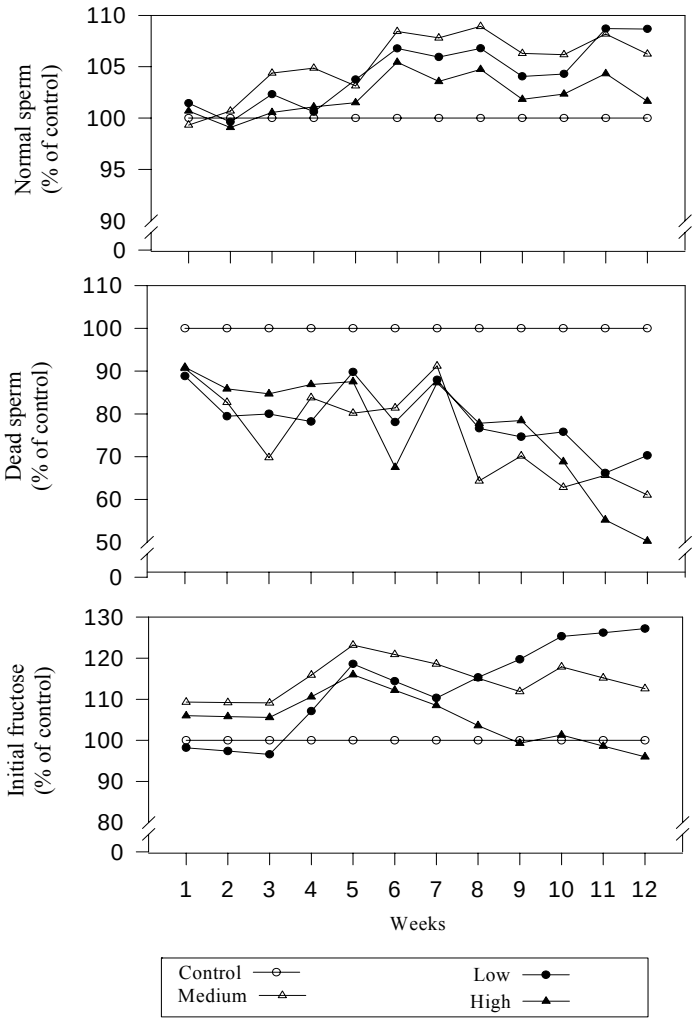

Figure3: Effect of different doses of folic acid on normal, dead sperm and initial fructose as % of control for male rabbits.

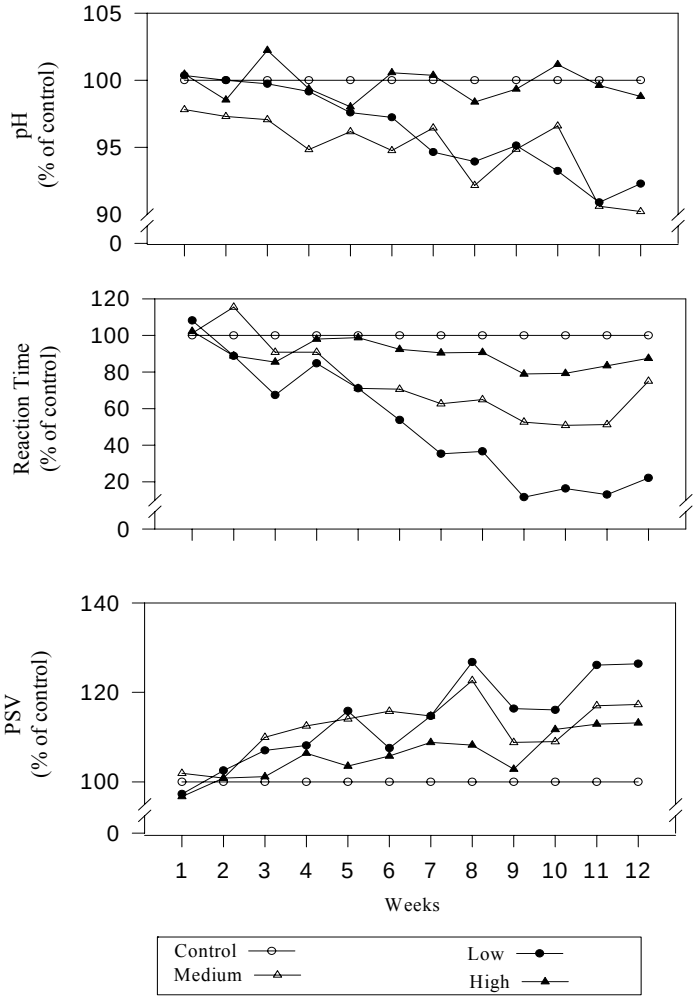

Figure 4: Effect of different doses of folic acid on pH, reaction time and packed sperm volume (PSV) as % of control for male rabbit.

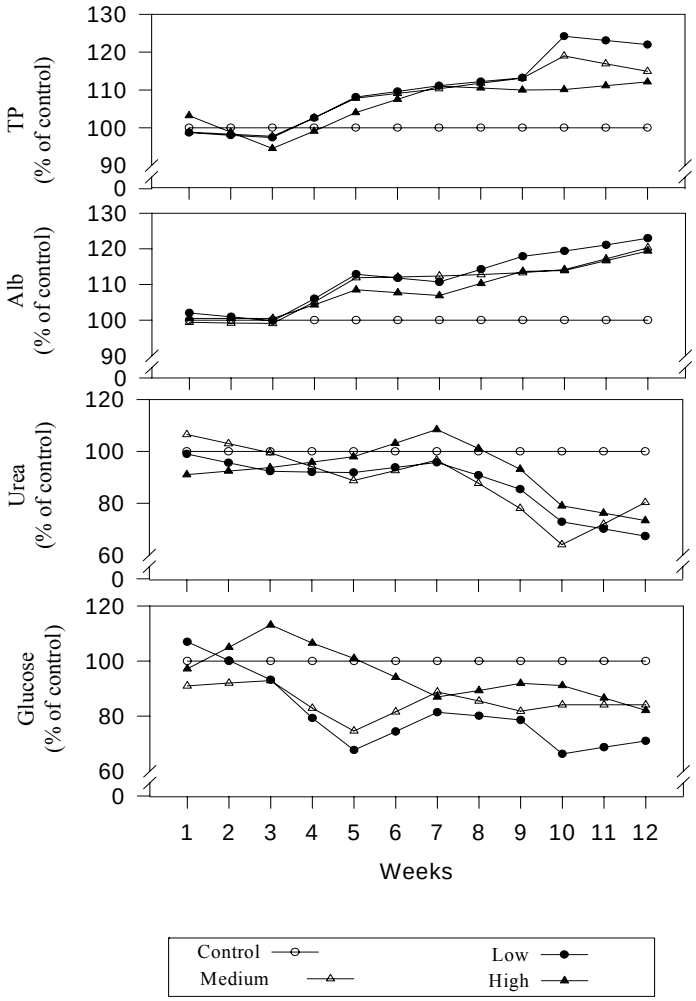

Figure 5: Effect of different doses of folic acid on seminal plasma total protein (TP), albumin (Alb), urea and glucose as % of control for male rabbits.

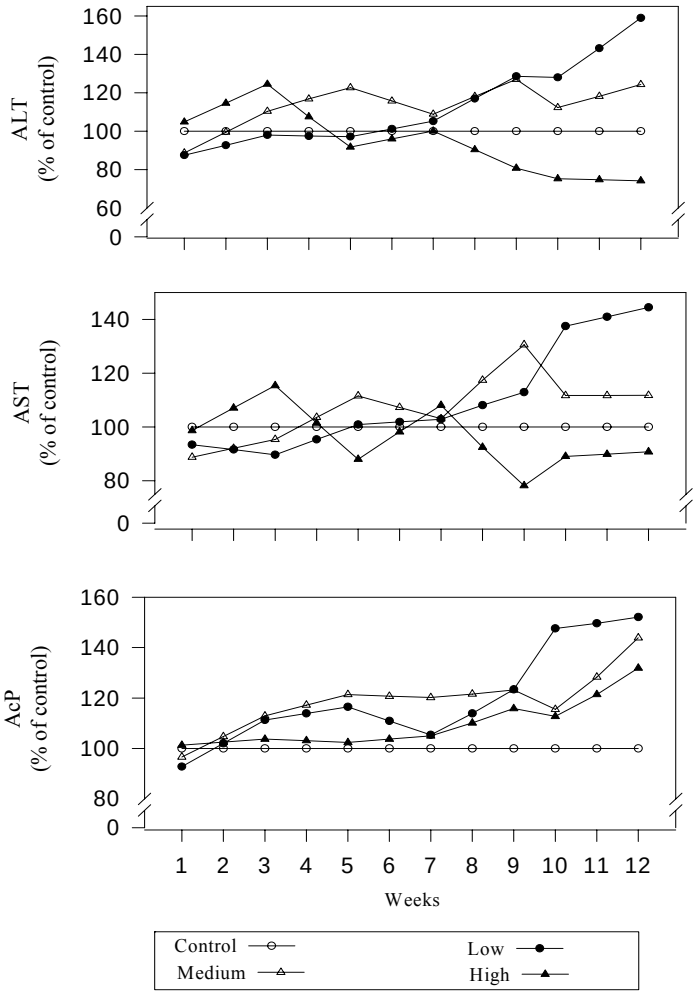

Figure 6: Effect of different doses of folic acid on seminal plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and acid phosphatase (AcP) as % of control for male rabbits.

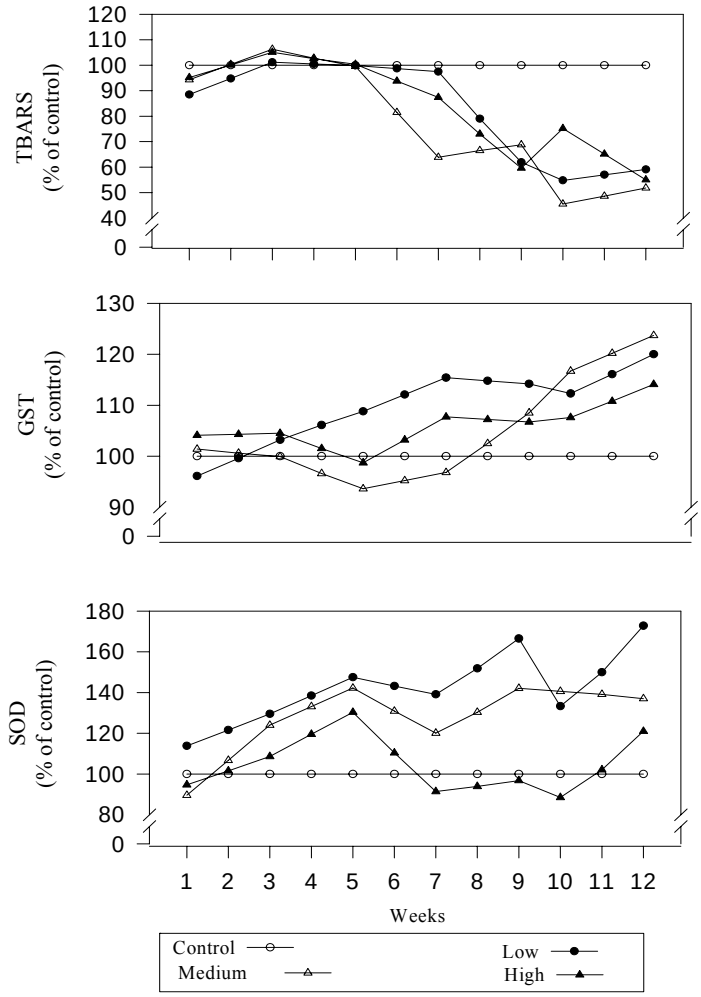

Figure 7:Effect of different doses of folic acid on seminal plasma thiobarbituric acid-reactive substances (TBARS), Glutathion peroxidase (GST) and superoxide dismutase (SOD) as % of control for male rabbits.

REFERENCES

Alvarez, J.G.; Touchstone, J.C.; Blasco, L. and Storey, B.T.(1987). *Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen Toxicity. J. Androl., 8: 338-348.*

AOAC, (1990). *OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL AGRICULTURAL CHEMISTS, 13th Ed. Benjamin, Franklin Station, Washington, D.C.*

Audet, I.; Laforest, J.P.; Martineau, G.P. and Matte, J.J. (2004). *Effect of vitamin supplements on some aspects of performance, vitamin status, and semen quality in boars. J Anim Sci., 82:626-633.*

Blom, E. (1950). *A one-minute live-dead sperm stain by means of eosin-nigrosin. J. Fertil. Steril., 1: 176-177.*

Brouwer I.A.; Dusseldorp, M.v.; West, C.E. and Steegers-Theunissen R.P.M. (2004). *Bioavailability and bioefficacy of folate and folic acid in man. Nutrition Research Reviews, 14: 267 – 293.*

Chen, Q.; Ng, V.; Mei, J. and Chia, S.E. (2001). *Comparison of seminal vitamin B12, folate, reactive oxygen species and various sperm parameters between fertile and infertile males Wei Sheng Yan Jiu., 30: 80-82.*

Ciereszko, A. and Dabrowski, K. (1995). *Sperm quality and ascorbic acid concentration in rainbow trout semen are affected by dietary vitamin C: an across-season study. Biol Reprod., 52:982–988.*

Comhaire, F.H. and Mahmoud, A. (2003). *The role of food supplements in the treatment of the infertile man. Reprod. Biomed. Online., 7: 385-391.*

Correa, J.R. and Zavos, P.M. (1996). *Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. Theriogenology, 46: 12225-12232.*

Costabile, R.A. (1993). *The effects of cancer and cancer therapy on male reproductive function. J Urol., 149:1327–1330.*

Dabas, Y.P.S.; Verma, M.C. and Tripathi, S.S. (1982). *Biochemical studies on Red Dane and Murrah bull seminal plasma. Cheiron, 11: 184-187.*

Dandekar, S.P.; Nadkarni, G.D.; Kulkarni, V.S. and Punekar, S. (2002). *Lipid Peroxidation and Antioxidant Enzymes in Male Infertility. Journal of Postgraduate Medicine, 48: 186-190.*

Dhami, A.J. and Kodagali, S.B. (1987). *Correlation between biochemical and enzymatic constituents of semen of Suerti buffalo bulls. J. Anim. Sci., 57: 1283-1286.*

Dhami, A.J.; Sahni, K.L.; Mohan, G. and Tripathi, R.P. (1994). *Comparative evaluation of initially static and motile semen ejaculate*

from Frieesian and Murrah buffalo bulls for physico-morphological, biochemical, enzymatic and mineral constituents of seminal plasma. Indian Journal of Animal Sciences, 64: 926- 932.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1977). *Albumin standards and the measurement of serum albumin with bromocresol green. Clinic. Chem. Acta., 31: 87-96.*

Habig, W.H.; Pabst, M.J. and Jakoby, W.B. (1974). *Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.*

Henry, R.J.; Cannon, D.C. and Winkelman, W. (1974). *CLINICAL CHEMISTRY PRINCIPLES AND TECHNIQUES, 11th ed. Happer and Row Publishers, p. 1629.*

Herbert, V. (1999). *Folic Acid. In: Shils M, Olson J, Shike M, and Ross AC, ed. NUTRITION IN HEALTH AND DISEASE. Baltimore: Williams and Wilkins.*

Holland, M.K.; Alvarez, J.G. and Storey, B.T. (1982). *Production of superoxide and activity of superoxide dismutase in rabbit epididymal spermatozoa. Biol. Reprod., 27: 1109-1118.*

Holm, J.; Hansen, S.I. and Hoier-Madsen, M. (1991). *A high-affinity folate binding protein in human semen. Biosci. Rep., 11:237– 242.*

Holm, J.; Hansen, S.I. and Hoier-Madsen, M. (1993). *High-affinity folate binding in human prostate. Biosci. Rep., 13: 99 –105.*

Jacob, R.A. (2000). *Folate, DNA methylation, and gene expression: Factors of nature and nurture. Am. J. Clin. Nutr., 72: 903-904.*

Landau, B.; Singer, R.; Klein, T. and Segenreich, E. (1978). *Folic acid levels in blood and seminal plasma of normo- and oligospermic patients prior and following folic acid treatment. Experientia, 34:1301–1302.*

Mann, T. (1948). *Fructose content and fructolysis in semen: Practical application in the evaluation of semen quality. J. Agric. Sci. (Camb.), 38: 323-331.*

Misra, H.P. and Fridovich, I. (1972). *The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.*

Moss, D.W. (1984). *In: Methods of Enzymatic Analysis, 3rd Ed. Bergmeyer, H.U., Ed.; Verlag-Chemie, 4: 92-106.*

Plante, M.; de Lamirande, E. and Gagnon, C. (1994). *Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. Fertil. Steril., 62:387-93*

Reitman, S. and Frankel, S.A. (1957). *Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clinic. Pathol., 28: 56-63.*

SAS. SAS User's Guide: *Statistics, version 5 Edition SAS Inst., Inc., Cary, NC, USA, 1986.*

Sharma, R.K. and Agarwal, A. (1996). *Role of reactive oxygen species in male infertility. Urology, 48: 835-850.*

Smith, J.T. and Mayer, D.T., (1955). *Evaluation of sperm concentration by the haemocytometer method. Fertil., 6: 271-275.*

Stanger, O. (2002). *Physiology of Folic Acid in Health and Disease Current Drug. Metabolism, 3: 211-223.*

Stanger, O.; Semmelrock, H.J.; Wonisch, W.; Bos, U.; Pabst, E. And Wascher, T.C. (2002). *Effects of folate treatment and homocysteine lowering on resistance vessel reactivity in atherosclerotic subjects. J. Pharmacol. Exp. Ther., 303:158-162.*

Steel, R.G.D. and J.H. Torrie (1980). *PRINCIPLE AND PROCEDURES OF STATISTICS. A. Biochemical Approach (2nd Ed.) Mc Gvaus-Hill Booh Company, New York, USA.*

Tappel, A.L. and Zalkin, H. (1959). *Inhibition of lipid peroxidation in mitochondria by vitamin E. Arch. Biochem. Biophys., 80: 333-336.*

Verma, A. and Kanwar, K. C. (1999). *Human spermatozoal motility and lipid peroxidation. Indian. J. Exp. Biol., 37: 83-85.*

Wagner, C. (1995). *Biochemical role of folate in cellular metabolism. In: Bailey, L.B., ed. Folate in Health and Disease. New York: Marcel Dekker, Inc., 23-42.*

Wallock, L.M. (2001). *Low seminal plasma folate concentrations are associated with low sperm density and count in male smokers and non.smokers. Fertil. Steril., 75: 252-9.*

Wallock, L.M.; Tamura, T.; Mayr, C.A.; Johnston, K.E.; Ames, B.N. and Jacob, R.A. (2001). *Low seminal plasma folate concentrations are associated with low sperm density and count in male smokers and nonsmokers. Fertility and Sterility 75: 252-259.*

Wong, W. Y.; Merkus, H. M.; Thomas, C.M.; Menkveld, R; Zielhuis, G. A. and Steegers-Theunissen, R. P. (2002). *Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertil. Steril., 77: 491-498.*

Yousef, M.I.; Abdallah, G.A. and Kamel, K.I. (2003). *Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci., 76: 99-111.*

Zhang, J. P. (2000). *Effect of smoking on semen quality of infertile men in Shandong. China. Asian. J. Androl., 2: 143-146.*

الملخص العربي

تأثر اضافة حامض الفوليك على جودة السائل المنوى والمعايير البيوكيميائية لذكور الارانب

كامل ابراهيم كامل
معهد بحوث الانتاج الحيواني – مركز البحوث الزراعية – وزارة الزراعة

اجريت هذه الدراسة على عدد ٢٤ من ذكور الارانب النيوزيلاندى الابيض بغرض دراسة تأثير جرعات مختلفة من حامض الفوليك على خصائص السائل المنوى من حيث الجودة والتغيرات الحادثة فى خصائصه البيوكيميائية. قسمت التجربة الى اربع مجاميع: المجموعة الاولى وهى المجموعة الضابطة،اما المجموعة الثانية والثالثة والرابعة تناولت عن طريق الفم جرعات من حامض الفوليك مقدرها ٤٠ (الجرعة المنخفضة)، ٨٠ (الجرعة المتوسطة)، ١٦٠ (الجرعة العالية) ميكروجرام لكل كليوجرام وزن جسم على التوالى يوم بعد يوم لمدة ١٢ اسبوع.

وقد اوضحت النتائج حدوث زيادة معنوية فى المجاميع المعاملة بالجرعة المنخفضة والمتوسطة من حامض الفوليك على حجم القذفة، درجة الحموضة، الرغبة الجنسية، تركيز الحيونات المنوية، العدد الكلى للحيونات المنوية فى القذفة، الحركة الكلية للحيونات المنوية، عدد الحيونات المنوية المتحركة لكل قذفة، حجم السائل المنوى المعبأ، عدد الحيونات المنوية الطبيعية (الغير شاذة)، وعدد الحيونات المنوية المتحركة والغير شاذة لكل قذفة. بينما انخفضت معنويا عدد الحيونات المنوية الميتة وتركيز الفركتوز. بينما المعاملة بالتركيز العالى لم يؤثر معنويا على معظم المعايير السابقة مقارنا بالمجموعة الضابطة.

المعاملة بالجرعات الثلاثة من حامض الفوليك سبب زيادة معنوية فى البروتين الكلى، والالبيومين والانزيمات اسبرتيت ترنزامينيز والانيين ترنزامينيز والكالاين فوسفاتيز فى البلازما المنوية.

كان لتأثير المعاملة بحامض الفوليك معنوى فى خفض تركيز الاصول الحرة (الشوارد) فى البلازما المنوية وزيادة نشاط انزيمات مضادات الاكسدة مثل الجلوتاثيون-اس-ترانزفيريز والسوبر اوكسيد دزميوتيز.

ومن هذه النتائج يتضح اهمية دور حامض الفوليك فى تحسين خصائص السائل المنوى لذكور الارانب خلال موسم التلقيح.

وقد حققت الجرعة المنخفضة من حامض الفوليك اعلى استجابة بتحسين مواصفات جودة السائل المنوى.