Effect of L-carnitine administered to pregnant mice on some reproductive properties of their male offspring

Muhammad-Baqir M-R. Fakhrildin
Department of Clinical Reproductive Physiology, High Institute of Infertility Diagnosis and ART , Al-Nahrain University, Baghdad- IRAQ .
Corresponding author e-mail: art_mbmrfd@yahoo.com).

Abstract:

Background
Carnitines are widely distributed in nature and their potential health benefits have been popularized. The carnitines exert a substantial antioxidant action. It is often sold as a nutritional supplement. The use of carnitine showed some promise in treatment of male infertility.

Objectives
This study was designed to investigate the effects of L-carnitine (LC) administered to pregnant mice on some reproductive parameters of male offspring.

Methods:
Ninety mature male offspring mice (age: 6 weeks) were divided originally into two groups depending on the pregnant mothers were administered either 0.5 mg/Kg L-carnitine (Treated group) or distilled water (Control group) throughout pregnancy period. Whole body and testicular weight, diameter of seminiferous tubules, thickness of spermatogenic cell layers, levels of serum gonadotropins and sperm parameters were assessed.

Results
In the present study, weight of whole body and testes of the male mice offspring group where mothers treated with 0.5 mg/Kg L-carnitine throughout gestation period was significantly increased ($P<0.05$) as compared to the control group. Further, significant increment ($P<0.05$) was observed in the thickness of spermatogenic cell layers and diameter of seminiferous tubules for the treated group as compared to the control group. Also, levels of serum follicle stimulating hormone (FSH) and Leutinizing hormone (LH) in the treated group showed significant elevation ($P<0.05$) as compared to the control group. Sperm parameters for the treated group were enhanced significantly ($P<0.05$) as compared to the control group.

Conclusion
Administration of low dose of L-carnitine to pregnant mice improved reproductive performance of male offspring. Further molecular studies are recommended to investigate the effects of L-carnitine administered to pregnant mice on quality and quantity of acrosomal enzymes for male offspring.

Key words:L- Carnitine, Mice, Pregnancy, Gonadotropins, Sperm.

Introduction

L-carnitine (-hydroxy- -trimethyl-aminobutyrate) was discovered in 1905 and is considered a cofactor involved in different biological pathways. Unlike most cofactors, LC is not a vitamin and is derived from the amino acids lysine and methionine (1). Carnitine is required for the transport of medium and long chain fatty acids into the mitochondria (2). Furthermore, carnitine facilitates transport of fatty acids across mitochondrial membranes, thereby improving the availability of fatty acids for beta-oxidation (3), regulating the free CoA to AcylCoA ratio and scavenging potentially toxic organic (4). Carnitine is a naturally occurring compound that is both synthesized in the body and ingested, principally as a component of meat. It has been applied in pharmacological amounts for a number of disorders, including decreased sperm motility (5).

Normally, the male genital tract contains several compartments that maintain the highest free carnitine concentrations in the body involving epididymes, seminal plasma and spermatozoa. The high concentration was found in the luminal fluid from the

distal head epididymis where the spermatozoa become motile (6). However in most mammalian species, the origin of free carnitine in seminal fluid is mainly epididymal tissue (7). Furthermore, male mice pretreated with LC before being exposed to electric and magnetic fields had their sperm concentration and motility restored at a faster rate following the exposure compared with non-treated male mice (8).

High concentrations of LC and its metabolite acetyl-L-carnitine are found in the sperm, and both have a crucial role to play in sperm energy metabolism (9). LC is supplied to the body through both endogenous synthesis and food intake. The human body synthesizes about 20 mg of LC every day. Moreover, the major sites of LC biosynthesis are the liver and kidneys (10). Evidence indicates LC is absorbed in the intestine by a combination of active and passive diffusion (11).

The heart, skeletal muscle, liver, kidneys and epididymes have specific transport systems for carnitine that concentrate carnitine within these tissues (12). Therefore, this study was designed to investigate the effects of L-carnitine administered to pregnant mice on level of serum gonadotropins, sperm parameters and testicular and epididymal histological changes of male offspring.

Materials and methods

Preparation of L-carnitine dose:

Tablet of L-carnitine (L-carnitine tartrate 1000mg/tablet; Harbin Yeekong Inc.; Australia) was crushed and dissolved in 100 mL of distilled water, therefore, 0.05 mL contains 0.5 mg/Kg of L-carnitine. Throughout gestation period, female mice of treated group were orally administered 0.5 mg/Kg of L-carnitine. While female mice of control group were orally administered similar amount of distilled water.

Animals:

Ninety healthy mature male offspring mice (age: 6 weeks) were divided originally into two groups depending on the pregnant mothers were administered either 0.5 mg/Kg L-carnitine (Treated group; No. 47) or distilled water (Control group; No. 43) throughout pregnancy period and obtained from Animal House Unit at Institute of Embryo Research and Infertility Treatment / Al-Nahrain University and kept in an air conditioned room with temperature 24 + 2 oC and exposed to 12-14 hours day light program. Water and food was locally prepared and consists of available constituents which fulfill the dietary requirements.

Measurements:

When male offspring reach adult stage (6 weeks age), whole body weight was assessed. Then, blood samples were taken under moderate anesthesia by heart puncture using 21-gauge needle attached to 2mL syringe and put in Eppendorf tubes (1.5 mL) and left for 10 minutes. For gonadotropins (FSH and LH) assay, serum was separated from blood by centrifugation (2500 RPM for 8 minutes) and preserved at -20 oC until time of test using Radioimmunoassay.

Also, each male was sacrificed by cervical dislocation and testes and epididymes were obtained. Then, fatty tissues surrounding epididymes and testes were removed. Both caudal of epididymes were minced using a microsurgical scissor until getting homogenized in 1 mL of sperm preparation medium, and spermatozoal suspension was incubated in air incubator at 37 oC until examination. Sperm concentration, sperm motility (%), progressive motile sperm (%), normal sperm morphology (%) and sperm agglutination (%) were assessed according to WHO procedure (13).

Testes were weighted and prepared for histological study according to Junquera and Carneiro (14). Diameter of seminiferous tubules and thickness of spermatogenic cell layers were measured.

Statistical analysis:

All results are expressed as mean±standard error of mean (SEM). Statistical tests were applied depending on available crude data using Statistical Package for Social Science (SPSS; version 14).

Also, t-test was applied to assess differences between the control and treated groups. P value of less than 0.05 was considered statistically significant (15).

Results

In the present study, the whole body weight for male mice offspring of the male mice offspring group where mothers treated with 0.5 mg/Kg L-carnitine throughout gestation period was significantly increased ($P<0.05$) as compared to the control group (Table 1). Similarly, significant increment ($P<0.05$) was assessed in the weight of mouse testes for the treated group when compared to the control group. From the same table, diameter of seminiferous tubules for the treated group was significantly increased ($P<0.05$) as compared to the control group. Furthermore, significant increment ($P<0.05$) was observed in the thickness of spermatogenic cell layers for the treated group as compared to the control group (Table 1).The levels of serum gonadotropins (FSH and LH) for male mice offspring where mothers administered L-carnitine during gestation period presented in the figure (1). Levels of serum FSH and LH in the treated group appeared significant elevation ($P<0.05$) as compared to the control group.

Table 1: Mouse body and testicular weights, diameter of seminiferous tubules and thickness of spermatogenic cell layers for male offspring# where mothers administered L-carnitine throughout gestation period

Groups	Sperm parameters			
	Body weight (G)	Testicular weight (G)	Diameter of Seminiferous Tubules	Thickness of spermatogenic cell Layers
Control group	16.40 ± 0.07	2.81 ± 0.01	13.50 ± 0.09	1.70 ± 0.01
Treated group	18.63 * ± 0.08	3.40 * ± 0.01	15.02 * ± 0.11	2.10 * ± 0.11

Significant difference (P<0.05) between control and treated groups.
Number of male offspring groups (Control= 43; Treated= 47).

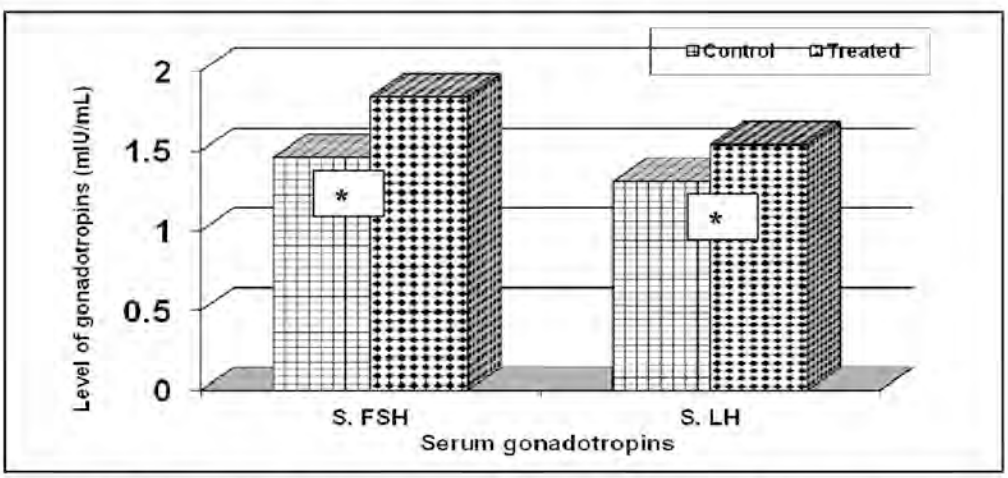


Figure1: Levels of serum gonadotropins for male offspring# where mothers administered L-carnitine throughout gestation period

* Significant difference (P<0.05) between control and treated groups.#: Number of male offspring groups (Control= 43; Treated= 47).

Table (2) shows the parameters of mouse spermatozoa for male offspring where mothers administered 0.5 mg/Kg L-carnitine throughout gestation period. Significant increment (P<0.05) was noticed in the sperm concentration and the percentage of sperm motility for the treated group as compared to the control group. Similarly, the percentage of progressive sperm motility for the treated group was increased significantly (P<0.05) when compared to the control group. However, the percentage of normal sperm morphology for the treated group was elevated significantly (P<0.05) when compared to the control group. In contrast, the percentage of sperm agglutination for the treated group was significantly decreased (P<0.05) as compared to the control group (Table 2).

Table 2: Mouse sperm parameters for male offspring* where mothers administered L-carnitine throughout gestation period

Groups	Sperm parameters				
	Sperm concentration (..X 10*/mL)	Sperm motility (%)	Sperm progressive motility (%)	Normal sperm morphology (%)	Sperm agglutination (%)
Control group	48.02 ± 0.09	56.0 ±0.07	45.80 ± 0.16	38.03 ± 0.10	19.02 ± 0.12
Treated group	62.08* ± 0.01	78.12* ± 0.19	57.50* ± 0.14	65.04* ± 0.21	4.82* ± 0.08

* Significant difference (P<0.05) between control and treated groups.
*: Number of male offspring groups (Control= 43; Treated= 47).

Discussion

In mammals, L-carnitine (LC) is always present as a mixture of free and acylated L-Carnitine (LAC). Typically, in healthy humans, approximately 80-85% of LC exists as free form in the plasma. The LC plasma levels are regulated mainly by the kidneys, and are age and sex-dependent (16). Taken together, the present work was designed to investigate the effects of administration of L-carnitine to pregnant mice on some reproductive parameters of male offspring.

Tissue distribution and uptake of LC are at least partially controlled by hormones, particularly in the liver and epididymes (17).An increased weight of whole body and testes, diameter of seminiferous tubules and thickness of spermatogenic cell layers for male where mothers treated with LC throughout gestation period as compared to the control group. These observations may be explained through the physiological activities of LC directly and/or indirectly. The concentration of carnitine in different species and in different tissues varies over a wide range. The highest concentrations reported have been found in horseshoe crab muscle, and in rat epididymal fluid, in which carnitine can reach a concentration of 60 mM (18). Specifically, carnitine protects cell membrane and DNA against damage induced by free oxygen radicals. It also prevents protein oxidation and lactate oxidative damage (19). Mature spermatocytes contain over 1000 times more carnitine than CoA (20). These observations led to extensive studies on the function of carnitine in spermatogenesis and fertility. It has been shown previously that the development of the high activity of carnitine acetyltransferase in the spermatozoa coincides with the maturation of sperm cells (17).Perhaps, an enhancement of mouse sperm quality for treated offspring males group where their pregnant mothers administered 0.5 mg/Kg LC throughout gestation period may be return to the influence of balanced overproduction of gonadotropins (FSH and LH) as presented in figure (1). In addition to the other factors, sex hormones may also influence carnitine distribution. Female rats have been reported to have a significantly higher liver/plasma carnitine concentration ratio than male rats, and the high carnitine uptake and concentration in the epididymes depend on an androgen-induced transport mechanism (21). It was observed that the extremely high concentrations of carnitine in the cauda epididymes and the epididymal fluid and to determine that this high concentration depends on the action of androgen hormones. In the epididymis the androgens induce a carnitine transport system with the ability to concentrate carnitine in the epididymal lumen (17).In this study, sperm parameters for male offspring where mothers administered LC were enhanced significantly ($P<0.05$) as compared to the control group. These results were obtained through the different biological actions of L-carnitine. It was known that the sperm plasma membranes contain high levels of polyunsaturated fatty acids, an essential component of the phospholipid content in cell membrane, and L-carnitine possesses antioxidant properties (22). Actually, LC may work as an antioxidant to scavenge free radicals (23). Therefore, the mode of action of L-carnitine in increasing sperm concentration is through its antioxidant properties, thus extending the life span of sperm (24). Although supplementation of diets with LC increased sperm concentration (25). However, the concentration of L-carnitine in semen is closely linked to sperm quality (26). Previous results showed that this high concentration and high enzyme activity depend on an effect of testosterone. Therefore, only its function as a store for activated acetyl groups has been established as a general function of carnitine in spermatogenesis and fertility (17).Clinical studies suggest that LC supplementation over a period of 3 to 6 months can positively affect sperm concentration, total sperm count, percentage of motile sperm and the percentage of sperm with rapid progression (7,27). On the other hand, oral administration of L-carnitine (3 g daily for four months) resulted in significant improvements in sperm number, quality, and motility in patients with inadequate sperm (28).

Recently, LAC is a bioactive production from LC and they both participate in the energy metabolism, which positively affects sperm motility, maturation and the spermatogenic process (29).The percentages of sperm motility and progressive sperm motility were increased significantly ($P<0.05$) for treated group as compared to the control group. These results should be interpreted in the light cautiously as available ATP needed for sperm motility. It was known that L-carnitine plays a vital role in transporting long chain fatty acids across the inner mitochondria membrane to produce energy through â-oxidation (17), which is the most efficient metabolic pathway for energy production (30). Thus, carnitine serves several critical physiologic functions. It is essential for the oxidation of long chain fatty acids, an important source of energy in the male reproductive tract. It also serves to export potentially toxic fatty acid metabolites from the sperm's cells. In general, the highest concentrations of carnitine are found in epididymal fluid of the male reproductive tract, where concentrations can exceed 2000 times that found in blood plasma (5). However, the primary mechanism of carnitine action is apparently attributable to its role as a cofactor in the transformation of free long-chain fatty acids into acylcarnitines for subsequent transport into the mitochondrial matrix (31).Briefly, carnitine therapy (with L-carnitine and/or L-acetyl-carnitine) showed some considerable positive effects in improving sperm quality (29). In another double-blind study, 100 infertile males were supplemented with 2 g L-carnitine daily or placebo for two months, followed by a two months

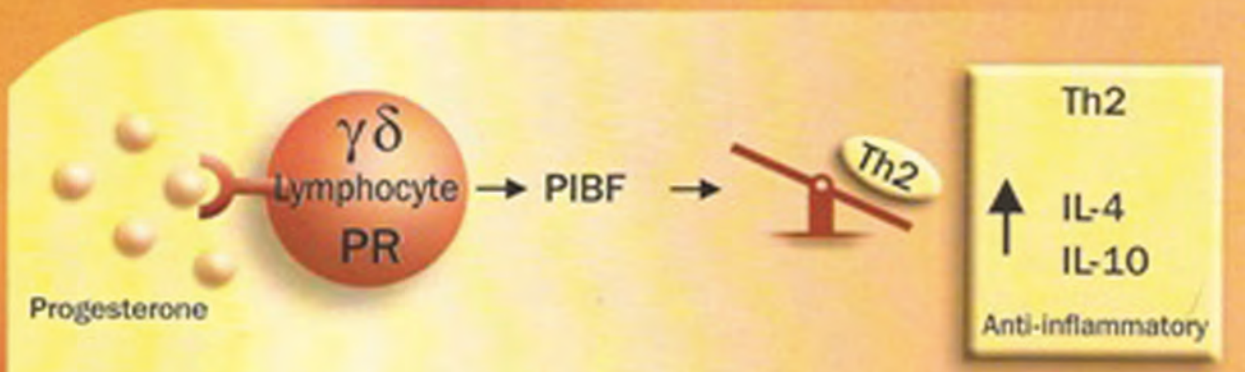
washout period, and finally two months on the opposite treatment. Statistically significant improvements in sperm count and motility were observed in the L-carnitine group (27). The same researchers conducted a second study on 56 infertile males and found the combination of L-carnitine (2 g daily) and acetyl-L-carnitine (1 g daily) led to significant improvement in sperm motility (32). As shown in a recent study, the administration of LC may be effective in improving pregnancy rate and sperm kinetic features in patients affected by male infertility (29). In conclusion, administration of low dose of L-carnitine to pregnant mice improved reproductive performance of male offspring. Further molecular studies are recommended to investigate the effects of L-carnitine administered to pregnant mice on quality and quantity of acrosomal enzymes for male offspring.

References

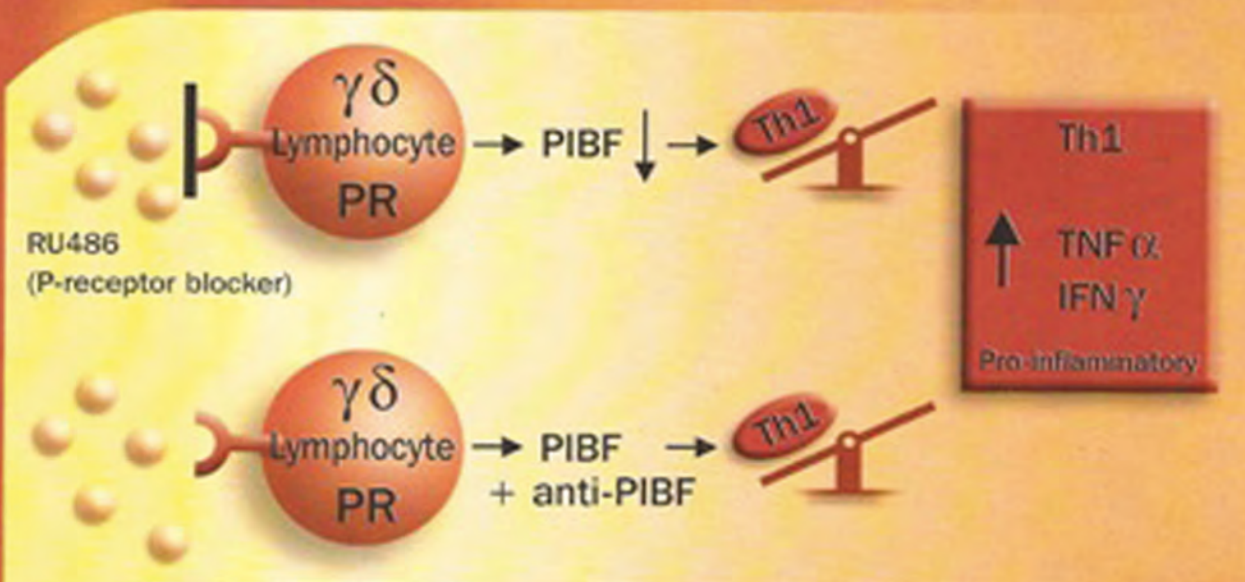
1. Harmayer J. The physiological role of L-carnitine. Lohman Information International. 2002; 27: 15-22.
2. Brody T. Fatty acids: regulation of energy metabolism. Nutritional Biochemistry. Academic Press, San Diego, CA, USA. 1994; Pp: 181-183.
3. Putet G. Lipid metabolism of the micropremie. Clin. Perinatol. 2000; 27: 57-69.
4. Scaglia F. and Longo N. Primary and secondary alterations of neonatal carnitine metabolism. Semin. Neonatol. 1999; 23: 152-161.
5. Jeulin C. and Lewin, L. Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. Hum. Reprod. Update. 1996; 2: 87-102.
6. Hinton B., Snoswell A. and Stechell B. The concentration of carnitine in the luminal fluid of the testis and epididymes of the rat and some other mammals. J. Reprod. Fertil. 1979; 56: 105-111.
7. De Rosa M, Boggie B., Amalfi B., Zarrilli S., Vita A., Colao A. and Lombardi G. Correlation between seminal carnitine and functional spermatozoal characteristics in men with semen dysfunction of various origins. Drugs R. D. 2005; 6: 1-9.
8. Ramadan L., Abd-Allah A. and Aly H. Testicular toxicity effects of magnetic field exposure and prophylactic role of Q10 and L-carnitine in mice. Pharma. Res. 2002; 46: 363-370. 9. Brooks D. In: Carnitine Biosynthesis, Metabolism and Functions. Frenkel R. and McGarry J. (eds.). New York, Academic Press, USA. 1980; P: 219.
10. Borum PR. Carnitine. Ann. Rev. Nutr. 1983; 3: 233-259.
11. Li B., Lioyd MI. and Gudjonsson H. The effect of internal carnitine administration in human. Am. J. Clin. Nutr. 1992; 55: 838-845.
12. Baker H., Frank O., DeAngelis B. and Baker ER. Absorption and excretion of L-carnitine during single or multiple dosings in humans. Int. J. Vitam. Nutr. Res. 1993; 63: 22-26.
13. World Health Organization (WHO): Part 1: Semen Analysis. In: WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th Edition. WHO Press, Geneva, Switzerland. 2010; Pp: 7-160.
14. Jungueira LC. and Carneiro J. Basic Histology. 11th edition. McGraw-Hill, USA. 2005; Pp: 418-440.
15. Ott L. Multiple comparisons. In: An Introduction to Statistical Methods and Data Analysis. Ott L. (ed.). PWS-Kent Publishing Company. Massachustts, USA. 1988; Pp: 437-466.
16. Evans AM. and Fornasini G. Pharmacokinetics of L-Carnitine. Clin. Pharmacokinet. 2003; 42: 941-967.
17. Bremer J. L-carnitine-Metabolism and functions. Physiol. Rev. 1983; 63: 1420-1480.
18. Brooks DE., Hamilton DW., and Mallek AH. Carnitine and glycerophosphorylcholine in the reproductive tract of the male rat. J. Repeal. Pert. 1974; 36: 141-169.
19. Arduini A. Carnitine and its acyl esters as secondary antioxidants. Am. Heart J. 1992; 123: 1726-1727.
20. Casillas ER. and Erickson BJ. The role of carnitine in spermatozoan metabolism: substrate-induced elevation in the acetylation state of carnitine and coenzyme A in bovine and monkey spermatozoa. Biol. Reprod. 1975; 12: 275-283.
21. Borum PR. Variation in tissue carnitine concentrations with age and sex in the rat. Biochem. J. 1978; 176: 677-681.
22. Speake BK., Murray AM. and Noble RC. Transport and transformations of yolk lipids during development of the avian embryo. Prog. Lipid Res. 1998; 37: 1-32.
23. Agarwal A., Prabakaran SA. and Said TM. Prevention of oxidative stress injury to sperm. J. Androl. 2005; 26: 654-660.
24. Neuman SL., Lin TL. and Hester PY. The effect of dietary carnitine on semen traits of White Leghorn roosters. Poult. Sci. 2002; 81: 495-503.
25. Zhai, W., Neuman SL., Latour MA. and Hester PY. The effect of dietary L-carnitine on semen traits of White Leghorns. Poult. Sci. 2007; 86: 2228-2235.
26. Jeulin C., Dacheux JI. and Soufir JC. Uptake and release of free L-carnitine by boar epididymal spermatozoa in vitro and subsequent acetylation rate. J. Reprod. Fertil. 1994; 100: 263-271.
27. Lenzi A., Lombardo F. and Sgro P. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil. Steril. 2003; 79: 292-300. 28. Vitali G., Parente R. and Melotti C. Carnitine supplementation in human idiopathic asthenospermia: clinical results. Drugs Exp. Clin. Res. 1995; 21: 157-159.
29. Zhou X., Liu F. and Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. Asia. Pac. J. Clin. Nutri. 2007; 16 (Suppl. 1): 383-390.
30. Borum PR. Regulation of the carnitine concentration in plasma. In: Frenkel RA, McGarry JD. (eds). Carnitine Biosynthesis, Metabolism and Functions. New York, USA: Academic Press, 1980; Pp: 115-126.
31. Jogl G., Hsiao YS. and Tong L. Structure and function of carnitine acyltransferases. Ann. NY. Acad. Scien. 2004; 1033: 17-29.
32. Lenzi A., Sgro P. and Salacone P. A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acetyl-carnitine treatment in men with asthenozoospermia. Fertil. Steril. 2004; 81: 1578-1584.

PIBF: the Link between the Endocrine and Immune System

Normally Progressing Pregnancy



Miscarriage



duphaston

Solvay Pharmaceuticals