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Influence of α-Tocopherol on Heat Stress-Induced Changes in the Reproductive Function of Swiss Albino Mice

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Abstract

The present study was carried out to investigate the influence of vitamin E (α-tocopherol) on heat stress-induced changes in the reproduction of Swiss albino mice. The evaluated parameters include: the estrous cycle, fertility, post-implantation losses of fetuses and estimation of progesterone levels in the serum. Eight groups of experimental mice (10 each) were used. Groups 1-4 (24 ˚C) consisted of a control and α-tocopherol (100, 200 and 400 mg/kg) treated groups. Groups 5-8 (42 ˚C) consisted of a positive control and α-tocopherol (100, 200 and 400 mg/kg) treated group. Heat-stress reduced significantly ($p > 0.001$) the number of fetuses and corpora lutea. There was also a significant decrease in the mean weights of fetuses ($p > 0.001$) and placenta ($p > 0.01$) in the heat-stress group with a decrease in their serum progesterone levels ($p > 0.01$). Heat-stress groups treated with high doses of α-tocopherol 200 and 400 mg/kg, showed protection against heat-stress related abnormalities. The results showed that α-tocopherol plays a role in protection against hyperthermia induced changes in the estrous cycle length, infertility, post-implantation losses and depletion in the serum level of progesterone.

Key words: α-Tocopherol, vitamin E, antioxidant, progesterone, reproduction, fertility.

Introduction

Maternal hyperthermia has been shown to be a potent teratogen in experimental animals and humans (Edwards, 1986 and Shepard, 1992). The character and intensity of teratogenic response to heat shock is dependent on the stage of embryonic development at the time of exposure, the degree of temperature elevation, and its duration. Developmental defects have been identified in the heart, CNS, neural tube, skeletal system, and the eyes of guinea pigs, rats and mice following exposure to hyperthermia (Edwards, 1967; Webster and Edwards, 1984; Shiota, 1988; Aoyama and Yamashina, 1994).

Pregnancy is a physiological state accompanied by a high metabolic need with an increase in oxygen demand (Spatling *et al.,* 1992). This leads to an augmentation of reactive oxygen species (ROS) generation (Halliwell and Gutteridge, 1990), and increasing lipid peroxidation levels. However, the increase in ROS production is usually protected by antioxidant vitamins (α-tocopherol and β-carotene) (Yu, 1994). α-Tocopherol has been shown to improve fetal outcome in diabetic rats (Cederberg and Eriksson, 2005).

It has become evident since the discovery of α-tocopherol, that this vitamin derivative is neccessary for maintaining the reproductive system (Evans *et al.,* 1936). The effect of α-tocopherol upon animals reproductive performance has been widely studied (Soderwall and Smith, 1962; Chavez and Patton, 1986; Grandhi *et al.,* 1993; Mavromatis *et al.,* 1999). This suggests that the effect of α-tocopherol may be due to the direct antioxidant effect on the follicle and embryonic development as well as through the hormonal effect during gestation period. α-Tocopherol is known as one of the best chain-breaking antioxidants to protect the cells against lipid peroxidation (Burton *et al.,* 1982).

Therefore, the aim of this research was to investigate the influence of α-tocopherol, on the heat stress-induced changes in the reproductive performance of Swiss albino mice.

Materials and Methods

Treatment

Three levels of α-tocopherol (100, 200 and 400 mg/kg diet) were mixed in regular rat diet (Purina chow) powder, obtained from Grain Silos and Flour Mills Organization, Riyadh branch.

Animals

Swiss albino female mice were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, weighing 20-22 gm were randomly assigned to different eight groups. The animals were maintained under standard conditions of temperature, humidity, and light (12 h dark, 12 h light). They were provided with Purina rat chow and free access to drinking water. The animals were divided into eight groups (10 mice in each group) and fed either the control or supplemented diet for a period of five weeks before mating and then continuously until the end of the experiment. Before mating, the heat stress group of animals was kept twice a week in a well maintained (humidity 50-55% and temperature 42 ˚C) incubator for ten minutes (Matsuzuka *et al.,* 2005). Animals were allowed *ad libitum* access to diet and water. Vaginal smears were obtained daily, each morning during the last two weeks of the pre-mating period. The smears were examined under a microscope and classified into four stages: pro-estrous, estrous, met-estrous and di-estrous. Vaginal plugs were checked daily for five days. The pregnant animals were divided into two categories. Category one animals were killed at day 4 of pregnancy and category two at day 18.

On day 4 and 18 of pregnancy the mice were killed using carbon dioxide inhalation, blood samples were collected by heart puncture and centrifuged for 10 min at 3,000 rpm. The serum samples were stored at -20 ˚C until analysis of progesterone levels. Numbers of corpora lutea (CL) in the ovaries were counted under the dissecting microscope. The post-implantation fetuses and placenta were weighed. Live, dead, normal and resorbed fetuses were counted.

Serum progestrone levels were estimated by using radio-immunoassay (RIA) kits, PROG-RIA-CT KIP 1459, BioSource Europe S.A., Belgium on LKB Mini GAMA counter, Finland. The sensitivity of this kid is 0.05 ng/ml with intra and inter-assays coefficients of variation of 6.8% and 10.1% respectively.

Statistical analysis

Results are expressed as mean values ± S.E., compared using unpaired studen´s t-test with significance levels at p< 0.05, 0.01 and 0.001 (Sokal and Rohif, 1981).

Results

Effects on estrous cycle

The frequency of pro-estrous, estrous, met-estrous, di-estrous did not show any significant difference in the animals fed with the differenent concentrations of α-tocopherol (100, 200 and 400 mg/kg) with and without heat-stress (Table 1). Different concentrations of α-

Table 1. Effect of α-tocopherol on heat stress-induced changes on the duration of estrous cycle phases.

Groups-Treatments (mg/kg diet)	Total number of days (Mean ±S.E.)				Mean estrous cycle length
	Pro-estrous	Estrous	Met-estrous	Di-estrous	
1-Control (Rat chow)	3.30 ± 0.37	4.20 ± 0.33	2.90 ± 0.28	3.20 ± 0.29	4.60 ± 0.42
2- α-Tocopherol (100)	3.60 ± 0.37	4.20 ± 0.39	2.70 ± 0.30	2.80 ± 0.25	4.60 ± 0.34
3- α-Tocopherol (200)	3.30 ± 0.30	4.10 ± 0.28	2.10 ± 0.23	2.70 ± 0.21	4.30 ± 0.45
4- α-Tocopherol (400)	3.70 ± 0.30	4.10 ± 2.8	2.10 ± 0.31	2.50 ± 0.17	4.70 ± 0.37
5- Heat stress (Rat chow)	3.10 ± 0.38	3.40 ± 0.27	1.90 ± 0.27	2.78 ± 0.30	2.10** ± 0.31
6 - α-Tocopherol (100) + Heat stress	3.4 ± 0.34	3.60 ± 0.31	1.80 ± 0.25	2.20 ± 0.25	2.80 ± 0.31
7- α-Tocopherol (200) + Heat stress	3.40 ± 0.31	3.30 ± 0.30	1.90 ± 0.35	2.60 ± 0.22	3.70* ± 0.34
8-α-Tocopherol (400) + Heat stress	3.60 ± 0.37	3.60 ± 0.31	1.90 ± 0.23	2.40 ± 0.31	4.20** ± 0.33

*P <0.01 and **P < 0.001.
Groups 2,3,4 and 5 were compared with group 1.
Groups 6,7 and 8 were compared with group 5.

tocopherol (200 and 400 mg/kg) increased significantly the mean estrous cycle length.

Effect of heat-stress and/or α-tocopherol on fetuses on day 18 of pregnancy

The mean number of fetuses significantly decreased in heat-stress group as compared to control group. Heat-stress significantly reduced the mean weight of both fetuses and placenta compared to the control. Treatment with α-tocopherol of 200 and 400 mg/kg protect the losses of fetuses and placenta weight (Table 2). Number of pregnant females increased significantly by doses of 400 mg/kg α-tocopherol. This treatment also increased the number of live fetuses and reduced the number of dead fetuses significantly when exposed to heat-stress (Table 3).

Effect of α-tocopherol and/or heat-stress on the number of corpora lutea

The numbers of CL were significantly decreased in heat-stress group and heat-stress plus α-tocopherol (100 mg/kg) supplemented groups as compared to controls (group without heat-stress). Animals fed with α-tocopherol diets of 200 and 400 mg/kg showed significant portection in

Table 2. Effect of α-tocopherol on heat stress-induced changes in the number of fetuses and weight of placenta, and fetuses at the time of slaughter (after 18 days of pregnancy).

Groups- Treatments (mg/kg diet)	Mean number of fetuses/mouse	Mean weight of fetuses (mg)	Mean weight of placenta (mg)
1- Control (Rat chow)	10.86 ± 0.34	723.86 ± 5.76	85.00 ± 4.61
2- α-Tocopherol (100)	11.00 ± 0.44	735.86 ± 4.37	74.43 ± 3.55
3- α-Tocopherol (200)	10.57 ± 0.49	733.00 ± 5.04	79.71 ± 3.26
4- α-Tocopherol (400)	10.71 ± 0.42	739.86 ± 2.65	79.43 ± 3.58
5- Heat stress (Rat chow)	7.86*** ± 0.40	685.41*** ± 4.32	65.14** ± 3.63
6- α-Tocopherol (100) + Heat stress	8.71 ± 0.52	697.14 ± 4.29	69.14 ± 3.26
7- α-Tocopherol (200) + Heat stress	9.43* ± 0.37	713.43** ± 5.87	78.86* ± 3.93
8- α-Tocopherol (400) + Heat stress	10.43** ± 0.57	733.14*** ± 3.28	85.14*** ± 3.22

*P<0.05, **P<0.01 and ***P<0.001.
Groups 2,3,4 and 5 were compared with group 1.
Groups 6,7 and 8 were compared with group 5.

Table 3. Effect of α-tocopherol on heat stress-induced changes in the fertility percent and mean implants per pregnant mice.

Groups - Treatments (mg/kg)	Pregnant females / Fertility percent	Mean Implants/pregnant female ± S.E.			Dead embryos (%)
		Total	Live	Dead	
1- Control (Rat chow)	26/30 (86.67)	10.17 ± 0.33	8.89 ± 0.37	0.42 ± 0.16	4.78
2- α-Tocopherol (100)	28/30 (93.33)	10.60 ± 0.85	8.34 ± 0.92	0.36 ± 0.13	4.86
3- α-Tocopherol (200)	27/30 (90.00)	10.37 ± 0.75	8.63 ± 0.98	0.53 ± 0.16	5.82
4- α-Tocopherol (400)	25/30 (83.33)	9.60 ± 0.65	9.13 ± 0.92	0.47 ± 0.13	4.86*
5- Heat stress (Rat chow)	15/30** (50.33)	7.40 ± 0.82*	4.67 ± 0.79***	0.97 ± 0.15*	9.27
6- α-Tocopherol (100) + Heat stress	18/30 (60.00)	8.88 ± 0.65	6.58 ± 0.56	0.86 ± 0.23	8.23
7- α-Tocopherol (200) + Heat stress	20/30 (66.67)	9.15 ± 0.44	8.25 ± 0.53**	0.63 ± 0.18	7.23
8- α-Tocopherol (400) + Heat stress	22/30* (73.33)	9.97 ± 0.54	9.34 ± 0.53***	0.52 ± 0.12*	5.13*

*P<0.05, **P<0.01 and ***P<0.001.
Groups 2,3,4 and 5 were compared with group 1.
Groups 6,7 and 8 were compared with group 5.

Table 4. Effect of heat-stress and/or α-tocopherol on number of CL and serum progesterone levels at days 4 and 18 of pregnancy.

Groups-Treatments (mg/kg diet)	Mean Number of Corpora lutea (CL) after 4 and 18 days of pregnancy	Serum progesterone levels (ng/ml)	
		4th day of pregnancy	18th day of pregnancy
1- Control (Rat chow)	11.07 ± 0.59 (14)	41.29 ± 4.60 (7)	53.01 ± 2.67 (7)
2- α-Tocopherol (100)	11.00 ± 0.71 (15)	43.31 ± 3.49 (7)	45.66 ± 1.64 (7)
3- α-Tocopherol (200)	11.14 ± 0.45 (14)	36.90 ± 2.30 (7)	49.40 ± 2.37 (7)
4- α-Tocopherol (400)	11.47 ± 0.47 (15)	38.66 ± 3.40 (7)	49.23 ± 2.68 (7)
5- Heat stress (Rat chow)	8.21 ± 0.47*** (15)	37.91 ± 3.92 (7)	39.36 ± 2.26** (7)
6- α-Tocopherol (100) + Heat stress	9.20 ± 0.57 (15)	39.48 ± 3.71 (7)	40.82 ± 2.17 (7)
7- α-Tocopherol (200) + Heat stress	10.25 ± 0.58* (15)	34 ± 1.93 (7)	43.89 ± 1.44 (7)
8- α-Tocopherol (400) + Heat stress	10.87 ± 0.55** (15)	38.67 ± 3.94 (7)	47.69 ± 1.81* (7)

*P<0.05, **P<0.01 and ***P<0.001.
Groups 2,3,4 and 5 were compared with group 1.
Groups 6,7 and 8 were compared with group 5.

Figures in parentheses denote the number of animals used.

CL numbers against the heat-stress (Table 4).

Effect of α-tocopherol and/or heat-stress on progesterone level

The serum progestrone levels at day-4 of pregnancy were not affected either by α-tocopherol (100, 200 and 400 mg/kg) supplementations or by heat-stress. At day-18 of pregnancy, heat-stress significantly decreased the progesterone concentrations in untreated mice as compared to controls (Table 4). Supplementation of α-tocopherol (400 mg/kg) diet showed significant protection against heat-stress as compared to the mice with heat-stress but un-treated.

Discussion

The results of the present investigation showed that the heat-stress reduces estrous cycle duration. (Roth, *et. al.,* 2000) reported immediate and delayed effects of heat-stress on the follicular development in cows where an increase in the number of large follicles and a surge in plasma follicular stimulating hormone was observed, suggesting that these alterations may have physiological significance leading to reduced fertility in cattle during the summer and autumn seasons.

There is a significant decrease in the mean number of fetuses in the heat-stressed group when compared to the controls and the α-tocopherol (400 mg/kg) fed group. This might indicate that the higher dose of α-tocopherol gave protection against hyperthermia. The decreased fertility due to heat-stress in the present study may be due to disruption in embryonic development through a combination of inappropriate cell death and/or altered cell proliferations. This is in agreement with the reports of Breen (1999) and Padmanabhan *et al.,* (2005), where maternal hyperthermia during critical stages of embryo development leads to intra-uterine growth retardation, cranio-facial malformations together with placental pathology as possible causes for the fetal growth retardations.

The results of the present study showed protection against heat-stress by the mice fed with higher doses (400 mg/kg) of α-tocopherol. α-Tocopherol is the most important fat-soluble, chain-breaking antioxidant. It also inhibits glutathione S-tranferase in concentration-dependent manner in human beings (Van Haaften *et al.,* 2001). Jishage *et al.,* (2005) have reported that α-tocopherol is indispensable for the proliferation and/or function of the placenta but not necessary for the normal growth of the embryo. This could be due to the fact that only fully developed placentas will be able to supply the required nutrients for the normal growth of the embryo. Previous studies showed that the antioxidants α-tocopherol and ascorbate improved fetal outcome in diabetic pregnant rats (Cederberg and Eriksson 2005).

Heat-stress significantly decreased the progesterone concentrations in mice as compared to controls at day-18 of pregnancy. Supplementation of α-tocopherol (400 mg/kg) diet showed significant protection against heat-stress as compared to the mice with heat-stress but untreated (Table 3). The seasonal differences in progesterone production in cows showed a significant increase in progesterone concentrations during winter than in summer (Wolfenson *et al.,* 2002). Rozenboim *et al.,* (2004) reported reduced progesterone in domestic turkey due to heat-stess. Results of previous studies in cattle exposed to heat stress are conflicting. Howell *et al.,* (1994) reported reduced progesterone levels. This might be attributed to lower plasma cholesterol concentration and the impairment of lipid metabolism which occurs under heat-stress. It is known that cholesterol is an indispensable precursor for de novo progesterone synthesis (Staples *et al.,* 1998). These alterations might be responsible for reduction of corpora lutea and progesterone in mice used in the present investigation exposed to heat stress.

It might be concluded that α-tocopherol might have a direct antioxidant effect on the follicle and embryonic development as well as through the normonal effect during the gestation period.

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تأثير الالفاتوكوفيرول على الصفات التناسلية للفئران السويسرية البيضاء تحت الاجهاد الحرارى المستحدث

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الملخص

تهدف هذه الدراسة لتحري تأثير فيتامين هـ (الفاتوكوفيرول) على الصفات التناسلية للفئران السويسرية البيضاء تحت الإجهاد الحراري المستحدث وتشمل الدراسة الصفات الآتية: دورة الشبق ، الخصوبة وفقد الأجنة ما بعد الانغراس ومستوى هرمون البروجستيرون في الدم. استخدمت في الدراسة ثمان مجموعات من الفئران المعملية (كل مجموعه مكونه من ١٠ حيوانات). مثلت المجموعات من (١–٤) عند درجة حرارة ٢٤ م° مجموعات الضبط والمعالجة (١٠٠، ٢٠٠، ٤٠٠ ملجم/كجم الفاتوكوفيرول) بينما مثلت المجموعات من (٥–٨) عند درجة حرارة ٤٢ م° مجموعات الضبط الايجابي والمعالجة (١٠٠، ٢٠٠ ، ٤٠٠ ملجم/كجم الفاتوكوفيرول). خفض الإجهاد الحراري معنويا (P > ٠,٠٠١) من أعداد الأجنة والأجسام الصفراء ، كما كان أيضا هناك انخفاضا معنويا على كل من وزن الأجنة (P > ٠,٠٠١)والمشيمة (P> ٠,٠١) ، وأيضا انخفاضاً في مستوي هرمون البرجستوون (P > ٠,٠١) بالدم. كذلك خففت الجرعات العالية من الالفاتوكوفيرول (٢٠٠ و ٤٠٠ ملجم/كجم) التأثير السلبي للإجهاد الحراري. وكما دلت النتائج على أن للالفاتوكوفيرول دوراً في الحماية ضد التغيرات التي تحدثها الحرارة الزائدة في طول دورة الشبق و انخفاض الخصوبة وفي الحد من فقدان الأجنة المنغرسة وفى المحافظة على مستوي هرمون البروجستيرون بالدم.
