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INVESTIGATION ON VITAMIN E AND LIPID PEROXIDE STATUS OF DOES BLOOD DURING PREGNANCY

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Introduction

The highly active oxygen substances - oxygen free radicals arising during endogeneus metabolic processes (Slater, 1976). The so-called antioxidant defense mechanism of the organism serves for preventing the oxidants formed within the organism or ingested with the feed from exerting their harmful effects (DeDuve and Hayaishi, 1978). The antioxidant defense mechanism includes, among other compounds, vitamin E (in membranes) and enzymes, superoxide dismutase, catalase and glutathione-peroxidase (in cytosol and in cell organelles) (Jones, 1982, Flohé et al, 1971). The pregnancy as a physiological process also cause some changes in free radical generating processes. For example prostaglandins are believed to have a major role in embrye implantation (Jones and Harper, 1984). They are synthetised by blastocytes and are involved in the vascular changes in the endometrium at the site of implantation. The synthesis of prostaglandins cause development of some free radical species, especially cyclic endoperoxides and one of the end product os prostanoid biosynthesis is malondialdehyde which also have effect on the lipid peroxidation (Hope et al, 1975).

Materials and Methods

New Zeeland White does (average two years old) were investigated during pregnancy. The animals were fed with commercial ratio for does ad libitum. The animals were kept in

common conditions according to the generally used technological rules.

Blood samples were taken from the ear vein and EDTA-Na_2 (0.2 mol/L) in a volume of 0.05 ml/ml blood was used as an anti-coaglant. The red blood cells (RBC) were separated from the plasma by centrifugation. For further investigations the RBC was washed twice with ice-cold isotenic saline then haemolysed in a nine-fold volume of redistilled water and by freezing and thawing.

The TBA-reactive plasma substances (malendialdehyde) was assayed according to Placer et al (1966). The vitamin E content of plasma was measured using the method of Bieri (1964). Catalase (E.C.1.11.1.6) activity wasmeasured kinetically and experessed in Bergmeyer units (B.U.) using the method of Beers and Sizer (1952). Glutathione-peroxidase (E.C.1.11.1.9) activity was measured by the direct assay of Szabó(1984). The substrates were reduced glutathione and cumene-hydroperoxide. The activity was expressed in units (U) which means the oxidation of glutathione in nanomoles at 25 °C per minutes. Enzyme activities were calculated to plasma protein content which was determined by the Biuret method (Weichselbaum,1946). The haemoglobin content of the RBC haemolysate measured by the cyanomethemoglobin method and the enzyme activities were calculated to the haemoglibin content of RBC haemolysate. The plasma pregesterone level for pregnancy diagnosis was measured after light petroleum extraction using enzyme-immunoassay method (Enzaklen-Preg EIA kit, Human Institute for Serobacterielogical Products and Research,Budapest). Mathematical evaluation of the differences were performed by the Student "t" test.

Results

The rate of lipid perexidation of plasma was measured as TBA-reactive substances, decreased significantly to the time of mating, increased again after it and remain at the same level up to birth (Table 1.). The vitamin E content of plasma increased one week prior to mating, increased again during the first part of pregnancy which was followed by a significant decrease,increased again to the time of birth (Table 1.).

The glutathione-peroxidase activity of plasma changed significantly as was found in the case of vitamin E (Table 1.). The glutathione-peroxidase activity of RBC haemolysate increased to the time of mating and after a marked decrease to the second week of pregnancy increased slowly but significantly to the time of birth (Table 2.). The catalase activity of RBC haemolysate showed similar changes as was found in the case of glutathione-peroxidase (Table 2.).

The progesterone content of plasma increased to the time of mating and reach the higher valu at the time of birth. After birth a marked decrease was found (Table 1.).

Discussion

The rate of lipid peroxidation showed increase after mating which could be explain by the findings that blastocytes accumulate and release prostaglandins and at the site of implantation inflammatory-like processes are ready (Jones and Harper,1984). These changes may cause development of free radicals also some TBA-reactive metabolites which are pass to the bleed stream after vascularisation of endometrium. The natural antioxidant vitamin E changed especially at the time of mating which may cause at frist the stress effect of mating and also possible some nutritional disturbances at that period. During the pregnancy marked changes was did not found and it means that the vitamin E content of ratio (30 mg/kg) was optimal or over-optimal for the animals.
The changes of glutathione-peroxidase activity of plasma and RBC haemolysate showed that the embrye implantation cause some lipid peroxidation or free radical generation changes which compounds as substrates could increase the glutathione-peroxidase activity.These explanations also supported by the changes as was found in the case of catalase. The glutathione-peroxidase activity changes different from some observations when no changes was found in reduced glutathione status of plasma during pregnancy (Granát et al,1978).

It can be concluded from the results of this investigation that after mating would be a slight increase of oxygen free radical generation and it may cause some changes in the

the vitamin E and lipid peroxide status of blood of does. The changes are in the physiological levels and the problems may cause clinical symptoms only undernutritional conditions especially low intake of vitamin E and antioxidant compounds for example trace elements (selenium,iron, zinc, manganese).

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Table 1. Lipid peroxide, vitamin E and pregesterone status of blood plasma of does

Time of sampling (weeks)	TBA-reactive substances (nmol/ml)	vitamin E (mmol/L)	Glutathiene-peroxidase (U/ g plasma protein content)	Pregesterone (nmol / L)
- 2	2.67±0.61	2.32±1.32	3.61±0.64	1.91±0.57
- 1	2.88±0.44	4.76±1.28^{xxx}	6.33±1.82^{xxx}	1.97±1.38
Mating	0.80±0.58^{xxx}	1.23±0.19^{xxx}	0.84±0.20^{xxx}	3.07±1.80
+ 1	3.47±0.91^{xxx}	4.40±0.61^{xxx}	2.78±1.35^{xx}	4.63±2.18
+ 2	3.25±0.87	4.24±1.12	2.34±0.42	7.45±4.83
+ 3	4.02±0.82	1.60±0.36^{xxx}	3.87±0.98^{x}	7.56±3.19
+ 4	3.01±0.49	2.13±0.27	2.21±0.77	7.72±3.62
Birth	2.83±0.55	3.03±0.66^{x}	2.17±0.84	5.63±1.65
+ 1	2.48±1.01	3.30±1.57	3.88±1.25^{x}	3.42±1.17

Levels of significance: x = $P<0.05$ xxx = $P<0.001$ means ± SD

Table 2. Antioxidant enzyme activities of RBC haemolysates of does (mean ± SD)

Time of sampling (weeks)	Glutathione-peroxidase (U/g haemoglobin content of RBC haemolysate)	Catalase (B.U./ g haemoglobin content of RBC)
- 2	11.00± 3.97	335.83±144.25
- 1	10.19± 2.48	231.40± 54.76
Mating	21.71±11.39^{x}	680.79±300.40^{xx}
+ 1	30.55±15.57	1021.74±432.78
+ 2	5.36± 0.96^{xxx}	166.30±28.61^{xxx}
+ 3	4.09± 1.03	136.10± 22.62
+ 4	10.37± 3.56^{xxx}	245.25± 61.04
Birth	11.21± 2.16	225.85± 45.91
+ 1	12.80± 4.01	310.22±132.97

Levels of significance: x = $P<0.05$ xx = $P<0.01$
xxx = $P<0.001$

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The lipid peroxide and vitamin E status of does blood was investigated. It was observed that the rate of lipid peroxidation (level of malondyaldehyde) of plasma decreased at the time of mating. The vitamin E level of plasma also decreased at the time of mating and decreased again during the second part of pregnancy to the birth which was followed by a slow increase. The glutathione-peroxidase activity of plasma showed similar changes as was found in the case of vitamin E. The glutathione-peroxidase activity of red blood cells increased in the first part of pregnancy and remain at the same low level after a marked decrease to the time of birth. The catalase activity of red blood cells showed similar changes as was found in the case of glutathione-peroxidase.
The results indicate that great attention should be paid to the changes of lipid-peroxidative processes in the body of does during pregnancy.

Es wurde der Lipidperoxidenstand und der Vitamin E-Gehalt im Blut der Mutterkaninchen wahrend der Trachtigkeit untersucht. Es konnte fergestellt werden, dass der Lipidperoxidengrad (Malondialdehyde-Inhalt) im Blutplasma nur im Zeitpunkt der Paarung wesentlich sinkt. Der Vitamin E-Gehalt im Blutplasma sankt auch bei der Paarung stark, und er fiel in der zweiten Halfte der Trachtigkeit bis zum Termin des Setzens wieder, dieser Senkung folgt dann ein kraftiger Zuwachs. Der Glutathionperoxidase- Aktivitat der Erythrozyten erhöhte sich wahrend der ersten Halfte der Trachtigkeit wesentlich und dann nach einer starken Senkung blieb sie bis zum Termin des Setzens auf dem gleichen Niveau. Die Katalase-Aktivitat der Erythrozyten anderte sich se wie derer Glutathionperoxidase-Aktivitat. Die Ergebnisse machen auf die Lipidperoxide-Prozesse im Organismus der Mutterkaninchen wahrend der Trachtigkeit aufmerksam.