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Oxidant/Antioxidant Status of Plasma in Arabian Mares with Uterine Lymphatic Cysts*

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Uterine cysts are one of important contributing factor to subfertility in the mare. There are no data concerning blood oxidant and antioxidant status in mares with lymphatic uterine cysts.

Therefore, this study was conducted to investigate plasma oxidant (malondialdehyde [MDA]) and antioxidant (glutathione [GSH], ß-carotene, vitamin E, glutathione peroxidase [GSH-Px], catalase [CAT]) status in mares with uterine cysts. For this purpose, blood samples were collected from 10 mares with uterine lymphatic cyst and 8 healthy Arabian mares. The age of the mares varied from 15 to 20 years.

There were no significant differences in the concentrations of vitamin E, GSH, MDA and activity of CAT and GSH-Px between groups. However, the concentration of plasma ß-carotene in the mares with uterine cyst was significantly lower (P<0.01) than in the healthy mares.

These results suggested that lower ß-carotene concentration may be one of factors which play role in the pathogenesis of uterine lymphatic cyst.

Key words: *Antioxidant, Mares, Beta carotene, Lipid Peroxidation, Uterine Cyst*

Lenfatik Uterus Kistli Arap Kısraklarında Plazma Oxidan-Antioxidant Değerleri

Uterus kistleri kısraklarda fertilite kaybına neden olan önemli faktörlerden biridir. Lenfatik uterus kisti olan kısraklardaki plazma oksidan-antioksidan dengesini inceleyen bir araştırmaya rastlanmamıştır.

Bu çalışmada, lenfatik uterus kisti bulunan kısraklarda plazma oksidan (malondialdehit [MDA]) ve antioksidan (glutatyon [GSH], ß-karoten, E vitamini konsantrasyonları ile glutatyon peroksidaz [GSH-Px] ve katalaz aktiviteleri) dengesi araştırıldı. Bu amaçla, yaşları 15-20 arasında değişen 10 uterus lenfatik kistli ve 8 sağlıklı Arap kısrağından kan örnekleri toplandı.

E vitamini, ß-karoten, GSH ve MDA konsantrasyonlarında gruplar arasında önemli bir farklılık gözlenmedi. Lenfatik uterus kistli kısraklardaki plazma beta-karoten seviyesinin ise sağlıklı kısraklarınkinden düşük olduğu tespit edildi (P<0.01).

Bu sonuçlar, düşük ß-karoten seviyelerinin uterus lenfatik kist patogenezinde rol alabileceğini düşündürmektedir.

Anahtar Kelimeler: *Antioksidan, Kısrak, Beta Karoten, Lipit Peroksidasyon, Uterus Kisti*

Introduction

Reproductive performance is considered to be one of the most important economic factors influencing productivity in modern equine breeding. Uterine cysts are a common form of uterine lesion, and probably an important reason for low pregnancy rates (1). The cysts are fluid-filled structures that can occur anywhere in the normal or chronically inflamed endometrium.

Cysts are characterized as either glandular or lymphatic. Lymphatic cysts arise from obstructed lymphatic channels and appear more commonly in multiparous mares with uterus that have undergone fibrotic changes associated with mixed endometrial disorders (2). The incidence of cysts increases with age and most affected mares are >10 years. The incidence of endometrial cysts in subfertile and older mares has been reported to be 55% (3). Cysts may affect vesicle fixation, conceptus mobility, and placentation. Improper implantation adjoining a cyst can result in inadequate blood flow and nutrient provision for the conceptus, leading to early embryonic loss. Multiple or large cysts may reduce placental exchange leading to inadequate placentation and potentially abortion (4). This suggests that the presence of uterine cysts may play an important role in the reduction of fertility of mares. Reactive oxygen species (ROS), which are produced as by products of normal metabolism, are capable of causing cellular damage, leading to cell death, and tissue injury. In organisms, extensive

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antioxidant defense system includes natural molecules such as urate, cysteine, vitamin E, vitamin C, β protective mechanisms have evolved to prevent ROS damage.-carotene, and enzymes including superoxide dismutase, CAT and GSH-Px (5). Oxidative stress has been defined as a disturbance in the pro-oxidant/antioxidant balance, resulting in several pathological processes such as inflammation, carcinogenesis, and ischaemia-reperfusion (6). ROS also play a number of significant, diverse roles in female reproductive biology including uterine environment, oocyte maturation and ovulation, corpus luteum function and regression (7). Accumulated evidence suggests that ROS have been associated with disorders of the reproductive system such as cervicitis, uterine myoma (8) and endometritis (9, 10).

Although uterine cysts are a contributing factor to subfertility in the mare, to our knowledge, there are no data concerning the effects of blood oxidant and antioxidant status in mares with lymphatic uterine cysts. Therefore, the present investigation was undertaken to compare plasma antioxidant status in normal mares and mares with uterine lymphatic cyst.

Materials and Methods

The study was performed in the ten mares with uterine lymphatic cysts and eight healthy purebred Arabian mares aged from 15 to 20 years. All mares showed regular ovulatuary cycles, however mares with uterine lymphatic cysts were barren. Uterine cysts were detected by transrectal real-time ultrasonography (6-8 MHz, Scanner 100LC Pie medical). The sizes of the cysts (>2.5 cm) indicated that these cysts were lymphatic in nature, based on the report by McKinnon et al. (11). Blood samples were collected on diostrus period at April by jugular venipuncture into vacutainers containing sodium EDTA and immediately centrifuged at 1500 g for 10 min.

Lipid peroxidation (MDA) level in plasma was estimated according to the method of Placer et al. (12) modified by Matkovics et al. (13) by monitoring thiobarbituric acid-reactive substances (TBARS). The quantification of TBRAS was determined by comparing the absorption to the standard curve of malondialdehyde equvalents generated by 1,1,3,3 tetramethoxypropane at 532 nm with spectrophotometer (Jainway 6100, UK). Vitamin E level was determined according to the method of Desai (14).

The plasma samples were saponified by the addition of KOH and 1% ascorbic acid, followed by heating at 70^{o}C for 30 min. After cooling the samples on ice, water and n-hexane were added, and then placed aside for 30 min to allow phase separation. The n-hexane extract was dried under nitrogen and redissolved by using methanol. Methanol extracts were measured at 536 nm after addition bathophenanthroline-ortho-phosphoric acid reagent. The levels of β-carotene in plasma samples were determined according to the method of Suzuki and Katoh (15). Two milliliters of n-hexane were mixed with 0.25 ml plasma and absorbance of hexane was measured at 453 nm in the spectrophotometer.

The GSH content in plasma was measured by using the method of Sedlak and Lindsay (16). The samples were precipitated with trichloracetic acid and then centrifuged at 1000 g for 5 min. The reaction mixture including supernatant, tris buffer (pH 8.9) and 5,5'dithio-bis-2-nitrobenzoic acid were kept at room temperature for 5 min, and read at 412 nm on the spectrophotometer.

GSH-Px activity of plasma was measured according to Lawrence and Burk (17). Briefly, the reaction mixtures including 0.1 ml plasma samples, tris buffer (pH 7.6), GSH and cumene hydroperoxide were incubated for 10 min at room temperature. Tricholoroacetic acid and 5,5-dithiobis-2 nitrobonzoic acid were added to the reaction mixtures and were kept at room temperature for 5 min, and absorbances at 412 nm were recorded. Catalase activity was determined according to the method of Goth (18). Reaction mixture including hydrogen peroxide and sodium-potassium buffer (pH 7.4) and 0.1 ml plasma samples were incubated at 37^{o}C for 1 min. Then ammonium molibdat was added to mixture and the yellow complex was measured at 405 nm against a blank by using a spectrophotometer.

Data were analyzed by using student's t test on SPSS software, 10.1.0 (SPSS Inc. Chicago, IL, USA). The results were expressed as mean ± SD. Differences were considered as significant when P< 0.05.

Results

In the mares with uterine lymphatic cysts, the mean concentration of plasma β-carotene was significantly lower (P<0.01) than in normal mares (Table 1). On the other hand, no significant differences in the concentration of vitamin E, GSH, MDA, activities of CAT and GSH-Px were observed.

Tablo 1. Mean (± SD) plasma oxidant (Malondialdehyde [MDA]) and antioxidant (Glutathione [GSH], ß-carotene, Vitamin E, Glutathione peroxidase [GSH-Px], Catalase [CAT]) status in healthy mares and mares with uterine lymphatic cysts.

	Healthy (n=8)	Cystic (n=10)
MDA (µmol/L)	1.0 ± 0.12	0.95 ± 0.26
GSH (µmol/L)	0.016 ±0.0027	0.015 ±0.0010
β -carotene (µmol/L)[a]	1.48 ± 0.19	1.09 ± 0.17*
Vitamin E (µmol/L)	6.77 ±1.32	6.26 ± 1.41
GSH-Px (IU/ g protein)	8.92 ±1.55	8.25 ± 1.04
CAT (kU/L)	26.12 ± 4.14	25.50 ± 5.43

*Results are expressed mean ± SD. *P<0.01 [a]: Seven healthy and nine cystic mares utilized.*

Discussion

The pathogenesis of uterus cyst depends on a multitude of factors including hormonal imbalance, uterine infections and senescence (4). The uteri of mares at the age of 15 onwards show increasingly age-related degenerative changes including accumulations of mononuclear cells and generalised fibrosis (19), epithelial hypertrophy (20), fibrous constrictions of the lymph drainage vessels causing dilatations and the formation of lymph-filled endometrial cysts (19).

It is known that ROS play a number of significant, diverse roles in female reproductive biology including events in the uterine environment, corpus luteum function and regression (7). An unbalanced prooxidant-antioxidant state has been postulated to be involved in the pathogenesis of a number of uterine disorders such as cervicitis, uterine myoma (8), and endometriosis (9, 10). However, our results failed to show imbalance between oxidant and antioxidant levels in mares used in this study except for β-carotene.

Plasma concentration of β-carotene in the mares with uterine lymphatic cyst was significantly lower ($P<0.01$) than in healthy mares. Differences in the levels of β-carotene between healthy mares and mares with uterine cysts could have been due to a number causes. Beta carotene, precursor of vitamin A is known to be necessary for different reproductive functions. It plays important roles in ovarian steroidogenesis and the uterus. β-carotene stimulated progesterone production in bovine luteal cells (21, 22). Lotthammer and Ahlswede (23) have also reported that β--carotene supplemented cows have a much lower incidence of ovarian cysts than unsupplemented cows. Some reports indicate that the uterus requires vitamin A for normal epithelial differentiation and for the maintenance of fertility (24). Vitamin A deficiency causes alterations in epithelial differentiation, proliferation of squamous cells and shedding of mucous cells. Dietary retinoic acid, vitamin A metabolit permits the replacement of squamous metaplastic cells by mucus-secreting cells and thus, reestablishes normal epithelial morphology and function (25). Oestrogen-dependent keratinisation of the vagina and cervical epithelium occurs during the oestrus phase of the ovarian cycle in rodents. Vitamin A administration was found to reduce the oestrogen-induced proliferation of rat uterine stromal and myometrial cells (24) and inhibits the progression of rat mammary carcinoma (26). These observations indicate that vitamin A can alter the responses of several oestrogen-sensitive tissues.

Lymphatic cysts may be associated with a normal uterus or with chronic inflammation of the uterus (27). β-carotene has been reported to possess immune modulator activities in humans and animals (28). Carotenoids stimulate the phagocytic and bacteria-killing ability of blood neutrophils and peritoneal macrophages. Vitamin A deficiency decreases antigen-specific secretory immunoglobulin A concentrations and mucosal-associated immune cell function (29). Michal et al. (30) have also reported that dairy cows fed β-carotene had lower incidence of metritis compared with non-supplemented cows. The genital tract may be susceptible to infections as a result of a dysfunction of protective epithelia due to β-carotene deficiency (25). Briefly, β-carotene may prevent uterine cyst formation by regulating ovarian steroid production, immune cell function and epithelial differentiation through its biological actions other than its antioxidant properties. On the other words, age-related degenerative changes was shown increasingly in the uteri of mares at the age of 15 onwards (19), meanwhile lower β-carotene concentration may accelerate the age-related degenerative diseases such as uterine cyst in mares.

In conclusion, it was observed no imbalance between plasma oxidant and antioxidant levels except for β-carotene in mares with uterine lymphatic cyst. Lower β-carotene concentration may be one of factors which expedite age-related uterine degeneration such as lymphatic cyst in mares. Antioxidants such as β-carotene and vitamin A may be advice to prevent possible age-related degenerative changes in uterine of the mares at the age of 15 onwards.

This study may be considered as a preliminary study because of the small number of animals. Further studies are required to investigate the role of β-carotene in the pathogenesis of uterine cysts.

References

1. Eilts BE, Scholl DT, Paccamonti DC, et al. Prevalence of endometrial cysts and their effect of fertility. Biol Reprod Monog 1995; 1: 527–532.
2. Morrow DA. Current Therapy in Theriogenology. Philadelphia: WB Saunders 1980: 174.
3. Tannus RJ, Thun R. Influence of endometrial cysts on conception rate of mares. J Vet Med A 1995; 42: 275-283.
4. Stanton MB, Steiner JV, Pugh DG. Endometrial cysts in the mare. J Equ Vet Sci 2004; 24: 14-19.
5. Serviddio G, Loverro G, Vicino M, et al. Modulation of endometrial redox balance during the menstrual cycle: relation with sex hormones. J Clin Endoc Metabol 2002; 87: 2843-2848.
6. Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. Free Rad Biol Med 2000; 29: 323-333.
7. Riley JCM, Behrman HR. Oxygen radicals and reactive oxygen species in reproduction. Pro Soc Exp Biol Med 1991; 198: 781-791.
8. Chiou JF, Hu ML. Elevated lipid peroxidation and disturbed antioxidant enzyme activities in plasma and erythrocytes of patients with uterine cervicitis and myoma. Clin Biochem 1999; 32: 189-192.
9. Ota H, Igarashi S, Hatazawa J, Tanaka T. Endometriosis and free radicals. Gyne Obstet Invest 1999; 48: 29-35.

10. Yaralioglu-Gurgoze S, Cetin H, Cen O, Yilmaz S, Atli MO. The changes of malondialdehyde levels and glutathione peroxidase activity in purebred Arabian mares with endometritis. Vet J 2005; 170: 135-137.

11. McKinnon, AO, Squires EL, Carnavale E et al. Diagnostic ultrasonograpy of uterine pathology in the mare. Proc Am Ass Eq Pract 1987; 605-622.

12. Placer ZA, Cushman L, Johnson BC. Estimation of products of lipid peroxidation (malonyldialdehyde) in biological fluids. Anal Biochem 1966; 16: 359- 64.

13. Matkovics B, Szabo L, Varga I. Determination of enzyme activities in lipid peroxidation and glutathione pathways. (in Hungarian). Lab Diagn 1988; 48: 248-250.

14. Desai ID. Vitamin E analysis methods for animal tissues. Method Enzym 1984; 105:138-47.

15. Suzuki J, Katoh N. A simple and cheap method for measuring vitamin A in cattle using only a spectrophotometer. Jp J Vet Sci 1990; 52: 1282-4.

16. Sedlak J, Lindsay RHC. Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellmann's reagent. Anal Biochem 1968; 25: 192-205.

17. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Com 1976; 71: 952-958.

18. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991; 196:143–57.

19. Allen WR, Antczak DF. Reproduction and Modern Breeding Technologies in the Mare. In: Bowling AT, Ruvinsky A. (Editors) The Genetics of the Horse. NY: CAB International 2000: 314-316.

20. Kenney RM. Cyclic and pathological changes of the mare endometrium as detected by biopsy with a note on early embryonic death. J Am Vet Med Assoc 1978; 172: 241-262.

21. Talavera F, Chew BP. Retinol, retinoic acid and β-carotene ration on progesterone secretion by bovine luteal cells. J Dairy Sci 1987; 70: 119.

22. Weng BC, Chew BP, Wong TS, Park JS, Kim HW, Lepine AJ. β-Carotene uptake and changes in ovarian steroids and uterine proteins during the estrous cycle in the canine. J Anim Sci 2000; 78: 1284-1290.

23. Lotthammer KH, Ahlswede L. Undersuchungen über eine spezifische, vitamin A-unablangige wirkung des β-carotins auf die fertilitat des rindes: 3. Mitteilung: Blutserumuntersuchungen. Deut Tierärztl Woch 1977; 84: 220-226.

24. Boettger-Tongand HL, Stancel GM. Retinoic acid inhibits estrogen-induced uterine stromal and myometrial cell proliferation. Endoc 1995; 136: 2975-2983.

25. Ponnamperuma RM, Kirchhof SM, Trifiletti L, De Luca LM. Ovariectomy increases squamous metaplasia of the uterine horns and survival of SENCAR mice fed a vitamin A-deficient diet. Am J Clin Nutr 1999; 70: 502-508.

26. Moon RC, Mehta RG. Chemoprevention of experimental carcinogenesis in animals. Prev Med 1989; 18: 576-591.

27. Blikslager AT, Tate LP, Weinstock D. Effects of neodymium: yttrium aluminum garnet laser irradiation on endometrium and on endometrial cysts in six mares. Vet Surg 1993; 22: 351-356.

28. Chew BP. Role of carotenoids in the immune response. J Dairy Sci 1993; 76: 2804-2811.

29. Semba RD. Vitamin A, infection and immune function. In: Calder PC, Field CJ, Gill HS. (Editors) Nutrition and Immune Function. New York: CABI Publishing 2002:151-169.

30. Michal JJ, Heirman LR, Wong TW, et al. Modulatory effects of dietary beta-carotene on blood and mammary leukocyte function in periparturient dairy cows. J Dairy Sci 1994; 77: 1408-1421.