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AGE-INDUCED OXIDATIVE STRESS IN RABBIT BUCKS: PROTECTIVE EFFECT OF MELATONIN

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ABESTRACT

The present study aimed investigate the effect of age on the physiological status of the rabbit bucks and to determine if the antioxidant properties of melatonin can protect aged rabbit males from oxidation stress. Forty five mature New Zealand White (NZW) rabbit bucks (15, 1 year-old and 30, 4 years-old) were used in the present work. They were classified into three groups each of 15 bucks (a control and two treatments; T1 and T2). The control group included: mature bucks without melatonin supplementation (Adult), while T1 and T2 included aged bucks without melatonin supplementation (Aged), and aged bucks supplemented with 25 mg melatonin/kg for 8 weeks (Aged + Mel), respectively. Semen quality, some blood hematology, blood and seminal biochemical plasma in two ages of rabbit bucks, and the effect of melatonin supplementation on these traits were studied. The achieved results showed that most of the studied traits were adversely affected in aged rabbit bucks without melatonin supplementation. On the other hand Melatonin supplementation to aged males for 8 weeks, significantly increased sperm concentration, sperm motility, WBCs, blood and seminal plasma of GST and SOD. Also, melatonin supplementation decreased abnormal and dead sperms, as well as blood and seminal plasma of TBARS. In conclusion, it could be recommended that melatonin supplementation at a level of 25 mg/kg body weight to aged rabbit bucks was effective in minimizing the oxidative stress which was induced with the advance of age, specially for semen quality parameters, blood hematology, blood plasma of cholesterol and triglyceride, and blood and seminal plasma of TBARS, GST, and SOD.

INTRODUCTION

Aging is defined by biological and demographic parameters characterized by an impairment of function (Sampson et al., 2007). It is associated with a decline in immune function (Srinivasan et al., 2005). Aging has different effects on the reproductive system: in the testes, spermatogenesis and steroidogenesis decrease with old ages (Levy *et al.,* 1999, Zirkin and Chen, 2000). and in the epididymal epithelium, some striking segment-specific changes occur at the histological and biochemical levels prior to the major loss of spermatogenesis. In male Japanese quail, Ottinger *et al.,* (2002) reported a loss of fertility, increase of morphological abnormalities in the testes, and a higher incidence of Sertoli cell tumors during aging. Also, the age is considered as an impairment of body function over time caused by accumulation of molecular damage in DNA, proteins and lipids. The accumulating damage may be eventually manifested in age-related health issues, such as decreased fertility (Sampson *et al.,* 2007).

It is well known that, fertility is a measurable feature that reflects semen quality (Sexton, 1983). Such decreasing fertility problem could be solved by melatonin supplementation which may participate to some extent in solving such problem. Knowledge of the molecular mechanisms involved in aging is required for the development of strategies to preserve the quality of life of increasingly aging population. Degenerative changes associated with aging have been related to progressive damage by reactive oxygen and nitrogen species in those situations in which the antioxidative defense system fails to eliminate them (Perdo, et al., 2006).

Recently, melatonin, the main hormone of the pineal gland, was proposed as a protective agent against macromolecular destruction associated with longevity (Reiter et al., 1996). The protective effects of melatonin could be related to its ability to synchronize circadian rhythms and thereby to reduce the biological stress, as well as to its direct free radical scavenging activity and its indirect antioxidants properties (Reiter et al., 2002). The objective of this study is to investigate the effect of rabbit males age on the physiological status and to determine if the antioxidant properties of melatonin can protect aged rabbit males from oxidation stress caused by aging.

MATERIALS AND METHODS

Animals and Housing:

Forty five mature New Zealand White (NZW) rabbit bucks (15, 1 year-old and 30, 4 years-old) were used in the present work. The rabbit bucks which healthy and clinically free of external and internal parasites were raised in flat deck batteries with universal specifications. The batteries were accommodated with feeders and automatic fresh water drinkers and were efficient for hygienic control. Feeding was carried out according to NRC (1977), recommendations. The rabbit bucks were divided into three groups, each 15 bucks nearly equal in the body weight.

Experimental Procedure:

Rabbits were assigned to the control group and two treatments: T1 and T2. The control group included: mature bucks without melatonin supplementation (Adult), while T1 and T2 included aged bucks without melatonin supplementation (Aged), and aged bucks supplemented with 25 mg melatonin/kg for 8 weeks (Aged + Mel), respectively.

Measurements:

Semen collection was carried out weekly from all males throughout the 8 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. The percentage of motile sperms was estimated by visual examination under low-power magnification (10x) using a phase-contrast microscope with heated stage (Blom, 1950). A weak eosin solution (Smith and Mayer, 1955) was

used for the evaluation of sperm concentration by the improved Neubauer haemocytometer slide. Assessments of abnormal and dead spermatozoa were performed using an eosin-nigrosine blue staining mixture.

Blood samples were collected from each rabbit in clean tube with heparin. Plasma was obtained by centrifugation of the blood samples at 3500 rpm for 20 min for later analysis. Blood hematological parameters (RBCs, Hb, Ht, and WBCs) were determined. Blood plasma samples were subjected to biochemical analysis using specific kits. Blood plasma was submitted for determination of glucose, cholesterol, and triglycerides. Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4c, and was stored at -20c until later analysis. Blood and 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 by using the method of Tappel and Zalkin (1959). Superoxide dismutase (SOD) activity was measured according to Misra and fridovich (1972).

RESULTS

Semen Characteristics:

Data of sperm parameters are presented in Table (1). Sperm motility and sperm concentration were significantly decreased in aged rabbit bucks (4 years-old) by 19.44% and 14.83%, respectively, compared to adult males (1 year-old). Abnormal and dead sperms were significantly increased in aged animals by 33.54% and 24.75%, respectively, compared to adult ones.

Melatonin supplementation to aged rabbit bucks increased sperm motility and concentration by 15.52% and 13.27%, respectively, and decreased the abnormal and dead sperms by 23.22% and 17.41%, respectively, as compared with unsupplemented aged males. Ejaculate volume was not affected by age or by melatonin supplementation.

Table (1): Effect of age and melatonin supplementation on semen characteristics of rabbit males.

Traits \ Treatments	Adult	Aged	Aged +Mel	S.E	Significant
Ejaculate volume (ml)	0.80	0.85	0.84	0.07	N.S
Sperm motility (%)	0.72 ^{a}	0.58 ^{b}	0.67 ^{a}	0.08	**
Sperm concentration (x10^6/ml)	343.2^{a}	292.3 ^{b}	331.1 ^{a}	2.65	**
Abnormal sperms (%)	15.8 ^{b}	21.1 ^{a}	16.2 ^{b}	1.11	**
Dead sperms (%)	19.8 ^{b}	24.7 ^{a}	20.4 ^{b}	1.15	**

Means within column for each item having different superscript differ significantly ** ($p \leq .01$)

Blood Hematological Parameters:

The results of some hematological parameters are presented in Table (2). Generally, the higher RBCs, Hb, Ht, and WBCs were noticed with aged rabbit bucks compared to adult bucks. These differences were significantly only with WBCs. Melatonin supplementation insignificantly

increased RBCs, Hb, and Ht, and significantly increased WBCs compared to the other group.

Table (2): Effect of age and melatonin supplementation on some blood hematological parameters of rabbit males.

Traits \ Treatments	Adult	Aged	Aged +Mel	S.E	Significant
RBCs ($x10^6$)	7.01	6.75	7.19	0.58	N.S
Hb (g)	12.86	12.32	13.0	1.12	N.S
Ht (%)	38.52	38.41	38.66	2.45	N.S
WBCs ($x10^3$)	7.59 ^{b}	5.95 ^{c}	8.15 ^{a}	0.78	*

Means within column for each item having different superscript differ significantly *(p ≤ .05)

Biochemical of Blood Plasma:

The effect of rabbit bucks age and melatonin supplementation are presented in Table (3). The results showed that cholesterol, triglycerides, and BARS values were higher by 21.54%, 29.54%, and 27.84%, while the values of GST and SOD were lower by 25.63% and 21.52%, respectively, in aged rabbit bucks compared with the adult ones. The effect of melatonin supplementation was recorded where aged rabbit bucks received melatonin showed significant decrease in cholesterol, triglycerides, and TBARS by 20.42%, 19.11%, and 13.71%, respectively, and significant increase in GST and SOD by 23.95% and 21.09%, respectively, as compared with aged males without melatonin supplementation. Glucose value was not significantly influenced by age or by melatonin supplementation.

Table (3): Effect of age and melatonin supplementation on biochemical blood plasma of rabbit males.

Traits \ Treatments	Adult	Aged	Aged +Mel	S.E	Significant
Glucose (mg/dl)	121.56	123.17	122.41	3.48	N.S
Cholesterol (mg/dl)	81.62 ^{b}	99.18 ^{a}	78.93 ^{b}	1.29	**
Triglyceride (mg/dl)	72.00 ^{b}	93.27 ^{a}	75.45 ^{b}	2.33	**
TBARS (n mol/ml)	0.97 ^{b}	1.24 ^{a}	1.07 ^{b}	0.12	*
GST (µmol/hr)	3.20 ^{a}	2.38 ^{b}	2.95 ^{a}	0.25	*
SOD (u/ml)	21.93 ^{a}	17.21 ^{b}	20.84 ^{a}	1.34	**

Means within column for each item having different superscript differ significantly *(p ≤ .05) ** (p ≤ .01)

Biochemical of Seminal Plasma:

Seminal plasma data of bucks as influenced by age and melatonin supplementation are illustrated in Table (4). The effect of age *per se* was recognized, where aged bucks showed significant increase in TBARS (49.15%) and decrease in GST (23.66%) and SOD (21.39%) as compared to the adult bucks. Melatonin supplementation significantly decreased TBARS by 17.61%, while GST and SOD were increased by 26.0% and 8.74%, respectively, compared to aged bucks.

Table (4): Effect of age and melatonin supplementation on biochemical semen plasma of rabbit males.

Traits \ Treatments	Adult	Aged	Aged +Mel	S.E	Significant
TBARS (n mol/ml)	1.18 [c]	1.76 [a]	1.45 [b]	0.08	**
GST (µmol/hr)	1.31 [a]	1.00 [b]	1.26 [a]	0.12	**
SOD (u/ml)	25.34 [a]	19.92 [b]	21.66 [b]	1.07	**

Means within column for each item having different superscript differ significantly ** (p ≤ .01)

DISCUSSION

As previously mentioned, the aim of the present study was to show the effect of rabbit males age on physiological status and to determine if the antioxidant properties of melatonin can protect aged rabbit males from oxidation stress. The obtained data showed that sperm motility and concentration were significantly decreased, while abnormal and dead sperms were increased in response to age progress. These results are in line with those found by Plas et al., (2000), Kidd et al., (2001), and Kuhnert and Nieschlag (2004). They described age-related decline in sperm count and motility, indicating decreases in sperm count by 22%, sperm motility by 37%, and morphological abnormalities spermatozoa by 18%. Sampson et al., (2007) reported that age-associated changes in the hypothalamus-pituitary-gonadal axis play a pivotal role in the aging male reproductive tract, resulting in altered testicular function and change in semen output. These may be related to altered steroidogenesis of the aging testis and morphological compromises of the aging spermatozoa.

The increase of sperm parameters in the current study was anticipated in melatonin supplementation. It could be conducted that sperm motility and concentration were significantly increased with melatonin supplementation. The abnormal and dead sperms were significantly decreased with melatonin supplementation. The current results are in agreement with findings obtained by Shang et al., (2004), who reported that melatonin can protect sperm mitochondria from the damage through its effective antioxidative potentiality.

lso, similar results were achieved by Gavella and Lipovac., (2000), who demonstrated that melatonin supplementation enhanced and improved semen quality. They described the ability of melatonin to suppress experimentally induced lipid peroxidation in sperm membrane.

The decrease of WBCs count was observed with aged male rabbits compared to adult ones. Melatonin supplementation significantly increased WBCs. Srinivasan et al., (2005) reported that, aging is associated with a decline in immune function, where many hormones that are associated with the maintenance of immune function also declined with advancing age. They added that melatonin immunoenhancing properties have been attributed to a direct action on the immunocompetent cells such as granulocyte-macrophage cells, NK cells and lymphocytes.

The current study showed that plasma cholesterol, triglycerides, and TBARS values were increased, while GST and SOD were decreased in aged rabbit bucks compared to the adult ones. Melatonin supplementation decreased cholesterol, triglycerides, and TBARS values and increased those of GST and SOD. The results of Yamada et al., (2004) revealed no change in the plasma glucose levels during rabbits aging. Also, Raghavendra and Kulkarni (2001) observed in mice that age-associated decline in glutathione level and enhanced lipid peroxidation, which was significantly reversed by administration of melatonin. The results of Pilaczynska et al., (2004) confirmed that melatonin plays an important role in the blood antioxidant defense system in elderly men. Similar results were observed by Gonca et al., (1999) in rat. The authors suggested that increased levels of lipid peroxidation products may have a role in aging, and added that exogenous melatonin may delay the aging process of tissues through its free radical scavenging effects.

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ضغوط الأكسدة الناتجة عن تقدم عمر ذكور الأرانب: الدور الوقائى لمادة الميلاتونين

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تهدف هذه الدراسة الى توضيح التأثيرات السلبية للتقدم فى العمر على الحالة الفسيولوجية لذكور الأرانب من خلال دراسة بعض صفات السائل المنوى وبعض صفات صورة الدم وكذلك بعض صفات بلازما الدم وبلازما السائل المنوى. وعلى الجانب الأخر دراسة مدى امكانية تحسين هذه الصفات عن طريق امداد الحيوانات بمادة الميلاتونين ولقد استخدم فى هذه الدراسة 45 من ذكور الأرانب من عمرين مختلفين (15 منهم عمر عام واحد & 30 منهم عمر 4 سنوات)، تم اسكانها فى أقفاص فردية، حيث تم تقسيم الأرانب الكبيرة العمر (4 سنوات) الى مجموعتين (كل مجموعة 15 ذكر) قدم لأحدهما 25مليجرام / كجم وزن حى من مادة الميلاتونين.وبذلك أصبح عدد المعاملات ثلاثة: الأولى (ذكور ناضجة) عدد الحيوانات بها 15 من الذكور عمر عام واحد، الثانية (ذكور معمرة) عدد الحيوانات بها 15 من الذكور المعمرة (4 أعوام) والثالثة (ذكور معمرة + ميلاتونين) عدد الحيوانات بها 15 من الذكور المعمرة التى قدم لها الميلاتونين لمدة 8 أسابيع. تم التسجيل لصفات جودة السائل المنوى ، وصفات بلازما السائل المنوى، وعمل صورة دم وكذلك قياس بعض صفات بلازما الدم. ولقد أظهرت النتائج انخفاض معظم الصفات تحت الدراسة فى حالة الذكور المتقدمة فى العمر (4 سنوات) والتى لم يقدم لها مادة الميلاتونين. ولقد أدت اضافة مادة الميلاتونين فى مياه الشرب للحيوانات المتقدمة فى العمر لمدة 8 أسابيع الى زيادة تركيز وحيوية الاسبرمات، كما أدى استخدام الميلاتونين الى انخفاض نسبة الاسبرمات المشوهة والميتة، و كذلك زيادة عدد كرات الدم البيضاء، والانزيمات المضادة للأكسدة وذلك بالمقارنة بالمجموعة المعمرة والتى لم يقدم لها الميلاتونين. مما سبق يتضح أن اضافة 25 جرام/كجم وزن حى من مادة الميلاتونين الى الحيوانات المعمرة قد أدت الى تحسين صفات جودة السائل المنوى، ورفع الحالة المناعيةعن طريق التأثير على انزيمات الأكسدة بالجسم .