LINE AND BIRTH SEASON EFFECTS ON OXIDATIVE STRESS PARAMETERS IN TESTIS OF MATURING RABBITS

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ABSTRACT

Oxidative stress is a factor related to the male reproductive function. Differences between breeds have been found for testis size, seminiferous tubule diameter, number and size of interstitial and germ cells, etc. Traits related to the redox system could also be affected by genetic factors. The existence of differences between lines for these traits could lead to differences in reproductive maturation and fertility.
The present paper has investigated the age-related changes of the superoxide anion ($O_2\cdot^-$) radical formation, superoxide dismutase activity (SOD), catalase activity (CAT) and thiobarbituric acid reacting substances (TBARs) level in testis of rabbits. The existence of differences between lines selected for different aptitudes (Caldes for growth rate and Prat for litter size) and the effect of birth season for these traits were assessed.
Major changes in the oxidative metabolism were observed at an early age and could be explained by the concomitant changes in testicular structure and function. Both lines showed similar developmental profiles and levels for all the variables studied. Environmental conditions affected both lines in the same manner. Significant seasonal variations were found in $O_2\cdot^-$, SOD and CAT. Future studies should take into account differences between seasons for a more precise analysis.

Key words: Rabbit testis, Antioxidant enzymes, TBARs, Development.

INTRODUCTION

The role of oxidative stress in the male reproductive function has recently gained considerable attention, since standard semen analysis seems not to provide a complete diagnosis of sperm quality and production (Sikka, 2001). Several authors have found expression and activity of Cu-Zn SOD, Mn SOD, CAT, and Se-Glutation peroxidase (Gpx) (Gu and Hecht, 1996; Samanta *et al.*, 1999) and $O_2\cdot^-$ generation (Georgellis *et al.*, 1988) in testes of various mammals. The antioxidant enzyme system of male germ cells shows a different pattern with respect to other organs, with high activity and mRNA levels of SOD and low CAT and Gpx (Gu and Hecht, 1996; Samanta *et al.*, 1999) and it has been hypothesised that hydrogen peroxide could be involved in the regulation of normal germ cell division and differentiation (Samanta *et al.*, 1999; Peltola *et al.*, 1992).

Environmental, physiological and genetic factors can affect normal spermatogenesis as well as sperm function, thereby altering male fertility. Concerning genetic factors, differences between breeds have been found for some morphologic traits of testis (in developing rabbits, García-Tomas *et al.*, 2007; in mature bulls, Tegegne *et al.*, 1991). Regarding the effect of environmental factors on rabbit male reproduction, it has been studied through seminal parameters (Theau-Clement *et al.*, 1995), morphologic traits of testis and epididymis (García-Tomas *et al.*, 2007) and serum testosterone (Chiericato *et al*., 1994). However, the information currently available for the effects of breed and environment on oxidative stress is very scarce or even non-existent. The aim of this study was to investigate the effects of line and birth season on the age-related changes of some oxidative stress parameters in maturing rabbit testis.

MATERIALS AND METHODS

Animals

The study involved two rabbit lines (Caldes and Prat). The Caldes line is a sire line selected on the basis of growth rate by individual selection (Gómez *et al.*, 1999); the Prat line is a maternal line selected on the basis of litter size at weaning (Gómez *et al.*, 1996). Animals were born in two different periods: December, with an average outdoor temperature of 8.5ºC, and May to July, with a medium outdoor temperature of 22.7ºC.

Males were placed in individual cages at 75 days old, they were raised with a photoperiod of 16 hours light/day and fed restricted with commercial rabbit pellets. Eight bucks per line (Caldes, Prat) and birth season were sampled at 4, 8, 10, 14, 16, 20 and 33 weeks of age.

Tissues sampling and preparation. Oxidative stress parameters

Rabbits were killed by intravenous administration of potassium pentobarbital 18%. Right testes were removed quickly and placed in ice-cold saline. After removal of the tunica albuginea, the testes were stored at –80 º C until the measurement of oxidative stress indicators was carried out. After thawing, testes were weighed and homogenised with a polytron (PT 3100 Polytron Kinematica AG) at 4ºC in 1:10 (w/v) phosphate buffer pH 7.4 (1.59 mM $NaH_2PO_4.2H_2O$, 8.8 mM $Na_2HPO_4.2H_2O$ and 140 mM NaCl). After that, homogenates were sonicated for 3 minutes using an ultrasound-bath.

Protein estimation of samples was made according to the Biuret method with bovine serum albumin as the reference protein.

The formation of O_2^- was evaluated in testis aliquots (2.5 mg) by chemiluminescence (Tarpey *et al.*, 1999). The O_2^- generation was expressed as relative light units (RLU)/mg testis/min.

To assay antioxidant enzymes, homogenates were centrifuged at 416 g for 10 minutes at 4ºC. Superoxide dismutase and CAT activities were measured in the supernatant of testis homogenates. Superoxide dismutase activity (cytosolic Cu/Zn) was assayed according to the procedure of Marklund (1985), results were expressed as units of enzyme activity/mg protein. Catalase activity at 25ºC was measured according to the procedure of Aebi (1984), results were expressed in K/mg protein.

Lipid peroxidation was estimated in the crude homogenate as TBARs by the thiobarbituric acid (TBA 1%) method of Yagui (1984). Before reaction with TBA at 90ºC for 60 minutes, samples were incubated in tricloroacetic acid (TCA 20%) at 4ºC for 90 minutes in order to minimize interaction with proteins. The values of TBARS were expressed as nmol of MDA/mg protein.

Statistical analyses

Data from testis were subjected to analysis of variance by using GLM procedures of SAS v.8 (SAS, 2001) according to a model which included the fixed factors of line (two levels: Caldes, Prat), birth season (two levels: cold, warm), age (6 levels SOD, CAT and TBARs, and 5 levels for O_2^-) and the double and triple interactions between the fixed factors. When interactions line x age and birth season x age were significant, the variables were analysed at each age with the same model as before to observe differences between lines or between birth seasons at a specific age.

RESULTS AND DISCUSSION

Figure 1 shows the age-related changes of studied variables according to the line. Our general results showed that the two lines studied had similar levels of oxidative stress parameters. However, statistical analyses showed interaction between line and age for TBARs and O_2^-. It is likely that these

interactions indicate a different rate of maturation between lines at pubertal stage.

The main changes in the SOD and CAT activities and lipid peroxidation (TBARs) observed at young ages (8 from 16 weeks), in our study, could be explained by the concomitant changes in testicular structure and function (Iczkowski *et al.*, 1991). Ihrig *et al*. (1974) reported the level of CAT activity in rabbit testis from prepubertal (4 weeks) to mature stages (34 weeks), and described a developmental profile of CAT similar to the profile shown in our work. Superoxide dismutase has been described as essentially localized in different sperm cells (Bauché *et al.*, 1994) and regulated during spermatogenesis (in mouse, Gu and Hecht, 1996). The developmental profile of SOD in our study can be due to the increase in the germ cell population during sexual maturation. In the present work, TBARs were high at young age and after that an important decrease was observed as it has also been described in rat testis (Samanta *et al.*, 1999). The lipid peroxidation showed may be due to a great production of ROS during young ages, as well as to the qualitative and quantitative changes occurring in phospholipids and fatty acid composition in the testis during maturation (Davis *et al.*, 1966).

In the present study we have observed an increase of SOD and a decrease of CAT with the age. Other authors showed similar age related changes for SOD and CAT in rat testis (Peltola *et al.*, 1992; Samanta *et al.*, 1999). Metabolism of O_2^- is presumably controlled to some extent by the induction of antioxidant enzyme activities. Dismutation of O_2^- by SOD generates H_2O_2, which in turn is converted to water by CAT. Low CAT activity could be related to the need for an increase in H_2O_2 levels during spermatogenesis (Samanta *et al.*, 1999; Peltola *et al.*, 1992). From our results the rise in the ratio SOD/CAT observed at the end of puberty stage, which was mainly due to a decrease in CAT, could be explained by a requirement of H_2O_2 in the last steps of spermatogenesis.

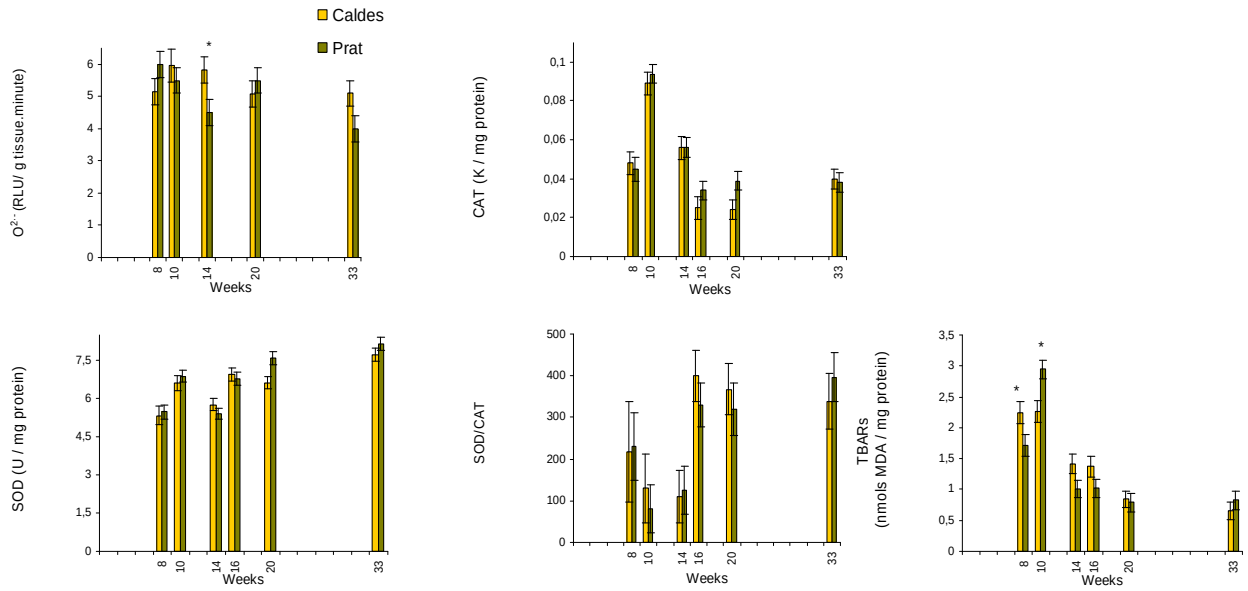

Figure 1: Superoxide anion (O_2^-) generation, superoxid dismutase activity (SOD), catalase activity (CAT) and thiobarbituric acid reacting substances (TBARs) concentration in Caldes and Prat bucks. The age points represent the mean of 10 to 16 animals per rabbit line, SEM are represented by bars. *indicate significant different groups at the 5% level

Figure 2 shows the age-related changes of studied variables according to the birth season. In our study, O_2^-, SOD and CAT activities showed seasonal differences, but all the variables studied had a significant interaction between season of birth and age. In this sense it has been found a different development pattern between males born in different seasons for all the variables. Bello-Klein *et al.* (2000) have reported seasonal variations for these traits in heart and liver in rats. No information is available for the environmental effects on testes oxidative stress.

In rabbits born in the warm season the prepuberty stage (8 weeks of age) was associated to low level

of CAT and to high level of lipid peroxidation and high SOD/CAT ratio that could be related to a negative effect on the testis and epididymis growth, which was delayed during the warm season (García-Tomás *et al.*, 2007). At puberty stage rabbits born in the warm season had high CAT, high TBARS and low SOD/CAT ratio, which could be related to low percentage of seminiferous tubules with presence of elongate spermatids and spermatozoa (García-Tomás *et al.*, 2007). At mature stage (33 weeks of age) maintenance of TBARs was observed in animals born in the cold and in the warm seasons, although the lipid peroxidation was high in rabbits born in the cold season. In animals born in the cold season the steady O_2^- jointly with the increment in SOD could explain the important rise in CAT and the stable levels of TBARs. Whereas in animals born in the warm season constant SOD together with the drop in O_2^- could mean a less need of CAT to maintain TBARs levels.

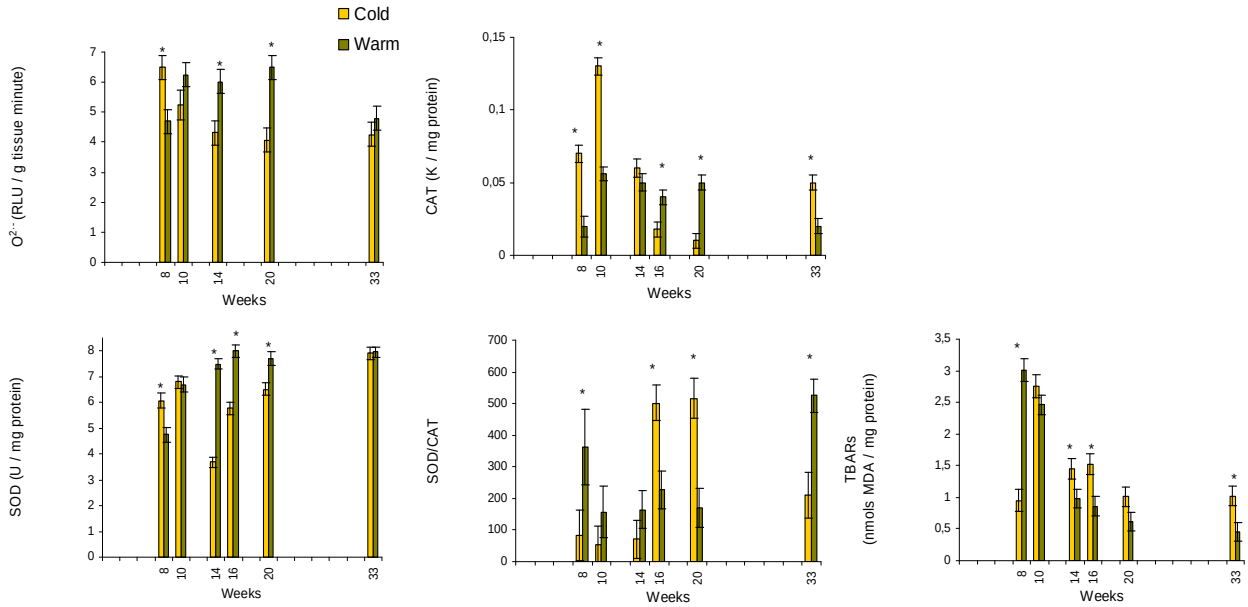

Figure 2: Superoxide anion (O_2^-) generation, superoxid dismutase activity (SOD), catalase activity (CAT) and thiobarbituric acid reacting substances (TBARs) concentration in bucks born in cold and warm season. The age points represent the mean of 10 to 16 animals per rabbit line, SEM are represented by bars. * indicate significant different groups at the 5 % level

CONCLUSIONS

In conclusion, changes in the oxidative metabolism in rabbit testis were studied for the first time. Both lines showed similar developmental profiles and levels for all the variables studied, presenting major changes at young ages and being affected by environmental conditions in the same manner. Significant seasonal variations were found in CAT, SOD and O_2^-. Future studies should take into account differences between seasons for a more precise analysis.

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