

# Effect of percutaneous transthoracic lung biopsy on oxidative metabolism in sheep

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This study aimed to assess the effect of percutaneous transthoracic lung biopsy on the oxidative metabolism of sheep by measuring the oxidative stress markers of superoxide dismutase (SOD), total glutathione (GSH-t), peroxidase (GSH-Px) and thiobarbituric acid reactive substances (TBARS) in the red cells of these animals. Blood samples were collected from 20 clinically healthy sheep prior to, and 30 min after, percutaneous transthoracic lung biopsy. After biopsy, there was a significant decrease ( $p < 0.05$ ) in SOD and GSH-Px activity, with no significant change ( $p \geq 0.05$ ) in GSH-t and TBARS concentrations. These results showed that percutaneous transthoracic lung biopsy did not significantly affect the oxidative metabolism of sheep 30 min after the procedure, which may be used widely in this species without causing serious tissue damage.

## Introduction

Respiratory diseases represent a serious threat to sheep production, accounting for high morbidity and mortality rates.<sup>1,2</sup> The precise diagnosis of several respiratory diseases requires methods complementary to physical examination for the establishment of adequate prophylactic strategies.<sup>3</sup> Lung biopsy is an invasive procedure for diagnostic elucidation and consists of removing a small sample of lung tissue for gross and microscopic examination. It includes laboratory sample processing and slide preparation followed by microscopic analysis and description.<sup>4</sup> The tissue sample may also be submitted for microorganism culture and antibiotic sensitivity testing.<sup>5</sup>

Over the past decades, numerous studies have aimed at investigating the role of reactive oxygen species (ROS) in animal respiratory tract diseases.<sup>6,7,8,9</sup> Lung biopsy tissue samples have been used successfully to assess ROS-induced lung parenchymal damage in humans,<sup>10,11</sup> mice<sup>11,12</sup> and sheep<sup>13,14</sup>. However, the question of whether lung biopsy might contribute to oxidative imbalance by allowing the formation of a large number of ROS remains unaddressed. *In vivo* ROS are formed by the catalytic action of enzymes during the transport of electrons during normal metabolism, which could be via the electron chain transport in the mitochondria or during inflammatory states.<sup>15</sup> When inflammation occurs, phagocytic cells release a large amount of ROS during the respiratory burst. These liberated ROS are probably the primary cause of inflammation-induced tissue damage. In addition, ROS act as inflammatory mediators exacerbating inflammatory response.<sup>16,17</sup>

The most important reactive oxygen metabolites are the superoxide radical ( $O_2^{\bullet}$ ), hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $OH^{\bullet}$ ).<sup>18</sup> Most ROS are extremely reactive, highly toxic and capable of reacting with several organic molecules,<sup>15</sup> but the balance between ROS generation and elimination is maintained by a complex antioxidant defence mechanism, which comprises enzymes such as superoxide dismutase (SOD), catalase (CAT) and the glutathione cycle enzymes.<sup>19</sup>

Nonetheless, ROS levels may rise as a result of increased intracellular ROS production or antioxidant defence mechanism deficiencies. An imbalance between oxidant and antioxidant molecules is called oxidative stress.<sup>20,21</sup> Oxidative stress may induce lipid peroxidation and the severe tissue lesions which result in damaged cell organelles, nucleic acids, lipids and proteins.<sup>21</sup> Given that lung biopsy is an invasive diagnostic method, it is likely to cause an acute inflammatory response increasing ROS production by phagocytic cells. Taking into account the high rate of respiratory diseases in the ovine species, and the evidence showing that ROS play a leading role in the development of respiratory disorders,<sup>6,7,8,9</sup> it is important to determine whether the use of an invasive semiologic method such as lung biopsy interferes with oxidative metabolism and thus aggravates the inflammatory process inherent to the disease. Therefore, the aim of this study was to measure oxidative stress biomarkers in clinically healthy sheep undergoing percutaneous transthoracic lung biopsy.

## Materials and methods

### Animals

This study included 20 (9 male and 11 female) clinically healthy Santa Inês sheep aged between 150 and 190 days (mean = 175 days) and weighing 23 kg – 44 kg (mean = 32 kg), which were raised for slaughter in commercial abattoirs. Routine clinical examination was performed immediately before lung biopsy to ensure that all animals were clinically healthy prior to testing.

### Lung biopsy

Lung biopsy was performed at the abattoir prior to slaughter and with the animals in a standing position and restrained by an assistant. The seventh right lateral intercostal space, approximately 5 cm above the olecranon, was chosen as the puncture site as it corresponds to the topographic position of the diaphragmatic lobe of the right lung. The biopsy site was clipped, disinfected with 1% iodine-povidine (Iodopovidona Tópico; Rioquímica, São José do Rio Preto, Brazil) and anaesthetised with 5 mL of 2% lidocaine (Lidovet; Bravet, Mogi Mirim, Brazil). A few minutes later, a 1 cm incision was made with a scalpel blade into the skin and subcutaneous tissue through the intercostal space. A semi-automatic biopsy needle (Soft tissue cannula BD 16 G × 15 cm; Euromed Cateteres, Lagoa Santa, Brazil) was introduced immediately cranial to the rib at right angles to the body surface, avoiding intercostal nerves and vessels. One lung tissue sample per animal was collected, according to the techniques described by Finn-Bodner and Hathcock<sup>22</sup> and Braun et al.<sup>23</sup> After lung biopsies were completed, all animals were slaughtered for commercial purposes.

### Blood sample collection and processing

In order to minimise the effects of stress caused by restraining the animals, blood samples were collected immediately after this procedure. Jugular venous blood samples (5 mL) were collected from each animal in heparin-containing vacutainer tubes. Similarly, other blood samples were drawn from all animals 30 min after clinical examination and lung biopsy. The samples were stored at 10 °C on scale ice in isothermal containers and sent to the Gynaecology and Obstetrics Experimental Research Laboratory of Botucatu Medical School at the São Paulo State University in Brazil, for the assessment of SOD, total glutathione (GSH-t), glutathione peroxidase (GSH-Px) and thiobarbituric acid reactive substances (TBARS).

Blood samples were centrifuged (Centrifuge 5804E; Eppendorf, Hamburg, Germany) at 185 g for 10 min at room temperature. Red cells were obtained after washing in phosphate-buffered saline (Solução tampão salina-fosfato; Ceaquim, Botucatu, Brazil) at 4 °C and centrifuged three times at 1575 g for 15 min. Upon each repetition, plasma, white cells and platelets were removed by micropipette aspiration. The washed red cells (50 µL) were haemolysed in 1900 µL deionised water and shaken by inverse stirring to obtain the

haemolysate that was used for the assessment of oxidative stress biomarkers (SOD enzymatic activity, as well as red cell and TBARS concentrations). To determine the enzymatic activity of GSH-Px and GSH-t concentration, the washed red cells (50 µL) were haemolysed in 950 µL stabilising solution (2.7 mM ethylenediaminetetraacetic acid [EDTA] and 0.7 mM 2-mercaptoethanol) (Solução tampão salina-fosfato; Ceaquim, Botucatu, Brazil)<sup>24</sup> and shaken by inverse stirring to obtain the haemolysate. All the haemolysate samples were frozen and stored at -80 °C until analysis six months after collection.<sup>24</sup>

### Biochemical analysis

The SOD, GSH-t, GSH-Px and TBARS activities were determined in triplicate by spectrophotometric colorimetry, according to the modified technique described by Souza et al.<sup>24</sup> Haemoglobin was assessed by using 20 µL haemolysate diluted and shaken in 2 mL Drabkin solution (Ceaquim, Botucatu, Brazil). After a 10 min rest, absorbance was measured at 546 nm with a spectrophotometer (SP 220; Bioespectro, São Paulo, Brazil) and expressed in g/dL.<sup>24</sup>

Lipid peroxides were estimated in washed red cells using thiobarbituric acid (TBA) (SIGMA, St. Louis, USA). Briefly, 1 mL of washed red cells were added to the test tube containing 1 mL of 3% sulphosalicylic acid (5-sulfosalicylic acid hydrate 95%; SIGMA, St. Louis, USA), agitated for 10 s, centrifuged at 11 000 rpm for 3 min and kept in rest for 15 min. The sample was diluted to 500 µL of 0.67% TBA solution. The mixture was heated to 80 °C for 30 min and absorbance was measured at a 535 nm wavelength. The results were expressed as nM of thiobarbituric acid reactive species (TBARS) per gram of haemoglobin (nM/g Hb).<sup>24</sup>

The SOD activity was determined from its ability to inhibit the pyrogallol auto-oxidation. The reaction mixture (1 mL) consisted of 5 mM Tris (hydroxymethyl) aminomethane (pH 8), 1 mM EDTA, bidistilled water and 20 µL of the sample. The reaction was initiated by the addition of pyrogallol (final concentration of 0.2 mM) (Pyrogallol; Shynth, Dracena, Brazil) and absorbance was measured with a spectrophotometer at 420 nm (25 °C) for 5 min. Enzymatic activity units were defined as SOD units able to produce 50% of pyrogallol oxidation inhibition. All data were expressed in units of SOD per milligram of haemoglobin (UI/mg Hb).<sup>24</sup>

The GSH-t, which consists of reduced and oxidised glutathiones, was enzymatically determined using 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) and glutathione reductase (GSH-Rd) in the presence of a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), forming 2-nitro-5-thiobenzoic acid. A mixture consisting of 1290 µL of distilled water, 200 µL of Tris-HCl buffer (1 M, pH 8, 5 mM EDTA), 200 µL of 10 UI/mL GSH-Rd (SIGMA, St. Louis, USA), 200 µL of 2 mM NADPH (SIGMA, St. Louis, USA) and 100 µL of 12 mM DTNB (SIGMA, St. Louis, USA) was added to 10 µL of the sample. Activity was measured at 412 nm on a spectrophotometer. One unit of activity was

equal to the micromolar of substrate reduced per gram of haemoglobin ( $\mu\text{M}/\text{g Hb}$ ).<sup>24</sup> To obtain the standard curve for the total glutathione quantification, the same test was carried out replacing the haemolysate sample by 10  $\mu\text{L}$  of buffer solution 1:1000 oxidised glutathione (GSSG) at a concentration of 0.03  $\mu\text{M}$ . The absorbance was determined in a spectrophotometer at 412 nm. The concentration of GSH-t was expressed in micromolar substrate reduced per gram of haemoglobin (mM/Hb).

Finally, the GSH-Px was assessed by monitoring NADPH oxidation. The mixture consisted of the addition of 1.3  $\mu\text{L}$  of distilled water, 200.0  $\mu\text{L}$  of Tris-HCl buffer (EDTA 1M, pH 8, 5 mM), 200.0  $\mu\text{L}$  of 10 UI/mL GSH-Rd, 200.0  $\mu\text{L}$  of NADPH (2 mM), 40.0  $\mu\text{L}$  of GSH (0.1 M) (L-glutathione oxidised; SIGMA, St. Louis, USA), to 40  $\mu\text{L}$  of haemolysate. The mixture was agitated in a vortex mixer (Vortex AP-56; Phoenix, Araraquara, Brazil) for 10 s. Next, 20.0  $\mu\text{L}$  of T-Butyl hydroperoxide (7 mM) (SIGMA, St. Louis, USA) was added and the solution was maintained at 37 °C for 10 min. Absorbance was determined with a spectrophotometer at a wavelength of 340 nm. The GSH-Px activity was expressed in enzymatic activity units per gram of haemoglobin (UI/g Hb).<sup>24</sup>

## Statistical analysis

Data were analysed using the PASW v.17.0.2 software program (SPSS Inc., Chicago, USA). The analysis of SOD and GSH-Px enzymatic activities, as well as GSH-t and TBARS concentrations in sheep red cells, prior to and 30 min after lung biopsy, was performed using Wilcoxon's non-parametric test. The significance level was set at 5%.

## Ethical considerations

This study was approved by the Animal Experimentation Ethics Committee of Botucatu Veterinary and Animal Science School at the São Paulo State University, Brazil (Protocol No. 20/2007 – CEEA).

## Results

The SOD, GSH-t, GSH-Px and TBARS values found in sheep red cell haemolysates obtained prior to and 30 min after lung biopsy are shown in Table 1. Thirty minutes after biopsy,

there was a statistically significant reduction in the activities of SOD ( $p = 0.006$ ) and GSH-Px ( $p = 0.001$ ) in the red cells of the animals that underwent the procedure. No significant difference was observed in the red cell concentrations of GSH-t and TBARS after lung biopsy.

## Discussion

Oxidative stress occurs when there is a disturbance in the equilibrium between ROS production and the presence of antioxidant agents.<sup>20,21</sup> SOD is the first line of defence against oxidative stress and is active in catalysing  $\text{O}_2^{\bullet}$  dismutation to  $\text{H}_2\text{O}_2$  and oxygen. In this study, biopsy-induced  $\text{O}_2^{\bullet}$  release caused a reduction in SOD values 30 min after the procedure. Thus, as the neutralisation of the deleterious effects of this radical was attempted, SOD activity was affected.  $\text{H}_2\text{O}_2$  production is neutralised by the action of CAT and/or by a cascade of glutathione-mediated reactions, known as the glutathione reduction cycle. In this cycle, GSH-Px uses glutathione as an electron donor substrate and reduces  $\text{H}_2\text{O}_2$ , thus forming GSSG. After being oxidised, GSSG is recycled to GSH by GSH-Rd. The cycle of glutathione reduction is also the primary mechanism for the removal of lipoperoxides produced by lipid peroxidation.<sup>25,26</sup>

In this study, GSH-Px activity was affected by the variation in  $\text{H}_2\text{O}_2$  induced by SOD, indicating that the cycle of glutathione reduction was activated. However, despite the decreased GSH-Px activity, GSH-t remained unchanged after biopsy, demonstrating that biopsy-induced oxidative stress was suppressed by glutathione action. Because glutathione is most abundant in mammalian red cells in its reduced form and GSH was most probably consumed because of its antioxidant properties, the fact that GSH red cell concentrations remained high contributed to the maintenance of GSH-t levels even after lung biopsy. In addition, GSH-t values might have remained high as a result of a compensatory increase in GSH production by other organs, such as the liver, to help the organism fight against a rise in ROS production.<sup>27</sup>

Malondialdehyde (MDA) is a marker for lipid peroxidation used to measure TBARS. TBARS blood levels are associated with increased lipid peroxidation and oxidative damage.<sup>28</sup> This study revealed that percutaneous transthoracic lung biopsy did not induce lipid peroxidation, as confirmed by the fact that no changes in red cell TBARS levels were detected 30 min after biopsy.

**TABLE 1:** Superoxide dismutase, total glutathione, peroxidase glutathione and thiobarbituric acid reactive substances values in sheep red cells prior to and 30 min after lung biopsy.

Variables	Time point	Mean	Median	Standard deviation	p-value
SOD (UI/mg Hb)	T0 <sup>a</sup>	1841.778	1682.294	± 623.621	0.006
	T30 <sup>b</sup>	1195.937	1072.720	± 750.333	
GSH-t ( $\mu\text{M}/\text{g Hb}$ )	T0	33.895	29.108	± 15.325	0.940
	T30	33.124	28.443	± 13.703	
GSH-Px (UI/g Hb)	T0	0.819	0.745	± 0.397	0.001
	T30	0.510	0.376	± 0.491	
TBARS (nM/g Hb)	T0	262.167	252.925	± 110.510	0.135
	T30	221.978	213.024	± 77.370	

SOD, Superoxide dismutase; GSH-t, total glutathione; GSH-Px, peroxidase glutathione; TBARS, thiobarbituric acid reactive substances; Hb, haemoglobin.

<sup>a</sup>, Before the procedure as a control.

<sup>b</sup>, After the procedure to quantify changes occurring during the 30 min.

Local inflammatory responses are characterised by increased blood flow and vascular permeability, vessel dilation and accumulation of inflammatory cells. The cells primarily involved in the acute phase of inflammation are neutrophils, whilst monocytes and macrophages and some lymphocyte strains migrate to the inflammation site in the late phase. These cells produce ROS during the respiratory burst as a response to phagocytic stimuli. Moreover, several aspects of the inflammatory process are regulated by mediators such as cytokines, histamine, leukotrienes, prostaglandins and ROS as well,<sup>29</sup> which are produced by cells of the immune system and endothelial cells.

Commonly, the body's ability to defend itself against the action of ROS is assessed by measuring the activities of SOD, GSH-Px and CAT. Some authors suggest that the increase in cytokine production that occurs in the early inflammatory process is associated with impaired SOD, GSH-Px and CAT activities, which increase oxidative stress and local cell damage induced by increased cell apoptosis.<sup>30</sup> According to McIntyre et al.<sup>31</sup>, the extent of inflammatory events and consequent injuries is related directly to the intensity of the initial stimulus and the immune mechanisms which are activated during the process. The tissue aggression caused by the introduction of the biopsy needle triggered and exacerbated the inflammatory response and the release of chemical mediators, culminating in oxidative damage, reflected in the decreased SOD and GSH-Px levels observed 30 min after biopsy.

According to some authors,<sup>32,33</sup> the interpretation of the results of oxidative stress studies should consider other factors such as the type of marker used, the method employed, the type of tissue investigated and, above all, the time elapsed after biopsy at which the marker was assessed. In humans submitted to intense exercise, Panza<sup>34</sup> observed no change in TBARS blood levels up to 15 min after exercise, whereas Goldfarb et al.<sup>35</sup> found increased plasma MDA only 48 h after intense training. Therefore, the fact that no signs of lipid peroxidation were observed until 30 min after lung biopsy in this study does not rule out the possibility that it might have occurred at another time during recovery. The same can be inferred about antioxidant agents. Transient and biphasic changes in enzymatic activity may occur,<sup>36,37</sup> as evidenced by an initial decrease in the consumption of viable antioxidant enzymes followed by an increase induced by the mobilisation of stocks in other organs to compensate for the constant exposure to increased oxidative stress.

## Conclusion

Based on these grounds, it is possible that a serial, long-term evaluation of SOD and GSH-Px activities and GSH-t and TBARS concentrations could reveal changes compatible with more intense oxidative stress and lipid peroxidation still unseen by the end of the period studied here. The results obtained in the present study suggest that percutaneous transthoracic lung biopsy is a safe procedure that causes no significant changes in oxidative metabolism up to

30 min after testing and thus may be used widely in routine veterinary practice.

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## Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

## Authors' contributions

A.A.S. (São Paulo State University) was the investigator responsible for this project, which was part of her Masters dissertation. R.C.G. (São Paulo State University) was the advisor of A.A.S. (São Paulo State University) and intellectual mentor of the project through all of its phases. A.A.S. (São Paulo State University), R.C.G. (São Paulo State University) and D.C.D. (São Paulo State University) were responsible for the experiment design, data analysis and manuscript writing. A.A.S. (São Paulo State University), D.O.L.F. (São Paulo State University) and B.P.S. (São Paulo State University) collected samples and gathered the results. A.D. (São Paulo State University) supervised the analytic methodology and revised the statistics.

## References

- Cutilip RC, Brogden AK, Lehmkühl HD. Changes in the lungs of lambs after intratracheal injection of lipopolysaccharide from *Pasteurella haemolytica* A1. *J Comp Pathol.* 1998;118:163–167. [http://dx.doi.org/10.1016/S0021-9975\(98\)80009-1](http://dx.doi.org/10.1016/S0021-9975(98)80009-1)
- Martin WB. Respiratory infections of sheep. *Comp Immunol Microb.* 1996;19:171–179. [http://dx.doi.org/10.1016/0147-9571\(96\)00002-1](http://dx.doi.org/10.1016/0147-9571(96)00002-1)
- Stone EA. Biopsy: Principles, technical considerations and pitfalls. *Vet Clin North Am Small Anim Pract.* 1995;25:33–34. PMID:7709562
- Porto N. [Percutaneous lung biopsy]. In: Silva LCC, editor. *Compendium on pulmonary disease.* São Paulo: Fundação FyK, 2003; p. 274–276. Portuguese.
- Braun U, Estermann U, Flückiger M, Sydler T, Pospischil A. Ultrasound-guided percutaneous lung biopsy in sheep. *Vet Rec.* 2000;146:525–528. <http://dx.doi.org/10.1136/vr.146.18.525>, PMID:11321214
- Art T, Kirschvink N, Smith N, Lekeux P. Indices of oxidative stress in blood and pulmonary epithelium lining fluid in horses suffering from recurrent airway obstruction. *Equine Vet J.* 1999;31:397–401.
- Deaton CM, Marlin DJ, Smith NC, Harris PA, Schroter RC, Kelly FJ. Antioxidant supplementation in horses affected by recurrent airway obstruction. *J Nutr.* 2004;134:2065–2067.
- Kirschvink N, Smith N, Fiévez L, et al. Effect of chronic airway inflammation and exercise on pulmonary and systemic antioxidant status of healthy and heaves-affected horses. *Equine Vet J.* 2002;34:563–571. <http://dx.doi.org/10.2746/042516402776180223>
- Ledwozyw A, Stolarczyk H. The involvement of polymorphonuclear leucocytes in the pathogenesis of bronchopneumonia in calves. VI. Superoxide dismutase and lipoprotein lipase activities. *Acta Vet Hung.* 1992;40:267–277. PMID:1305794
- Comhair SAA, Xu W, Ghosh S, et al. Superoxide dismutase inactivation in pathophysiology of asthmatic airway remodeling and reactivity. *Am J Pathol.* 2005;166:663–674. [http://dx.doi.org/10.1016/S0002-9440\(10\)62288-2](http://dx.doi.org/10.1016/S0002-9440(10)62288-2)
- Slade R, Stead AG, Graham JA, Hatch GE. Comparison of lung antioxidant levels in humans and laboratory animals. *Am Rev Respir Dis.* 1985;131:742–746. PMID:4003918
- Forman JH, Fisher AB. Antioxidant enzymes of rat granular pneumocytes. *Lab Invest.* 1981;45:1–6. PMID:6894775

13. Ikeda T, Murata Y, Quilligan EJ, Parer JT, Doi S, Park S. Brain lipid peroxidation and antioxidant in fetal lambs 72 hours after asphyxia by partial umbilical cord occlusion. *Am J Obstet Gynecol*. 1998;178:474–478. [http://dx.doi.org/10.1016/S0002-9378\(98\)70423-5](http://dx.doi.org/10.1016/S0002-9378(98)70423-5)
14. Malhotra SP, Reddy VM, Thelitz S, He Y, McMullan DM, Reimer FK. The role of oxidative stress in the development of pulmonary arteriovenous malformations after cavopulmonary anastomosis. *J Thorac Cardiovasc Surg*. 2002;124:479–485. <http://dx.doi.org/10.1067/mtc.2002.120346>, PMID:12202863
15. Halliwell B, Gutteridge JMC. *Free radical in biology and medicine*. 3rd edn. New York: Oxford University Press; 1999.
16. Art T, Franck T, Lekeux P, et al. Myeloperoxidase concentration in bronchoalveolar lavage fluid from healthy horses and those with recurrent airway obstruction. *Can J Vet Res*. 2006;70:291–296. PMID:17042382
17. Rahman I, MacNee W. Oxidative stress and regulation of glutathione in lung inflammation. *Eur Respir J*. 2000;16:534–554. <http://dx.doi.org/10.1034/j.1399-3003.2000.016003534.x>, PMID:11028671
18. Rahman I, Biswas SK, Kode A. Oxidant and antioxidant balance in the airways and airway disease. *Eur J Pharmacol*. 2006;533:222–239. <http://dx.doi.org/10.1016/j.ejphar.2005.12.087>, PMID:16500642
19. Wright DT, Cohn LA, Hongfei L, Fischer B, Li CM, Aalder KB. Interactions of oxygen radicals with airway epithelium. *Environ Health Persp*. 1994;102:85–90.
20. Kirschvink N, Lekeux P. Oxidants and airway inflammations. *Proceedings of the 3rd World Equine Airways Symposium; 2005 July 22; Ithaca, New York, USA*. New York: WEAS; 2005.
21. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39:44–84. <http://dx.doi.org/10.1016/j.biocel.2006.07.001>
22. Finn-Bodner ST, Hathcock JT. Image-guided percutaneous needle biopsy: Ultrasound, computed tomography, and magnetic resonance imaging. *Semin Vet Med Surg*. 1993;8:258–278.
23. Braun U, Estermann U, Feige K, Sydler Y, Pospischil A. Percutaneous lung biopsy in cattle. *J Am Vet Med Assoc*. 1999;215:679–681. PMID:10476716
24. Souza MSS, Sinzato YK, Lima PHO, Calderon IMP, Rudge MVC, Damasceno DC. Oxidative stress status and lipid profiles of diabetic pregnant rats exposed to cigarette smoke. *Reprod Biomed Online*. 2010;20:547–552. <http://dx.doi.org/10.1016/j.rbmo.2010.01.002>, PMID:20153698
25. Kinnula VL, Crapo JD. Superoxide dismutase in the lung and human lung disease. *Am J Respir Crit Care Med*. 2003;167:1600–1619. <http://dx.doi.org/10.1164/rccm.200212-1479SO>, PMID:12796054
26. Kinnula VL. Focus on antioxidant enzymes and antioxidant strategies in smoking related airway disease. *Thorax*. 2005;60:693–700. <http://dx.doi.org/10.1136/thx.2004.037473>, PMID:16061713
27. Machefer G, Groussard C, Ranou-Bekono F, et al. Extreme running competition decrease blood antioxidant defense capacity. *J Am Coll Nutr*. 2004;23:358–364. PMID:15310740
28. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*. 1990;186:421–431. [http://dx.doi.org/10.1016/0076-6879\(90\)86135-1](http://dx.doi.org/10.1016/0076-6879(90)86135-1)
29. Huerrer MR, Gounon P. Inflammation: Patterns and new concepts. *Res Immunol*. 1996;147:417–434. [http://dx.doi.org/10.1016/S0923-2494\(97\)84407-0](http://dx.doi.org/10.1016/S0923-2494(97)84407-0)
30. Linke A, Adams V, Schulze PC, Erbs S, Gielen S, Fiehn E. Antioxidative effects of exercise training in patients with chronic heart failure increase in radical scavenger enzyme activity in skeletal muscle. *Circulation*. 2005;111:1763–1770.
31. McIntyre TM, Modur V, Prescott SM, Zimmerman GA. Molecular mechanisms of early inflammation. *J Thromb Haemost*. 1997;78:302–305. PMID:9198170
32. Volland NBJ, Shearman JP, Cooper CE. Exercise-induced oxidative stress: Myths, realities and physiological relevance. *Sports Med*. 2005;35:1045–1062. <http://dx.doi.org/10.2165/00007256-200535120-00004>, PMID:16336008
33. Witt EH, Reznick AZ, Viguie CA, Starke-Reed P, Packer L. Exercise, oxidative damage, and effects of antioxidant manipulation. *J Nutr*. 1992;122:766–773. PMID:1514950
34. Panza VSP, Wazlawik E, Schütz GR, Comim L, Hecht KC, Da Silva EL. Consumption of green teas favorably affects oxidative stress markers in weight-trained men. *Nutrition*. 2008;24:433–442. <http://dx.doi.org/10.1016/j.nut.2008.01.009>, PMID:18337059
35. Goldfarb AH, Bloomer RJ, McKenzie MJ. Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Med Sci Sports Exerc*. 2005;37:234–239. <http://dx.doi.org/10.1249/01.MSS.0000152887.87785.BE>
36. Denek SM, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol*. 1989;257:163–173.
37. Mills PC, Higgins AJ. Oxidant injury, nitric oxide and pulmonary vascular function: implications for the exercising horse. *Vet J*. 1997;135:125–148. [http://dx.doi.org/10.1016/S1090-0233\(97\)80034-2](http://dx.doi.org/10.1016/S1090-0233(97)80034-2)