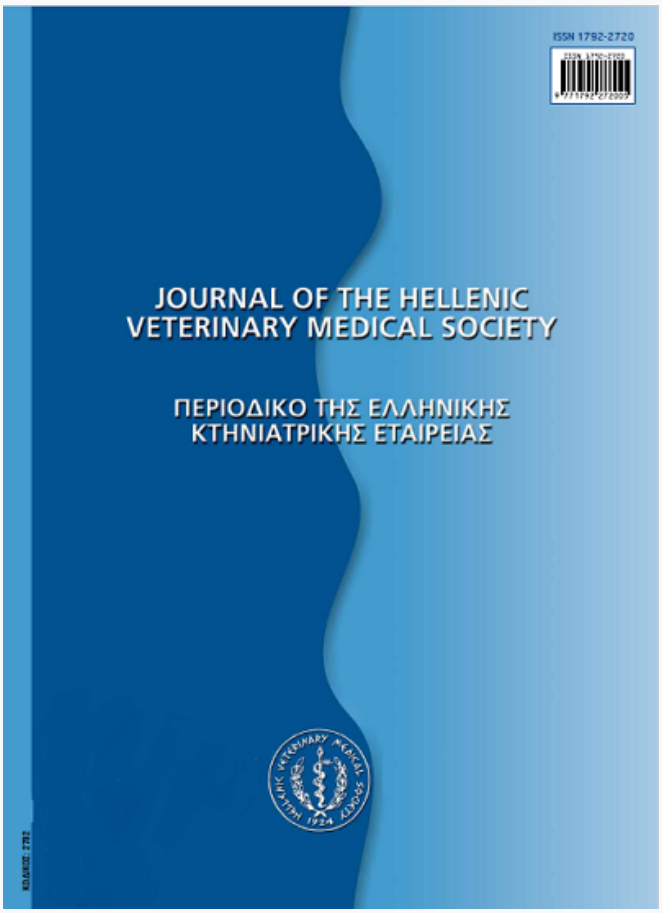
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 3 (2017)



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doi: 10.12681/jhvms.15544

To cite this article:

FIDAN, A. F., YENI, D., AVDATEK, F., ÖZÇINAR, Ü., & HAZMAN, Ö. (2018). Changes in oxidative stress markers during electro-ejaculation procedure in merino rams. *Journal of the Hellenic Veterinary Medical Society*, *68*(3), 479–486. https://doi.org/10.12681/jhvms.15544

J HELLENIC VET MED SOC 2017, 68(3): 479-486
ΠΕΚΕ 2017, 68(3): 479-486

Research article
Ερευνητικό άρθρο

Changes in oxidative stress markers during electro-ejaculation procedure in merino rams

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ABSTRACT. Electro-ejaculation is a technique used to collect semen from rams. The aim of the study was to evaluate the stress response and the oxidant/antioxidant levels during electro-ejaculation (EE) procedure in merino rams. To assess the effect of this technique, six 3-4 years old merino rams were subjected to semen collection by EE. The technique was applied one time in previously untreated animals. The studied parameters [Heart and respiratory rate, rectal temperature, white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb) levels, hematocrit (Hct) value, blood malondialdehyde (MDA) and glutathione (GSH) concentrations, plasma cortisol, nitric oxide (NO), glucose, cholesterol and triglyceride concentrations as well as plasma total antioxidant status (TAS) and total oxidant status (TOS)] were measured before and immediately after electrical stimulation. Heart and respiratory rate, WBC, MDA, glucose, cholesterol and triglyceride concentrations as well as plasma TOS and cortisol concentrations were dramatically increased after EE procedure, whereas rectal temperature, TAS and GSH concentrations were significantly decreased. These results demonstrate that MDA, GSH, TAS and TOS were the most powerful markers for evaluating the oxidant/antioxidant status in merino rams. Furthermore, EE procedure was a stressful situation leading to an oxidative stress, which can be amplified by increased glucocorticoid secretion.

Key words: Merino rams, electro-ejaculation, oxidative stress, physiological parameter, hematological parameters

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Date of initial submission: 10.10.2016
Date of revised submission: 17.11.2016
Date of acceptance: 29.11.2016

INTRODUCTION

Animals are exposed to a large number of stressors and the ability of the animal to fight against the stressors is important for maintenance of their health and productivity (Rahal et al., 2014). Suffering, pain and stress are being uncomfortable conditions for animals and animal welfare targets a life away from these. Currently, absence of stress response is accepted as an indicator for welfare in animals (Cingi et al., 2012). Stress has generally been defined as a reflex response that occurs unavoidable when animals are exposed to adverse environmental situations; furthermore, it is the cause of many inadequate outcomes, ranging from discomfort to death (Dantzer and Mormède 1983).

One of the most important and routine procedure for semen collection in veterinary medicine is electro-ejaculation (EE) (Whitlock et al., 2012). EE is the word generally used since 1936 to denote the obtaining of semen by electrical stimulation with electrodes in the rectum (Brindley, 1981). According to Damián and Ungerfeld, EE is a stressful technique that may cause pain and affects welfare in rams (Damián and Ungerfeld, 2011). The acute cortisol response is used to evaluate the animal well-being and nociceptive response to a particular stimulus in farm animals (Whitlock et al., 2012). The increase secretion of cortisol from the adrenal cortex in animals during EE procedures has been previously reported (Ortiz-de-Montellano et al., 2007; Damián and Ungerfeld, 2011). Cortisol is often used in stress and welfare assessments, and to measure acute pain (Ortiz-de-Montellano et al., 2007). Under normal circumstances, only ten percent of circulating cortisol is free and active. At body temperature, 90% of plasma cortisol is bound to proteins, of which 70% are corticosteroid binding globulins and albumin (Granger et al., 2009). However, the free cortisol concentrations have been reported to reach 20 to 30% during the stress response (Rijnberk and Mol, 1997).

Oxidative stress is defined as an imbalance between production of free radicals and reactive oxygen species (ROS). ROS are products of normal cellular metabolism and play vital roles in stimulation of signaling pathways in plant and animal cells in response to changes of intra and extracellular environmental conditions (Reuter at al. 2010). Under normal circumstances, the generated ROS are neutralized by the antioxidants present in the body; the generated ROS and the present antioxidants are in equilibrium. However, ROS overproduction or inadequate antioxidant defense affect this equilibrium and oxidative stress is favored by the ROS upsurge. These radicals often interact with various cellular components and macromolecules, and cause metabolic, structural, and functional damage that may lead to cell death (Fidan et al. 2009; Emecen et al. 2010).

The life and metabolic activity can be affected by the oxidative stress. Actually, ROS are produced as normal products of the cellular metabolism (Fidan et al., 2010). Oxidative stress occurs when the homeostatic processes fail and free radical generation is much beyond the capacity of the body's defenses, thus promoting cellular injury and tissue damage, leading to alterations of macromolecules, changes in intracellular calcium and intracellular pH, and finally to cell death (Cingi et al., 2012; Rahal et al., 2014); mainly throughout the free radical-mediated lipid attack called lipid peroxidation (LP). Since membrane phospholipids are the major targets of oxidative damage, LP is often the first parameter analyzed for proving the involvement of free radical damage; furthermore, the plasma MDA concentrations have been reported to directly correlate with the damage severity (Aleksandrovskii et al., 1988). Glutathione (GSH) plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events. Glutathione deficiency contributes to oxidative stress, and, therefore, may play a key role in aging and the pathogenesis of many diseases. Therefore, a decrease of the GSH concentrations may reflect depletion of the antioxidant reserve (Wu et al. 2004). Moreover, high glucocorticoid concentrations are known to decrease blood GSH and erythrocyte superoxide dismutase activity in rats (Orzechowski et al., 2000). Avci et al. (2008) reported that transport might induce oxidative stress via a decrease in GSH. Total antioxidant status (TAS) is a biochemical parameter suitable for evaluating the overall antioxidant status of serum and body fluids resulting from antioxidant intake and/ or production, and their consumption by increased levels of ROS production (Kayar at al. 2015). The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, giving an insight into the delicate balance between oxidants and antioxidants in vivo (Ghiselli et al. 2000; Kayar at al. 2015). When the oxidant/antioxidant balance is tilted towards oxidants and oxidative stress arises, there is a significant negative correlation between the TAS

and total oxidant status (TOS) values (Erel 2005). Nitric oxide (NOx) is formed from arginine by the action of NO-synthase (NOS) (Pryor et al. 2006). The role of NOx seems to be controversial. NOx is produced by constitutive NOS during vasodilating processes (eNOS) or during transmission of nerve impulses (nNOS). In the presence of stressors, NOx is produced by catalytic action of inducible NOS (iNOS) and is at higher concentrations. NOx can cause damage to proteins, lipids, and DNA either directly or after reaction with superoxide, leading to the formation of the very reactive peroxynitrite anion (Cingi et al., 2012; Rahal et al., 2014).

There was no published article investigating the effects of EE procedures on animal welfare using biomarkers of oxidative stress in merino rams. A new approach in determining the stress could be proposed by using the oxidative stress markers. Certain hematological, biochemical and clinical parameters, which have been proposed as useful stress indicators, were analyzed for this purpose. Therefore, the objective of this study was to evaluate the stress response and the oxidant/antioxidant levels during EE procedure in merino rams.

MATERIALS AND METHODS

The study was conducted on six, 3 to 4 years old, Merino Rams. Rams were maintained and managed at the Afyon Kocatepe University, Veterinary Faculty, Research and Application Farm (Turkey). Prior approval to conduct this study was obtained from the Animal Ethics Committee of Afyon Kocatepe University. The rams were fed a concentrate and alfalfa-based diet and had free access to water.

Semen samples from 6 rams were collected by AC-1 Electro-ejaculator (manufactured by Beltron Instruments, Longmont CO, USA). The EE procedure was applied one single time in previously untreated animals. A rectal probe with three ventrally oriented longitudinal electrodes (2,5 cm diameter and 25 cm length) was inserted following obstetrical lubricant application to both probe and anal sphincter to the rectum, and the rams were stimulated. The EE regime consisted of consecutive series of 5 sec pulses of similar voltage, each separated by 5 sec break. Each series consisted of a total of five pulses; the initial voltage was 1 V, which was increased in each series until a maximum of 9 V. At the end of the EE regime, 1-2 ml semen sample was collected by each ram. The EE procedure was carried out in a closed area, isolated from the area where the other rams were housed. Each animal was unable to see and hear the EE procedure of the other animals. After the procedure finished, the animals were allocated in another closed area.

Blood samples were collected from each animal, an hour before and just after accomplishment of the EE procedure, by puncture of the jugular vein, into heparinized and regular tubes for measuring white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) levels, hematocrit (Hct) value, total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NOx), Malondialdehyde (MDA), reduced glutathione (GSH), glucose, total cholesterol and triglyceride levels, as well as the plasma cortisol concentrations.

Rectal temperature, heart rate and respiratory rate were recorded an hour before EE, and just after EE. Heart rate was determined by auscultation and Respiratory rate by observation of abdominal-costal movements. Both parameters were recorded in all the animals by the same observers. The rectal temperature (RT) was determined with a conventional digital thermometer. Digital rectal thermometer was inserted about 2 cm into the rectum, and it remained until the audible beep was heard.

The WBC, RBC, Hct and Hb values were measured in whole blood by using an automatic blood count device (MS9-3, Melet Schloesing Laboratoires, Osny, France), within 6 h after sampling.

Blood MDA levels (or concentrations), an index of lipid peroxidation, were measured by the double heating method of Draper and Hardley (1990). Blood GSH concentration was measured as described by Beutler et al. (1963). Plasma TAS and TOS levels were determined using a commercially available kit (REL assay diagnostics, Mega Tip, Gaziantep, Turkey). Nitric oxide decomposes rapidly in aerated solutions to form stable nitrite/nitrate products. Plasma nitrite/nitrate concentration was measured by a modified method of Griess assay, described by Miranda et al. (2001). Glucose, total cholesterol and triglyceride levels were analyzed by using an auto-analyzer (Roche, Cobas C111, Basel, Switzerland). Plasma cortisol concentrations were determined using a Sheep cortisol ELISA kit (Sunred Biological Technology Co., Ltd, Catalogue No:201-07-0067, China).

Statistical analysis

All data for parametric variables are presented as mean ± Standard Error of Means (SEM). The comparisons of parameters were performed with the t-test. The variation factors were evaluated by the following formula: variation factor (%) = 100 [Ca–Cb] / Cb, where Ca and Cb were the concentrations observed after EE procedure and before EE procedure, respectively. Data were analyzed using the SPSS® for Windows computing program (Version 10.0), and $p<0.05$ was considered significant.

RESULTS

The heart and respiratory rates were significantly increased just after the EE procedure compared to the initial values ($p<0.05$, variation factor: 31,96% and 85,04% respectively), whereas the rectal temperature was significantly ($p<0.05$,variation factor: -0,99 %) decreased (Table 1).

The WBC levels and Hb concentrations were significantly increased just after the EE procedure compared to the baseline values ($p<0.05$, variation factor: 8,16 % and 6,70% respectively); whereas no significant difference was found in RBC levels and the Hct values (Table 2).

The biochemical markers before and after EE procedure are summarized in Table 3. The glucose, cholesterol, triglyceride and plasma cortisol concentrations were significantly and markedly increased ($p < 0.05$); the relevant variation factors were 22, 85 %, 15, 06 %, 19, 97%, 224, 01% respectively. Moreover, a remarkable and significant ($p < 0.05$) increase of the circulating MDA concentrations and TOS were observed after EE procedure compared to the initial values (factor of variation: 23, 64% and 44, 16% respectively). On the other hand, the blood GSH concentrations and TAS were significantly decreased by a factor of -5,47 and -23,64% respectively. The nitric oxide concentrations have not significantly differed.

DISCUSSION

Stress has been defined as a process of altered biochemical homeostasis produced by psychological, physiological, or environmental stressors (Rahal et al., 2014). The deterioration of biological functions caused by the stress can compromise animals' health state and life (Kagan and Levi, 1974; Moberg, 1987). When an animal is under stress, a stress response is initiated that involves first the sympathetic–adrenal-medulla axis resulting in catecholamine release and later the hypothalamic–pituitary–adrenal-cortex axis resulting in corticosteroid release. This response induces changes in hematological, biochemical, and clinical parameters, which have been proposed as useful stress indicators (Olvera et al., 2004). According to our knowledge, there is no report available on oxidative stress during EE procedure, but it has been shown that some other management procedures cause oxidative stress. Transport, dehorning and rectal palpation procedure has reported to induce significant increase in blood MDA concentrations, which indicate the occurrence of an oxidative stress in sheep and cattle (Avci et al., 2008; Fidan et al., 2010; Cingi et al., 2012).

Physiological parameters such as rectal temperature, heart and respiratory rates are known to be the

Table 1. Effect of electro-ejaculation on rectal temperature, heart and respiratory rates (Mean ±SEM)

Parameters	Before EE	After EE	Variation Factor (%)
Heart rate (beats/min.)	81,33±2,51	107,33±4,94*	31,96
Respiratory rate (cycles/min.)	35,66±1,4	66±7,26*	85,04
Rectal temperature (°C)	38,35±0,14	37,96±0,16*	-0,99

*$p < 0.05$. The variation factors were evaluated by the following formula: variation factor (%) = 100 [Ca – Cb] / Cb, where Ca and Cb were the concentrations observed after EE procedure and before EE procedure, respectively.

Table 2. Effect of electro-ejaculation on WBC, RBC, Hb and Htc values (Mean ±SEM)

Parameters	Before EE	After EE	Variation Factor (%)
White Blood Cell (mm^3)	8416,66±893,46	9104,16±694,51*	8,16
Red Blood Cell ($10^6/mm^3$)	11,21±0,48	11,45±0,41	2,18
Hemoglobin (g/dL)	11,93±0,32	12,73±0,25*	6,70
Hematocrit (%)	34,45±1,3	34,79±1,17	0,99

*$p < 0.05$. The variation factors were evaluated by the following formula: variation factor (%) = 100 [Ca – Cb] / Cb, where Ca and Cb were the concentrations observed after EE procedure and before EE procedure, respectively.

Table 3. Effect of electroejaculation on biochemical and oxidant/antioxidant parameters (Mean ±SEM)

Parameters	Before EE	After EE	Variation Factor (%)
Cortisol (ng/ml)	5,63±0,76	18,26±4,75*	224,01
Glucose (mg/dl)	55,8±3,19	68,55±3,43*	22,85
Cholesterol (mg/dl)	44±4,55	50,62±4,62*	15,06
Triglyceride (mg/dl)	18,19±1,88	21,83±1,64*	19,97
Malondialdehyde (mmol/ml)	3,13±0,14	3,87±0,23*	23,64
Reduced glutathione (mg/dl)	17,77±0,46	16,8±0,19*	-5,47
Nitric oxide (µmol/l)	5,73±0,51	6,16±0,27	7,44
Total oxidant status (µmol H_2O_2/l)	38,72±8,71	55,83±7,48*	44,16
Total antioxidant status (mmol Trolox equiv/l)	0,67±0,08	0,51±0,1*	-23,64

*$p < 0.05$. The variation factors were evaluated by the following formula: variation factor (%) = 100 [Ca – Cb] / Cb, where Ca and Cb were the concentrations observed after EE procedure and before EE procedure, respectively.

most relevant on-the-spot diagnostic parameters of the state of an animal's health. These parameters are of importance in evaluating the adaptability of domestic animals to various environmental stress factors (Minka and Ayo, 2009). In the present study respiratory and heart rates were significantly increased following EE procedure, as previously reported by Boussena et al. (2013). This increase in respiratory and heart rates could reflect pain responses to EE.

According to our knowledge, a few studies have been devoted to the influence of EE on rectal temperature (RT) in animals. In a study on rams, Lindsay (1969) reported that EE resulted in virtually no rise in rectal temperature. On the other hand, it has been reported that the RT decreased during EE in deer (Fumagalli et al., 2012) and in ram (Boussena et al., 2013). In our study, RT after the EE procedure was found significantly reduced, but still within the physiological norms, as reported by Boussena et al. (2013) in rams. Thus, it is suggested that the temperature is not really affected by this technique.

The first stage of the stress response is the activation of the sympathetic nervous system, in which the stimulation of adrenal medulla and the release of catecholamines occur. The spleen contraction that is caused by the action of catecholamines on -adrenergic receptors located in the capsule frequently leads to an increase in hemoglobin concentration and packed cell volume (Avci et al., 2008; Fidan at al. 2010). Damián and Ungerfeld (2011) observed that the Hct, Hb and RBC concentrations decreased 120 min after EE in ram, but WBC levels did not change. In the present study, WBC levels and Hb concentrations were significantly increased just after the EE procedure compared to the baseline values, whereas no significant difference found in RBC and the Hct value. These changes in WBC levels could be explained by the effects of catecholamines on spleen contraction during the EE procedure.

Glucose concentration increases through increased rate of glycogenolysis and gluconeogenesis associated with the increase in catecholamine and glucocorticoids, which are released during stressful situations (Shaw and Tume, 1992). In this study, glucose concentration increased during the EE procedure probably through an increase of cortisol secretion. Our results are consistent with the results of previous studies conducted on stressed animals (Damián and Ungerfeld, 2011; Cingi et al., 2012).

The effect of stress on serum cholesterol seems to be variable. Thus, plasma cholesterol concentrations are likely to increase following a stress episode; Olvera (2004) has also confirmed such an increase. However, as cholesterol also plays a role in corticoid synthesis, plasma cholesterol concentrations are also likely to decrease during a stress period (Peinado et al., 1993). Both these increased and decreased serum cholesterol and triglyceride concentrations have been found to be related to stress (Marco and Lavin, 1999). In this study, there was an increase in cholesterol concentrations following the EE procedure. Triglyceride concentrations were also significantly higher after EE, a finding which supports that of Marco and Lavín (1999) in red deer during capture and handling operations.

Ortiz-de-Montellano et al., (2007) reported that the EE procedure has induced strong elevations of cortisone concentrations, in Criollo goats. Damián and Ungerfeld (2011) have demonstrated that plasma cortisol concentrations increase after EE, reaching maximum concentrations at 20 min and returned to basal concentrations 60 min after EE in rams. Similarly, significant increases in plasma cortisol concentrations following the EE procedure were recorded in the present study. Our results suggested that EE itself would be a stressful condition.

In the present study, the EE procedure has induced strong elevations of blood MDA concentrations. This result could be explained by the occurrence of free radicals due to the stress factors during the EE procedure. On the other hand there was a decrease in GSH value following the EE procedure, which indicates the occurrence of an oxidative stress. In parallel, the increased cortisol secretion evidenced here would also contribute to the GSH deficiency. Furthermore, we used the measurement of TAS and TOS to evaluate the systemic effect of oxidative stress. In the present study, while TAS value was significantly decreased, the rectal EE procedure has induced strong elevation in TOS value, which indicates oxidative stress.

Although there is no report available on NOx levels during EE procedure, it has been shown that some other veterinary procedures, cause an increase of NOx levels (Fidan et al., 2010; Cingi et al., 2012). In the

present study, although the NOx levels were not significantly modified, a relative increase was observed.

CONCLUSIONS

Electro-ejaculation has been successfully used to collect semen in a wide variety of animals. However, our results show that, the EE procedure would be considered a stressful condition, as evidenced by the strong increase in circulating cortisol, MDA and TOS concentrations associated with decrease in TAS and GSH concentrations. Furthermore, increased plasma cortisol concentrations in response to EE could be an additional factor responsible for the oxidative stress, which led to an increase in TOS and MDA and a decrease in plasma TAS and GSH. A more comprehensive identification of the physiological changes during EE could be beneficial for further researches, in terms of accurate management in rams practices and industry. Moreover, the determination of the oxidative stress parameters could provide novel approaches for the evaluation of the stress in merino rams during EE.

ACNOWLEDGEMENTS

The summary of this scientific work was presented at the thirty-second world veterinary congress (2015, Istanbul, Turkey).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aleksandrovskii IA, Poıurovskii MV, Neznamov GG, Seredeniia SB, Krasova EA (1998) Lipid peroxidation in emotional stress and neurotic disorders. Zhurnal Nevropatologii i Psikhiatrii Imeni S.S. Korsakova 88(11): 95-101.

Avcı G, Kucukkurt I, Fidan AF, Eryavuz A, Aslan R, Dundar Y (2008) The influence of vitamin C and xylazine on cortisol, lipid peroxidation and some biochemical parameters in transported sheep. Fırat University Journal of Health Sciences (Veterinary) 22(3):147-152.

Beutler E, Dubon OB and Kelly M (1963) Improved method for the determination of blood glutathione. Lab Clin Med 61:882-8.

Boussena S, Bouaziz O, Dehimi L, Hireche S, Aimeur R, Kabouia R (2001) The effects of electroejaculation on some physiological parameters (rectal temperature,respiratory and cardiac rates) in OuledDjellal breed. Slovak J Anim Sci 46(1):16-21.

Brindley GS (1981) Electroejaculation: its technique, neurological implications and uses. J Neurol Neurosurg Psychiatry 44:9-18.

Cingi, C.C. Durmus FB, Yasemin SK, Fidan AF (2012) Stress Response in Dairy Cows Related to Rectal Examination. Acta Scientiae Veterinariae 40:3 1053.

Damián JP, Ungerfeld R (2011) The stress response of frequently electroejaculated rams to electroejaculation: hormonal, physiological, biochemical, haematological and behavioural parameters. Reprod DomestAnim 46: 646–650.

Dantzer R, Mormède P (1983) Stress in farm animals: A need for a reevaluation. J Anim Sci 57:1 6-18.

Draper HH, Hardley M (1990) Malondialdehyde determination as index of lipid peroxidation. Meth Enzymol 186: 421-431.

Emecen Ö, Berçik Inal B, Erdenen F, Usta M, Aral H, Güvenen G (2010) Evaluation of oxidant/antioxidant status and ECP levels in asthma. Turk J Med Sci 40(6): 889- 895. doi:10.3906/sag-0906-6

Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry 38:1103–1111.

Fidan AF, Küçükkurt İ, Eryavuz A, Ciğerci İH, Yardimci M, Ozdemır A (2009) Effects of shearing procedures on oxidant-antioxidant status in Chios sheep. Revue Méd Vét 160(7):349-355.

Fidan AF, Pamuk K, Ozdemir A, Saritas ZK, Tarakci U (2010) Effects of dehorning by amputation on oxidantantioxidant status in mature cattle. Revue Méd Vét 161(11):502-508.

Fumagalli F, Villagra´ N M, Damián J.P, Ungerfeld R (2012) Physiological and Biochemical Parameters in Response to Electroejaculation in Adult and Yearling Anesthetized Pampas Deer (Ozotocerosbezoarticus) Males. Reprod Domest Anim 47(2):308–312.

Ghiselli A, Serafini M, Natella F, Scaccini C (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med 29:1106–1114.

Granger DA, Hibel LC, Fortunato CK, Kapelewski CH (2009) Medication effects on salivary cortisol: tactics and strategy to minimize impact in behavioral and developmental science. Psychoneuroendocrinology 34(10):1437-48.

Kagan AR, Levi L (1974) Health and environment - psychosocial stimuli: a review. Social Science and Medicine 8:225-241.

Kayar A, Dokuzeylul B, Kandemir F M, Kirbas A, Bayrakal A, Or M E (2015) Total oxidant and antioxidant capacities, nitric oxide and malondialdehyde levels in cats seropositive for the feline coronavirus. Veterinarni Medicina 60(5):274–281

Lindsay RD (1969) Sexual activity and semen production of rams at high temperatures. J Reprod Fert 18:1-8.

Marco I, Lavin S (1999) Effect of the method of capture on the haematology and blood chemistry of red deer (Cervuselaphus). Res Vet Sci 66:81-84.

Minka NS, Ayo JO (2009) Physiological responses of food animals to road transportation stress. Afr J Biotechnol 8:7415-7427.

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5:62-71.

Moberg GP (1987) Problems in defining stress and distress in animals. J Am Vet Med Assoc 191(10):1207-1211.

Olvera JRL (2004) Capture, restraint and transport stress in Southern chamois (Rupicaprapyrenaica). Modulation with acepromazine and evaluation using physiological parameters. PhD Thesis; UniversitatAutònoma de Barcelona. Barcelona, Spain.

Ortiz-De-Montellano M, Galindo-Maldonado F, Cavazos-Arizpe EO, Aguayo-Arceo AM, Torres-Acosta JFJ, Orihuela A (2007) Effect of electro-ejaculation on the serum cortisol response of Criollo goats (Capra hircus). Small Rum Res 69:228–231.

Orzechowski A, Ostaszewski P, Brodnicka A, Wilczak J, Jank M, Balasińska B, Grzelkowska K, Ploszaj T, Olczak J, Mrówczyńska A (2000) Excess of glucocorticoids impairs whole-body antioxidant status in young rats-relation to the effect of dexamethasone in soleus muscle and spleen. Horm Metab Res 32;174-180.

Peinado V I, Fernandez-Aries A, Viscor G, Palomeque J (1993) Haematology of Spanish ibex (Capra pyrenaicahispanica) restrained by physical or chemical means. Vet Rec 132: 580-583.

Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, Squadrito GL, Davies KJ (2006) Free radical biology and medicine: it's a gas, man!. Am J Physiol Regul Integr Comp Physiol 291(3):491-511.

Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S and Dhama K (2014) Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. Bio Med Research International 1-19. doi:10.1155/2014/761264.

Reuter S, Subash CG, Madan MC, and Bharat BA (2010) Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med 49(11):1603–1616

Rijnberk AD, Mol JA (1997) Adrenocortical function. In: Clinical biochemistry of domestic Animals. 5th edition, Kaneko J.J., Harvey J.W. and Bruss M.L. (eds.), Academic Press Inc., San Diego, USA: pp 553-570.

Shaw DF, Tume KR (1992) The assessment of pre-slaughter treatments of livestock by measurement of plasma constituents: A review of recent work. Meat Sci 32:311-329.

Whitlock BK, Coffman EA, Coetzee JF, Daniel JA (2012) Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology 78:737–746.

Wu G, Fang YZ, Yang S, Lupton JR, Turner ND (2004). Glutathione Metabolism and Its Implications for Health. The Journal of nutrition 134:489-92.