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Dept. of Theriogenology,
Fac. Vet. Med., Suez Canal Univ., Ismailia Egypt.

RELATION BETWEEN OXIDATIVE STRESS AND RETAINED PLACENTA IN BUFFALOES

(With One Table)

By

S.A. ATALLAH and S.A. MOUSTAFA

Dept. of Zoology, Fac. Science, Suez Canal Univ., Ismailia Egypt.

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العلاقة بين إجهاد الاكسده واحتباس المشيمة في الجاموس

سيد عبد النعيم عطاالله ، سهير عبدالله مصطفى

أجريت هذه الدراسة على ٣٠جاموسه في مزرعة كلية الطب البيطري بالإسماعيلية – تراوح عمر هذه الحيوانات بين ٤–٨ سنوات. قسمت الحيوانات الى مجموعتين منها ١٥ جاموسه عانت من احتباس المشيمه مقابل ١٥ جاموسه أخرى كان نزول المشيمه فيها طبيعيا. شملت الدراسة أخذ عينات دم وكذا بعض فلقات المشيمه من هذه الحيوانات في نفس يوم الولادة. بالنسبة لفلقات المشيمه فقد تم تجميدها في النيتروجين السائل ثم حفظها عند درجة حرارة ٧٠ تحت الصفر لحين إجراء قياس مستوى الجلوتاثيون ونواتج الاكسده فوق الدهنيه. أما مصل الدم فقد تم حفظه عند درجة حرارة ٢٠ تحت الصفر لحين إجراء قياس مستوى كل من الاستراديول ١٧ب– البروجيستيرون– الكورتيزول– الكفاءة الكلية المضادة للاكسده. وقد أوضحت النتائج ما يلي: ١– وجود ارتفاع عالي المعنوية في مستوى الجلوتاثيون ونواتج الاكسده فوق الدهنيه في فلقات المشيمه في حالات احتباس المشيمه مقارنة بمثيلاتها في المجموعة الضابطة. ٢–في حالات احتباس المشيمه لوحظ ارتفاع عالي المعنوية في مستوى الكورتيزول وكذا الكفاءة الكلية المضادة للاكسده في مصل الدم مقارنة بمثيلاتها في المجموعة الضابطة. ٣– وجود نقص ملحوظ في مستوى الاستراديول ١٧ب في الحيوانات التي تعانى احتباس المشيمه مقارنة بمثيلاتها في المجموعة الضابطة. ٤– لم تجد الدراسة أية فروق معنوية في مستوى البروجيستيرون في الحالات التي تعانى احتباس المشيمه مقارنة بمثيلاتها في المجموعة الضابطة. توصي الدراسة بضرورة أن تكون تعدية الحيوان كافيه لاحتياجاته كماً و نوعاً في المراحل المختلفة من الحمل خاصة الأخيرة منها والاهتمام بإضافة مضادات الاكسده إلى الأعلاف مثل السيلينيوم وفيتامين هـ كإجراء وقائي ضد إجهاد الاكسده بهدف التغلب على العديد من الاضطرابات التناسلية المرتبطة بإجهاد الاكسده أثناء الحمل والولادة والنفاس.

SUMMARY

This study was carried out at the Animal Farm, Faculty of Veterinary Medicine in Ismailia and was aimed to investigate any association between

oxidative status and placental retention in buffaloes. The materials involved collection of blood and placental tissue (cotyledons) samples from 15 multiparous buffalo-cows with retained placenta matched with 15 cases of the same parity with normal placental drop as controls. Placental samples were homogenized and the levels of lipid peroxidation products as well as the activity of glutathione were determined. Blood samples were immediately centrifuged and the concentrations of oestradiol-17β, progesterone, cortisol and total antioxidant capacity (TAC) were measured. The results revealed that oxidative stress could be a mediator for retained placenta in buffaloes. High significant ($P<0.01$) levels of lipid peroxidation products and glutathione activity were recorded in the retained placental tissues compared to that of not retained cotyledons. Serum total antioxidant capacity and cortisol showed high significant ($P<0.01$) values in buffaloes with retained placenta compared with those of the controls. A high significant ($P<0.01$) decrease in the serum levels of oestradiol-17β was found in the diseased group compared with the control group.

Key words: *Stress, retained placenta, buffaloes*

INTRODUCTION

Retained placenta is one of the most important postpartum disorders constituting a major problem in dairy animals. It has a subsequent ill-effect on the postpartum fertility and milk production of the cow resulting in recognizable financial losses (Joosten *et al.*, 1988; Laven and Peters 1996). Although the etiology of retained placenta has been the subject of numerous studies, the exact cause is not clear (Joosten and Hansen, 1992; Wischral *et al.*, 2001).

According to Miller *et al.* (1993); Brzezinska-Slebodzinsk *et al.* (1994) and Kankofer (2001a), oxidative stress due to uncontrolled increase of reactive oxygen species (ROS) could be a risk factor for retained placenta in cattle. ROS has a negative influence on steroidgenic (Staats *et al.*, 1988), arachidogenic acid cascade, enzymes and NADPH/NADP ratio (Golden and Ramdath, 1987). Such alterations may modify the concentration of steroid hormones and prostaglandins that could result in disturbance in reproduction (Heuwieser and Grunert, 1987; Horta, 1988).

Unfortunately, there is a lack of information concerning involvement of ROS imbalance during retained placenta in buffalo. Therefore, the present study aimed to threw some light on 1) Levels of lipid peroxidation

products and glutathione activity in buffaloes with and without retained placental tissues. 2) Serum levels of total antioxidant capacity (TAC) of retained and control buffalo-cows. 3) Serum levels of estradiol-17β , progesterone and cortisol in buffaloes with and without retained fetal membranes.

MATERIALS and METHODS

Animals and sampling:

This study was conducted at the Animal Farm, Faculty of Veterinary Medicine in Ismailia. During the green season from January to the mid of May, the main feeding stuffs used were 20 kg berseem and 7 kg concentrates/head daily, compared to concentrates 6 kg, derris 3 kg, green maize stem 20 kg and rice straw 2 kg during the dry season from May 15th till the end of December. All animals in the farm were kept under veterinary supervision and vaccinated against some infectious diseases and were free from brucellosis and tuberculosis. The material in this work, involved 15 buffalo-cows with retained placenta matched with 15 animals showed a normal placental drop. The fetal membranes were considered retained when they had not been expelled within 12h post partum (Grunert, 1983). Within 2-3h after calving, sampling comprised placental tissue (cotyledons) and blood from these animals. Fetal cotyledons (one per buffalo-cow) were manually collected from gravid horn on ice, washed with 0.9% NaCl, frozen in liquid nitrogen then stored at -70°C till assayed. The remainder of fetal membranes was left *in situ* until they were released spontaneously within 12h after parturition or removed manually after 24h if retained. Blood samples were collected in plain clean centrifuge tubes and centrifuged after clot for collection of sera, then stored at -20°C until assayed.

Biochemical analysis

Placental samples were homogenized in 4 volumes of 0.15 M KCl on ice for 30 seconds at a speed of 10.000 rpm by using an all-glass Ten-Broeck homogenizer. The whole homogenate was used to determine GSH activity according to the method of Tieze (1969).

The level of lipid peroxidation was estimated by the thiobarbituric acid (TBA) test in placental tissue homogenates (10% w/v in cold distilled water) according to the method described by Uchiyama and Mihara (1978). Briefly, aliquot (0.5 ml) of the homogenate were mixed with 1.0% phosphoric acid (3 ml, pH 2.0) and 0.6% TBA (1 ml) in airtight tubes and were kept in boiling water bath for 45 minutes. The samples were cooled in ice and butanol (5 ml)

was added along with through mixing of the mixture. The butanol phase was separated by centrifugation (1000 g) and transferred to glass cuvettes. The color of the TBA chromogen was measured at 520 nm and 532 nm using a spectrophotometer (Bauch and Lomb, Spectronic-20). The difference between absorbance at 520 nm and at 532 nm gave the TBA value, which primarily represents the malonaldehyde concentration and was taken as the measure of lipid peroxidation (Yonaha *et al.*, 1980; Sohal, 1981).

The serum levels of cortisol, progesterone and oestradiol-17β were determined as described by Hasler *et al.* (1976), Xing *et al.* (1983) and Kubosik (1984) respectively using validated radioimmunoassay (*Diagnostic Products Corporation, Los Angles CA). Serum total antioxidant capacity (TAC) was also measured as mentioned by* Stocks *et al.* (1974).

Statistical analysis

The obtained results were statistically analyzed using Statistical Analysis System "SAS" (1987).

RESULTS

All data concerning serum levels of oestradiol-17β, progesterone, cortisol, total antioxidant capacity (TAC) and placental tissue antioxidants e.g. glutathione, lipid peroxidation are presented in Table 1.

Retained placenta buffalo-cows displayed high significant ($P < 0.01$) values concerning the activities of glutathione (0.25±0.12 mmol/L) and lipid peroxidation (0.20±0.07) compared to low values (0.13 ±0.05 mmol/L) and (0.09±0.02) respectively for buffaloes with normal placental drop.

A highly significant ($P < 0.01$) variation in the serum levels of oestradiol-17β between buffaloes with (258.4±32.7 pg/ml) and without (380±38.9 pg/ml) retained fetal membranes was recorded. Similarly, high significant ($P < 0.01$) differences were found in the concentration of cortisol in buffaloes with retained placenta (2.7±1.2 ng/ml) compared to that (1.4±0.4 ng/ml) of not retained cases. A highly significant ($P < 0.01$) increase in the total antioxidant capacity (TAC) was observed in the sera of retained placenta buffalo-cows (9338.0 ±2372.04 mmol/L) compared to those (6226.9 ±3568.3 mmol/L) that showed normal placental drop.

No significant differences in the concentrations of progesterone between retained (0.29±0.09 ng/ml) and not retained placenta (0.26±0.04 ng/ml) buffalo-cows were noticed.

Table 1: Serum levels of estradiol-17β, progesterone, cortisol, TAC and placental tissue antioxidants in buffaloes with and without retained placenta.

Placental drop	No.	Lipid peroxidation products	GSH (mmol/L)	TAC (mmol/L)	Estradiol-17β (Pg/ml)	Progesterone (ng/ml)	Cortisol (ng/ml)
Retained	15	0.20±0.07[a]	0.25±0.12[a]	9338.0 ±2372.04[a]	258.4±32.7[a]	0.29±0.09	2.7±1.2[a]
Normal	15	0.09±0.02[b]	0.13±0.05[b]	6226.9 ±3568.3[b]	380.0±38.9[b]	0.26±0.04	1.4±0.4[b]

Different superscripts (a-b) mean highly significant ($p<0.01$) differences.
GSH : glutathione.
TAC : total antioxidant capacity.

DISCUSSION

Retention of fetal membranes (RFM) is a postpartum pathological condition, the etiology of which is multifactorial and is not yet fully understood. Understanding the pathological mechanisms underlying this disorder is paramount to ultimately developing prevention and therapeutic intervention. It has been documented that RFM is associated with the presence of uncontrolled elevated levels of reactive oxygen species (ROS) which may disturb physiological processes leading to expulsion of the placenta (Kankofer, 2001 a, b). When free radical generation exceeds the body's antioxidant production capacity, oxidative stress develops (Roth, 2000). Living organisms are equipped with defense mechanisms against ROS consisting of non-enzymatic and enzymatic components able to neutralize them and terminate the deleterious consequences of their action (Miller *et al.*, 1993; Castillo *et al.*, 2003).

The potential source of free radical generation in RFM remains unknown. It has become increasingly evident that, rather than being independent, postpartum reproductive diseases such as retained placenta, endometritis and cystic ovaries are among a constellation of intercorelated syndromes of a postpartum disease complex (Correa *et al.*, 1993; Lewis, 1997). Since oxidative stress is the etiological factor in a number of reproductive diseases including pre-eclampsia and retained placenta, it is conceivable to assume that the source of oxidative stress could be common in these diseases. Hypoxia – re-oxygenation was recorded to be a potent inducer of apoptotic changes in human placenta initiating oxidative stress and a possible etiological factor in pre-eclampsia (Hung, *et al.*, 2002). Therefore, it is tempting to speculate that deficient trophoblast invasion of the endometrial arteries, leads to an ischemia-reperfusion type of insult which could be the source of oxidative stress and possibly etiological factor in RFM. Xanthine oxidase has been implicated in post-ischemic-reperfusion injury via the generation of superoxide anion radicals (superoxide; $O2^{\cdot-}$) and hydrogen peroxide (Winyard *et al.*, 1994). Supporting to the hypothesized role of ischemia - reperfusion type of insult in post partum diseases (including RFM) is the findings of Poston and Raijmakers (2004) indicating enhanced enzymatic synthesis of superoxide by xanthine oxidase and NAD(P)H oxidase in trophoblast oxidative stress in pre-eclampsia and miscarriage. In addition, aberrant expression of xanthine oxidase in

eutopic and ectopic endometrium was reported to play a pathologic role in endometriosis (Tanaka *et al.*, 2001).

In the current study oxidative stress in buffaloes affected with improper release of fetal membranes was confirmed not only by alterations in the levels of serum TAC and placental GSH but also by the elevation of placental lipid peroxidation products levels. These disturbances may induce alteration of activities of enzymes which are involved in hormone metabolism. The observed GSH depletion in animals with RFM should disturb the redox status of the cells since it induces alteration in NAD/NADH and GSH/GSSG ratios. It has been reported that maintenance of the redox potential of the cell is a crucial factor in maintaining the synthetic capacity of the cell (Hazelton and Lang, 1980 ; Balin and Allen, 1986). Therefore, it is natural to consider that the disturbance in the redox status in buffalos with RFM may lead to an impairment in the synthetic capacity of one or more of the enzymes and hormones (and /or their receptors) affecting the proper placental separation and expulsion e.g. oxytocin and estradiol-17β. Indeed, the current results reveal a decrease in estradiol-17β levels in RFM animals compared to normal ones. Previous studies (Heuwieser and Grunert, 1987; Horta, 1988) indicating alterations in the levels of steroid hormones and prostaglandins during the retention of fetal membranes in cows give an additional support to this view. The impairment in the synthetic capacity of the cell may also affect collagen synthesis. Collagenization of placental connective tissue is a basic step in the loosening process that precedes placental separation (Grunert, 1986). The negative impact of oxidative stress upon the synthetic capacity of the cell has multiple phases. In addition to its effect on the redox status of the cell it could induce DNA damage which should directly affect the protein manufacturing machinery of the cell. Actually, the study of Kankofer and Schmerold, (2002) has confirmed the existence of oxidative lesions in cellular DNA in retained bovine placentas.

Based on the evident susceptibility of animals with RFM to lipid peroxidation (Table 1), it is expected that fatty acid molecules involved in steroid hormones synthesis will be subjected to the peroxidative damage under these conditions. Unsaturated fatty acids, mainly linoleic and arachidonic acid were reported to be susceptible to peroxidative damage (Surya *et al.*, 1990). They are precursors of biologically active substances like prostaglandins that play an essential role in the periparturient period. Moreover, microsomes were reported to be one of

the most important targets of lipid peroxidation (Itoh *et al.*, 1989), therefore, it is conceivable to assume that enzyme systems linked to microsomes will be highly susceptible to peroxidative damage. Actually steroidogenic enzymes are cytochrome P_{450} dependent and were reported to be highly susceptible to the lipid peroxidation damage of microsomes (Miller *et al.*, 1993).

Normally increasing estrogen and decreasing progesterone activities at term lead to multiple subtle changes leading to an increased prostaglandin synthesis and mainly to a rise in oxytocin receptor concentration in the myometrium and the deciduas (Husslein, 1984). These basic changes are necessary for subsequent placental separation and expulsion (Husslein, 1984). Consequently, the unchanged level of progesterone and decreased levels of estrogen detected in retained placenta (Table 1) could be a major factor contributing to the pathogenesis of RFM in buffaloes.

In addition to the mechanical factors involved in the normal separation and expulsion of placenta which are mainly hormonal–dependent, research has shown that the incidence of retained placenta is higher in cows with impaired immune function (Kimura *et al.*, 2002). It has been proposed that placental tissue becomes a dead foreign body at the time of parturition, which the body must recognize and "reject" and that retained placentas are the result of the immune system failing to recognize the fetal membranes as a foreign body (Gunnink 1984a, b). Retained placenta also has been found to be strongly associated with reduced chemotaxis of leukocytes (Gunnink, 1984a). The etiology of this immunosuppression is also multifactorial. Oxidative stress could be implicated in this immunosuppession. Supporting to this view is that selenium and vitamin E have been reported to play an important role in lymphocyte (Pollock *et al.*, 1994) and neutrophil (Boyne and Arthur, 1979 & 1981; Smith *et al.*, 1997) functions. On a theoretical basis, therefore, one would expect selenium and vitamin E deficiencies to increase rates of RFM. Actually, selenium and vitamin E have been reported to have an effect on uterine motility, and this effect has been posited to play a role in the pathogenesis of RFM in deficient animals (Segerson *et al.*, 1980). Deficiency of selenium and vitamin E could directly be linked to the existence of oxidative stress. It is well established that GSH peroxidase enzyme (GSH-Px) is selenium dependent (Balin and Allen, 1986; Brzezinska-Slebodzinska *et al.*, 1994), it would, therefore, seem logical to assume that GSH depletion in RFM buffalos (Table 1) reflects an altered activity of GSH-Px. The

study of Kankofer (2001b) reporting an increased activity of GSH-Px in cows with RFM supports this notion. Indeed, supplementation of selenium and vitamin E; which is a very important endogenous antioxidant; has been reported to have positive responses in prevention of RFM (Campbell and Miller, 1998).

Immunosuppression mediating the pathogenesis of RP could be related to altered hormonal levels. Increased cortisol secretion (Table 1) could be one factor. Cortisol has a direct and strong immunosuppressive effect through its protein catabolic effect on the lymphoid tissue (Guyton, 1991). Interestingly, is the finding that corticotropin releasing hormone (CRH) acting via type 1 CRH receptors in human myometrial cells, exerts an effect on the NOS/cGMP system, increasing cNOS expression (Aggelidou *et al.,* 2002). Nitric oxide (NO) has been recently postulated as having a role in the complex molecular interplay which regulates myometrial function during gestation (Hertelendy and Zakar, 2004). Moreover, evidence exists that generation of NO occurs in uterine tissues of several species, including the rat (Ogando *et al.,* 2003), guinea-pig (Jobling *et al.,* 2004), rabbit (Roberts *et al.,* 1993) and sheep (Magness *et al.,* 2005).

Weeks (2001) proposed that RFM could be caused by the persistence of one of the placental inhibitory factors that are normally reduced prior to the onset of labor, possibly progesterone or nitric oxide. Actually Nitric oxide synthase activity in pregnant uterus decreases on the last day of pregnancy (Roberts *et al.,* 1993). Therefore, it could be proposed that the observed increase in cortisol levels in buffalo with RP (Table 1) may reflect an increase in CRH since stress breaks through the negative feedback inhibition of cortisol on the hypothalamic release of CRH (Guyton, 1991). Based on this hypothesis, the possible increase in CRF in these animals may cause an up regulation of NOS. The resultant increase in NO levels may not only inhibit uterine contraction but also it could be an underlying cause of the chemotactic inhibition in these animals. NO released by the endothelium has been shown to inhibit the surface expression of many endothelial adhesion molecules including P-selectin, E-selectin and leukocyte adhesion molecule-1, thus attenuating interaction between cell adhesion molecules on the endothelium and on leukocytes (Jones and Lefer 2000). This effect should result in decreased neutrophil sequestration and immunosuppression.

Interestingly, is the finding that progesterone from corpus luteum does stimulate secretion of molecules from the endometrium that block

lymphocyte proliferation (Joosteen and Hensen, 1992), thus the persistence of unchanged progesterone levels in buffalos with RFM (Table 1) could be a contributing factor to the expected immunosuppression in these animals.

When considered together, the current results seem to extend prior results documenting oxidative stress as an integral and possibly causative part of the pathogenesis of RFM, and to our knowledge this is the only study to date demonstrating this topic in buffalo. However, it is important to emphasize that a greater understanding of mechanisms of RFM may help set a new direction toward the development of therapeutic strategies that aim to interrupt the stress sensitive pathways mediating this disorder. Therefore, the impact of nitric oxide system on the maternal and fetal circulation, the expression of endothelial and leukocyte adhesion molecules, pro-inflammatory cytokines and xanthine oxidase in RFM in buffalo warrant further investigation. In addition, the overwhelming evidence for oxidative stress in the placenta and the maternal circulation in RFM has led to the suggestion that antioxidant prophylaxis may prevent oxidant stress and so ameliorate or prevent the disease.

REFERENCES

Aggelidou, E.; Hillhouse, E.W. and Grammatopoulos, D.K. (2002): Up-regulation of nitric oxide synthase and modulation of the guanylate cyclase activity by corticotropin-releasing hormone but not urocortin II or urocortin III in cultured human pregnant myometrial cells. Proc. Natl. Acad. Sci., USA., 99(5): 3300-5.

Balin, A. and Allen, R.G. (1986): Mechanisms of biologic aging. The aging skin, 4 (3): 347-358.

Boyne, R. and Arthur, J.R. (1979): Alterations of neutrophil function in selenium-deficient cattle. J. Comp. Path., 89: 151-158.

Boyne, R. and Arthur, J.R. (1981): Effects of selenium and copper deficiency on neutrophil function in cattle. J. Comp. Path., 91: 271-276.

Brzezinska-Slebodzinska, E.; Miller, J.K.; Quigley, J.D. and Moore, J.R. (1994): Antioxidant status of dairy cows supplemented pre-partum with vitamin E and selenium. J. Dairy Sci., 77 : 3087-3095.

Campbell, M.H. and Miller, J.K. (1998): Effect of supplemental dietary vitamin E and zinc on reproductive performance of dairy cows and heifers fed excess iron. J. Dairy Sci., 81: 2693-2699.

Castillo, C.; Benedto, J.L.; Lopez-Alonso, M.; Miranda, M. and Herandez, J. (2003): Values of plasma lipid peroxides and total antioxidant status in healthy dairy cows: preliminary observation . Achieves of Animal breeding 46: 227-233.

Correa, M.T.; Erb, H. and Scarlett, J. (1993): Path analysis for seven postpartum disorders of Holstein cows. J. Dairy Sci., 76 : 1305-1312.

Golden, M.H. and Ramdath, N. (1987): Free radicals in the pathogenesis of Kwashiorkor. Proc. Nutr. Soc., 46 : 53-60.

Grunert, E. (1983): Etiology, pathogenesis and treatment of placental retention in the cow. Wein. Tieraerztl. Monatsschr., 70 : 230 – 235.

Grunert, E. (1986): Etiology and pathogenesis of retained bovine placenta. In: Morrow, D.A. (ed.), Current Therapy in Theriogenology. 2nd edition. W.B. Saunders, Philadelphia, 237-242.

Gunnink, J.W. (1984a): Retained placenta and leukocyte activity. Vet. Quart., 6: 49 - 51.

Gunnink, J.W. (1984b): Pre-partum leucocytic activity and retained placenta. Vet. Quart., 6: 52 - 54.

Guyton, A.C. (1991): The adrenocortical hormones In: Text Book of Medical Physiology. Edited by Wonsiewicz, M.J. and Hallowell, R. Saunders, Philadelphia, PA. W.S., pp. 855-866 .

Hasler, M.J.; Painter, K. and Niswender, G.D. (1976): An 125I – labeled cortisol radioimmunoassay in which serum binding proteins are enzymatically denaturated. Clin. Chem. 22: 1850-1854.

Hazelton, G.A. and Lang, C.A. (1980): Glutathione contents of tissues in the aging mouse. Biochem. J., 188 : 25-30.

Hertelendy, F. and Zakar, T. (2004): Regulation of myometrial smooth muscle functions. Curr. Pharm., 10 (20) : 2499-517.

Heuwieser, W. and Grunert, E. (1987): Steroidhormonprofile in der Nachgeburts-phase beim Rind. Dtsch. Tierarzt. Wochenschr, 104: 13-14.

Horta, A.E.M. (1988): Prostaglandin F2α and prostacyclin imbalance in cows with placental retention : new findings. Ann. Resh. Vet., 17: 395 – 399.

Hung, T.H.; Skepper, J.N.; Jones, S.C. and Burton, J.I. (2002): Hypoxia, re-oxygenation. A potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. Circ. Res., 90: 1274-1293.

Husslein, P. (1984): The importance of oxytocin and prostaglandins to the mechanism of labor in humans. Wien. Klin. Wochenschr. Suppl., 155: 1-32.

Itoh, S.; Yamagishi, F. and Matsuyama, Y.Y. (1989): Relationship between liver microsomal function and serum thyroid hormones in rats treated with carbon tetrachloride. Res. Commun. Chem. Pathol. Pharmacol., 65: 111-114.

Jobling, P.; Gibbins, I.L.; Lewis, R.J.; Ozols, D.I. and Morris J.L. (2004): Differential involvement of N-type calcium channels in transmitter release from vasoconstrictor and vasodilator neurons. Br. J. Pharmacol., 141 (6): 961-970.

Jones, P. and Lefer, J.D. (2000): Myocardial reperfusion injury: Insights gained from gene-targeted mice. News Physiol. Sci., 15: 303-308.

Joosten, I. and Hensen, E.J. (1992): Retained placenta : an immunological approach. Anim. Reprod. Sci., 28: 451 – 461.

Joosten, I.; Stelwagen, J. and Dijkhuizen, A.A. (1988): Economic and reproductive consequences of retained placenta in dairy cattle. Vet. Rec., 123: 53 – 57.

Kankofer, M. (2001a): The levels of lipid peroxidation products in bovine retained and non retained placenta. Prostaglandins, Leukotrienes and Essential Fatty Acids, 64 (1): 33-36.

Kankofer, M. (2001b): Anti-oxidative defense mechanisms against reactive oxygen species in bovine retained and not- retained placenta: activity of glutathione peroxidase, glutathione transferase, catalase and superoxide dismutase. Placenta, 22 : 466-472.

Kankofer, M. and Schmerold, I. (2002): Spontaneous oxidative DNA damage in bovine retained and nonretained placental membranes. Theriogenology, 57: 1929-1938.

Kimura, K.; Goff, J.P.; Kehrli, M.E. and Reinhardt, T.A. (2002): Decreased neutrophil function as a cause of retained placenta in dairy cattle. J. Dairy Sci., 85: 544–550.

Kubosik, N.D. (1984): Evaluation of direct solid phase radioimmunoassay for progesterone. Clin. Chem., 30: 384. Cited in diagnostic products corporation pamphlet.

Laven, R.A. and Peters, A.R. (1996): Bovine retained placenta: etiology, pathogenesis and economic loss. Vet. Rec., 139: 465-471.

Lewis, G.S. (1997): Uterine health and disorders. J. Dairy Sci., 80: 984-994.

Magness, R.R.; Chen, D.B.; Lopez, G.; Phernetton, T.M.; Zangl and Byers, M.J.A. (2005): Endothelial vasodilator production by ovine uterine and systemic arteries: ovarian steroid and pregnancy control of ER alpha and ER beta levels. J. Physiol., 15: 85-99.

Miller, J.K.; Brzezinska-Slebodzinska, E. and Madsen, F.C. (1993): Oxidative stress, antioxidants and animal function. J. Dairy Sci., 76: 2812 – 2823.

Ogando, D.; Farina, M.; Ribeiro, M.L.; Perez Martinez, S.; Cella, M.; Rettori, V. and Franchi, A. (2003): Steroid hormones augment nitric oxide synthase activity and expression in rat uterus. Reprod. Fertil . Dev., 15 (5): 269-74.

Pollock, J.M.; McNair, J.; Kennedy, S.; Kennedy, D.G.; Walsh, D.M.; Goodall, E.A.; Mackie, D.P. and Crockard, A.D. (1994): Effects of dietary vitamin E and selenium on in vitro cellular responses in cattle. Res. Vet. Sci., 56: 100-107.

Poston, L. and Raijmakers, M.T. (2004): Trophoblast oxidative stress, antioxidants and pregnancy outcome, a review. Placenta, 25: 72-78.

Roberts J.M.; Lykins, D.; Regenstein, A.C. and Sladek, S.M. (1993): Nitric oxide synthase activity in pregnant rabbit uterus decreases on the last day of pregnancy. Am. J. Obstet. Gynecol., 169 (5): 1285-91.

Roth, E. (2000): Oxygen free radicals and their clinical implications . Acta Chirugica Hungarica, 36: 302-305.

SAS (1987): Statistics, Version 6.11. Cary, NC: SAS Institute Inc.

Segerson, E.C.; Riviere, G.; Bullock, T.R.; Thimaya, S. and Ganapathy, S.N. (1980): Uterine contractions and electrical activity in ewes treated with selenium and vitamin E. Biol. Reprod., 23: 1020-1028.

Smith, K.L.; Hogan, J.S. and Weiss, W.P. (1997): Dietary vitamin E and selenium affect mastitis and milk quality. J. Anim. Sci., 75: 1659-1665.

Sohal, R.S. (1981): Effect of age and metabolic rate of lipid peroxidation in housefly Musca Domistica. Mech. Ageing Dev., 16: 159-168.

Staats, D.A.; Lohr, D.P. and Colby, H.D. (1988): Effect of tocopherol depletion on the regional differences in adrenal microsomal lipid peroxidation and steroid metabolism. Endocrinol., 123: 975-980.

Stocks, J.; Gutteridge, J.M.; Sharp, R.J. and Dormandy, T.L. (1974): Assay using brain homogenate for measuring the antioxidant activity in biological fluids. Clin. Sci. Med., 47: 215-222.

Surya, P.P.; Das, U.N. and Koratkar, R. (1990): Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. Prostaglandins, Leukotrienes and Essential Fatty Acids, 41: 27-35.

Tanaka, T.; Igarashi, S. and Ota, H. (2001): Xanthine oxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil. Steril., 75 (4): 785-790.

Tieze, F. (1969): Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Applications to mammalian blood and other tissues. Anal. Biochem. 27: 502-522.

Uchiyami, M. and Mihara, M. (1978): Determination of malonaldehyde precursor in tissues by thiobarbaturic acid test. Anal. Biochem., 86: 271-278.

Weeks, A.D. (2001): The retained placenta. Afr. Health Sci., 1 (1): 36-41.

Winyard, P.J.; Moris, C.J.; Winrow, V.R.; Zaidi, M. and Blake, D.R. (1994): Free radical pathways in the inflammatory response. In Free Radical Damage and its Control, eds. C.A. Rice-Evans and R.H. Burdon, 361-379, Elsevier Science, B.V.

Wischral, A.; Verreschi, I.T.N.; Lima, S.B.; Hayashi, L.F. and Barnabe, R.C. (2001): Pre-parturition profile of steroids and prostaglandin in cows with and without fetal membrane retention. Anim. Reprod. Sci., 67: 181-188.

Xing, S.; Cekan, C.Z. and Diczfalusy, U. (1983): Validation of radioimmunoassy for oestradiol-17ß by isotope dilution mass spectrometry and by a test of radiochemical purity. Clin. Chem. Acta, 135: 189. Cited in diagnostic products corporation pamphlet.

Yonaha, M.; Itoh, I.; Ohbayashi, Y. and Uchiyama, M.M. (1980): Induction of lipid peroxidation in rats by mercuric chloride. Res. Commun. Chem. Pathol. Pharmacol., 28: 105-112.