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CHANGES IN LIPID PEROXIDE PRODUCTION AND ANTIOXIDANT ACTIVITIES IN CORPORA LUTEA AND IT'S RELATION TO SERUM PROGESTERONE LEVELS IN BUFFALO-COWS

(With 3 Tables)

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التغيرات في الأكسدة الفوقية للدهون ونشاط مضادات الأكسدة فى الأجسام الصفراء وعلاقتها بمستوي البروجيستيرون في سيرم إناث الجاموس

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في هذا البحث تمت دراسة التغيرات في كل من الأكسدة الفوقية للدهون وأكسيد النيتريك ونشاط السوبر أوكسيد ديسميوتيز ومستوي كل من الجلوتاثيون وفيتامين هـ كمضادات للأكسدة ، وكذلك مستوي الدهون والكولسترول في نسيج الأجسام الصفراء لإناث الجاموس . تم تجميع ثمانية وأربعون جسم أصفر من مبايض إناث الجاموس بعد ذبحها في المجزر في إناء حفظ به ثلج ونقلها مباشرة إلي المعمل ، حيث تم تصنيفها طبقا للتشريح الظاهري إلي ثلاثة مجموعات : أجسام صفراء نامية ، أجسام صفراء كاملة النمو وأجسام صفراء متحللة . وقد وجدت هذه الدراسة أن الأكسدة الفوقية للدهون قد زادت بينما أكسيد النيتريك قد نقص في الأجسام الصفراء المتحللة عن مثيلتها في كل من الأجسام الصفراء النامية وكاملة النمو. كذلك وجد أن الأكسدة الفوقية للدهون مرتبطة سلبيا بكل من مستوي البروجيستيرون في السيرم ونشاط السوبر أوكسيد ديسميوتيز في نسيج الأجسام الصفراء عامة وقد كان هذا الارتباط معنويا في كل من الأجسام الصفراء النامية والكاملة النمو . وقد لوحظ أيضاً أن نشاط السوبر أوكسيد ديسميوتيز ومستوي الجلوتاثيون في أنسجة الأجسام الصفراء الكاملة النمو أعلي معنويا عن مثيلتها في كل من الأجسام الصفراء النامية والمتحللة . وقد ارتبط نشاط السوبر أوكسيد ديسميوتيز في نسيج الأجسام الصفراء النامية وكاملة النمو إرتباطاً إيجابياً بمستوي البروجستيرون في السيرم. كذلك وجد أن مستوي فيتامين هـ في الأجسام الصفراء المتحللة أعلي من محتوي هذا الفيتامين في كل من الأجسام الصفراء النامية والكاملة النمو . وقد وجد أنه بالرغم من أن الدهون الكلية في نسيج الأجسام الصفراء المتحللة كان أعلي من محتوي هذه الدهون في نسيج الأجسام الصفراء النامية وكاملة النمو إلا أنه لوحظ عدم وجود تغير معنوي في مستوي الكولسترول في أي من هذه

الأنسجة . وقد أستنتج من هذه الدراسة أن التغيرات في الأكسدة الفوقية للدهون وأكسيد النيتريك ومستوي مضادات الأكسدة هي عمليه ديناميكية منتظمة قد تلعب دورا هاما في تنظيم وظيفة الجسم الأصفر في إناث الجاموس .

SUMMARY

The changes in lipid peroxide and nitric oxide productions, antioxidant activities (superoxide dismutase, glutathione and vitamin E) and total lipids were studied in corpora lutea and its relation to serum progesterone levels in buffalo-cows. A total of 48 corpora lutea were dissected from ovaries obtained from local abattoir and classified according to physico-anatomy to developing, fully developed and regressing corpora lutea. In regressing corpora lutea, although, the total LPO production was higher ,the NO production was lower than in developing or fully developed CLs. Moreover, LPO production was negatively correlated with both SOD and serum P_4 levels , which were significant in fully developed or in regressing CLs. Superoxide dismutase activity and glutathione content were significantly higher in fully developed corpora lutea. SOD activity was significantly positively correlated with serum P_4 levels in developing or fully developed CLs. Vitamin E content tend to be higher in regressing CL than in other stages of development of CL. However, a marked increase of total lipid content was observed in regressing CL than in developing or fully developed CL, the cholesterol levels was unchanged. It's concluded that changes in lipid peroxide and nitric oxide production and antioxidant activities is a dynamic and regulated process, which could play an important role in regulating luteal function during estrous cycle in buffalo-cows.

Key Words: *Buffalo-Cows-Corpus luteum-Lipid peroxide-Serum progesterone*

INTRODUCTION

The corpus luteum (CL), one of the biological clocks of the estrous cycle and pregnancy, is known for its responsibility for progesterone (P_4) production and early pregnancy maintenance in mammals. Although, the histo-morphological appearance of the CL during estrous cycle and pregnancy has been well characterized (Singh and Roy, 1995; 1996 ; Fields and Fields, 1996, and Singh et al., 1997), the regulatory factors that influence growth, hormone production and regression of the CL remain to be clarified.

Recent studies by Sawada and Carlson (1991; 1994) reported that reactive oxygen species (superoxide, nitric oxide and hydroxyl radicals) increased in the CL during regression phase, which had anti-gonadotrophic and anti-steroidgenic actions in rat luteal cells (Gatzuli et al., 1991; Helsa et al., 1992, and Shimamura et al., 1995). Lipid peroxide which is induced by reactive oxygen species (Hochstein and Jain, 1981) also increased during the regression of CL (Sawada and Carlson, 1991). It is well known that reactive oxygen species and their products lipid peroxide damage the cell membrane (Slater, 1984) by cross-linking in proteins and lipids and by formation of the gel-phase lipid (Pauls and Thompson, 1980).

Protection against reactive oxygen species provided by enzyme degradation (catalase, superoxide dismutase, glutathione peroxidase), scavenging by antioxidants (vitamin E, C and A; reduced glutathione) and molecular repair (Aten, et al., 1992). These antioxidants (enzymes, vitamins and reduced glutathione) are present in the ovary and may be hormonally regulated (Laloraya et al., 1988, and Aten et al., 1992). In addition, Sugino et al.(1993), and Sugino and Kato, (1994) reported that antioxidant activities in the CL change in a similar manner to serum progesterone concentrations and suggested an important role of these antioxidants in regulation of luteal function.

An *in vivo* examination of the role of reactive oxygen species in cellular events is severely compromised by extremely short half-lifes of these metabolites. One indirect approach frequently used to characterize free radicals mechanisms in physiological processes is to measure the highly specific inhibitor to these radicals (Helsa et al., 1992, and Shimamura et al., 1995). Although, most of recent studies in experimental animals (rat and rabbit) have been published are concerned with the ovarian action of oxygen free radicals and antioxidant activities in the mechanical process of ovulation (Miyazaki et al., 1991) and in CL during pregnancy and normal luteolysis (Sawada and Calrson, 1991, 1994, and Shimamura et al., 1995). Yet little information about these vital agents is available for growth and regression of CL during estrous cycle in bovine species (Ndikum-Moffor, et al., 1995). The present study was designed to clarify the changes in lipid peroxide and nitric oxide productions; antioxidants activities and its relation to serum progesterone levels during the natural lifespan of the corpus luteum in buffalo-cow.

MATERIAL and METHODS

The genital organs were collected at local abattoir immediately after slaughter from 48 buffalo-cows, whose reproductive organs showed no macroscopic abnormality. The animal's age, ranged from 5 -7 years. Before slaughter, peripheral blood sample for progesterone determination was collected from each animal by jugular vein puncture and centrifuged at 3000 g. for 10 min., the serum was removed and stored at - 20 C° until assay. Immediately after slaughter, the ovaries were collected and transported within 1 to 2 h. to the laboratory in container containing ice bags (4C°).

These corpora lutea were classified into 3 groups based on physico-anatomy, to: (I) developing, (ii) fully developed, and (iii) regressing cyclic corpora lutea (Okuda, et al., 1988). The corpus luteum was dissected from each ovary, cleaned from adhering tissue and weighed, then stored frozen at - 20 C° until used for further processing. A suitable portion of CL (10% w/v) was homogenized in ice cold 0.1 M phosphate buffer solution adjusted to PH 7.4 in a glass homogenizer and stored frozen at - 20 C° until used for biochemical assays. A portion of homogenate was centrifuged at 8000 r.p.m for 20 min. and the supernatant (cytosol) was collected and used for determination of superoxide dismutase (SOD), nitric oxide (NO) and Vit. E. The another portion of homogenate was divided to two aliquots, the first aliquot used for measuring lipid peroxide (LPO) and glutathione (GSH), and the second used for determination of total lipid and cholesterol.

Protein concentration in homogenate and cytosol was determined by the method of Lowry, et al. (1951) using a commercial chemicals (Sigma Chemical Co.) Lipid peroxide in corpora lutea was assessed by the thiobarbituric reaction, which measures the secondary product of peroxidation, malondialdehyde (MDA) (Ohkawa, et al., 1979). The amount of LPO was expressed as nmol MDA/ mg protein. Nitric oxide activity was measured using Gries reagent with sodium nitrite as a standard according to Ding, et al., (1988), and the activity was expressed as nmol/ mg protein. Total SOD activity in the CL was measured by using the method describing by Misra and Fridovich (1972), based on the ability of SOD to inhibit the autoxidation of epinephrine at PH 10.2. Total SOD activity was expressed as ng / mg protein. The glutathione content of the CL was determined according to the method of Beutler, et al., (1963) and the amount of GSH was expressed as a µg / mg protein. Vit. E content of the CL was assessed by using Emmerie-Engle reaction (Roe, 1961) based on the reduction of

ferric to ferrous ions by tocopherol (Vit. E), then the ferrous ions form a red complex with α α dipyridyl. The amount of vit. E was expressed as μg / mg protein. Lipids was extracted according to method describing by Bligh and Dyer, (1939). The total lipids was determined by the method of Knight et al., (1972), and cholesterol content was measured using Cholesterol Kit (Diamond Diagnostics, Egypt). The amounts of total lipids and cholesterol were expressed as mg / g. wet tissue weigh. Serum P_4 concentration were determined by RIA method (Coat A- Count progesterone, Diagnostic Products Co. Los Angeles, U.S.A.).

Data were analyzed by analysis of variance and the new Duncan's multiple rang test. Differences were considered to be significant if $p < 0.05$. Pearson Correlation Coefficient was used to determine the relationship between serum P_4 concentrations and weight of CL as well as some biochemical constituents of corpora lutea in buffalo-cows.

RESULTS

The mean levels of some biochemical constituents of CL homogenate are presented in table 1. Although, the LPO contents gradually increased in different stages of development of cyclic corpora lutea, with significantly ($p < 0.05$) higher at regression stage of corpus luteum. There is a gradual decrease in NO production in CL with significantly ($p < 0.05$) higher in developing CLs than other stages of development of CL (Table 1). LPO levels showed negative correlation with SOD activities and serum P_4 concentrations at different stages of development of CLs, whereas, these correlations ($r = -0.4115$; $r = -0.5244$ and $r = -0.8325$; $r = -0.7644$) were highly significant ($p < 0.01$) for fully developed and regressing CL, respectively (Table 2).

The SOD activity and GSH content in CL homogenate were significantly ($p < 0.05$) higher in developed CL than in other stages of development of CLs (table 1). In contrast to this increase in SOD activities in developing and fully developed CL than regressing CL, the SOD activity showed a significant positive correlation with serum P_4 concentrations ($p < 0.05$, $r = 0.3328$ and $p < 0.01$, $r = 0.7710$), respectively (Table 2). The Vit. E content of CL homogenate tend to increase in regressing CL than in developing and fully developed corpora lutea, but this difference was not significant (Table 1).

The total lipid content in CL homogenate was higher (24.63±2.10) for regressing CL than (13.75 ± 1.42 and 16.15 ±2.52) for developing or

fully developed CLs, respectively, this difference was significant ($p < 0.05$) (Table 1). The average cholesterol contents of CLs homogenates were 2.95±0.85, 3.28±1.02 and 2.67±1.04 for developing, developed and regressing CLs, and these differences were not significant (Table 1).

The average weight of corpora lutea changed in parallel to serum P_4 concentrations, which significantly ($p < 0.05$) increased for fully developed CL than developing or regressing Cls (Table 3). In contrast to this change, the weight of CL showed highly significant ($p < 0.01$) positive correlation's ($r = 0.9852$; $r = 0.9394$) with serum P_4 concentrations in developing or fully developed Cls (Table 3).

DISCUSSION

The corpus luteum (CL) is a transient endocrine gland with an essential role in normal reproductive function (Niswender, et al.,1994). Knowledge of physiological and biochemical aspects of this gland during estrous cycle is of vital importance to understand the exact mechanisms controlling structural and functional development and regression of this very dynamic endocrine structure and improve the reproductive efficiency in bovine species.

To our knowledge, there is little comparable information about the free radicals and it's products as well as antioxidant activities in corpora lutea in bovine species. Therefore, the results of the present study were compared with the findings of previous studies on experimental animals. The present study showed that LPO and NO productions, antioxidant activities and total lipids contents are subject to marked changes during the course of development of CL in buffalo-cow. These data are consistent with previous findings during natural luteolysis of the CL in experimental animals (Aten, et al., 1992; Hesla, et al., 1992; Sugino, et al., 1993, and Shimamura, et al., 1995).

The present study revealed that LPO production by CL gradually increase during the course of development of CL in buffalo-cow. This marked increase in LPO production by regressing CL coincide with decrease in serum P_4 concentrations. These data are consistent with the previous findings that superoxide radical and lipid peroxidation which stimulate prostaglandins biosynthesis (Hemler, et al., 1979, and Aten, et al., 1992) increasing during regression phase of CL (Sawada and Carlson, 1989). Lipid peroxidation induced by superoxide radical (Fridovich, 1986) inhibited the luteinizing hormone-dependent component of steriodogenesis (Behrman and

Aten, 1991). The results of the present study also indicated that serum P_4 concentrations decreased in regression stage of CL. Moreover, during luteolysis, the corpus luteum undergo extensive tissue remodeling (Juengel, et al., 1994) which was regulated by the variations in the levels of metaloproteinases and their inhibitors. The luteal cells undergoing apopptosis becoming detached from extracellular matrix and begin to shrink (Kerr, et al., 1972, and Young et al., 1997). This lead to loss of positive interactions between luteal cells which cooperate to obtain peak P_4 secretion during luteal phase (Hansel, et al., 1991). It is not fully understood, however, why serum P_4 values were high, in spite of the increase of LPO production by fully developed corpus luteum. Similar finding was reported by Shimamura, et al.(1995) in mature CL during pseudopregnancy in rats. The inhibitory effect of LPO on P_4 production may be blocked by some factors in fully developed CL. Previous work by Sawada and Carlson (1994) found that P_4 secretion was increased by LH in spite of increase of superoxide radical generation by fully developed CL. In addition, the amount of LPO may be not enough to suppress the luteal function during mid luteal phase (Shimamura, et al., 1995). Moreover, the present study indicated also an increasing of superoxide dismutase (SOD) activity which is scavenges superoxide radical in developing CL or fully developed CL..

In the present study, the NO production was higher in developing CL and fully developed CL than in regressing CL. Previous work by Gospodarowicz and Thakral, (1978). reported that CL formation is accompanied by intense capillaries angiogenesis and a large increase in luteal blood flow. However, the regression of CL is accompanied by degeneration of luteal capillaries and decrease in blood flow (O'Shea, et al., 1977). The nitric oxide (endothelial-deived relaxing factor) is produced by endothelial cells lining the luteal vasculature which is an important mediator of vasodilator responses induced by several pharmacological agents (Ward and Peters, 1995). In addition, the later authors also reported that NO can react with superoxide (both are free radicals) to form nitrate (non-radical product), therefore, variation in production of NO and superoxide radical by endothelial cells lined the luteal vasculature may provide one mechanism that regulate vascular tone of corpus luteum. In contrast to the previous suggestion, the present study found that indirectly (level of LPO production) the level of superoxide radical production was minimum in developing CL and fully developed CL and reach maximum level in regressing CL. Moreover, Anthony et al., (1997) mentioned that the catalytic

activity of nitric oxide synthase (specific enzyme for catalyzing NO) increased in regression stage of CL.

Superoxide dismutase (SOD), the specific inhibitor of superoxide radical, has been isolated in several forms , which differ in their transition at the active center in their cell location (Fridovich, 1986). The result of this study demonstrated the existence of a dependent relationship between SOD activity, LPO production and serum P_4 levels in different stages of development of CL. The significant decline in SOD activity in regressing CL was matched by fall in serum P_4 levels as well as increase in LPO production. This coincide with previous findings in pseudopregnant rabbit (Hesla et al., 1992) or rats (Shimamura, et al., 1995). Oxygen free radicals created during mitochondrial electron-transport system and cytochrome P450 (Weiss, 1986) which may cause damage and decrease steriodogenesis if not detoxified by SOD and other scavenges. Moreover, Sugino, et al., (1993) reported that SOD, in the presence of catalse, apparently blocks the antisteriodogenic effect of reactive oxygen species.

Glutathione protects cells against oxidative damage and prolonging the biologic life of polyunsaturated fatty acids which are important membrane consituents. Nearly all glutathione being present as reduced glutathione with less than 5 % of the total as oxidized glutathione (Ward and Peters, 1995). The result of the present study indicated an increasing of GSH contents especially in fully developed CL than in developing CL or in regressing CL. This coincide with Shimamura et al., (1995) who found that increasing in GSH peroxide and SOD activities would have reduced reactive oxygen species and contributed to the maintenance of luteal function during mid-luteal phase. Moreover, GSH peroxide requires reduced glutathione (GSH) as co-enzyme (Atroshi, et al., 1986).

Aten, et al., (1992) showed an accumulation of ovarian vitamin E during luteal regression and after LH administered in the mid-luteal phase. Since luteal regression results in a time-dependent increase in LH secretion (Soodak, et al. 1988), the marked rise in vitamin E levels seen with natural luteal regression was most likely to an increase endogenous LH secretion. The results of this study indicated that vitamin E content tend to be higher in regressing CL than other stages of development of CL. The mechanism of LH simulation of vitamin E accumulation is not known, but may due to increase lipoprotein accumulation by the corpus luteum. Vitamin E is transported by lipoprotein in plasma (Halliwell and Gutteridge, 1989), LH is known to stimulate the accumulation of lipoprotein by the rat corpus luteum (Rajkumar, et al., 1985). The present study also demonstrated the increasing

in the total lipid content in regressing CL than in developing CL or fully developed CL. In addition, Fields and Fields (1996) reported that the presence of lipid droplets in luteal cells is a sign of impeding luteal regression in which the cells has a reduced capacity to convert cholesterol estres to steriod hormone.

In the present study, a significant correlation between the weight of CL and serum P_4 levels during luteal growth and regression was observed in buffalo-cows. This is in agreement with that reported by Assey et al., (1993) who found that CL size correlated with its ability to secrete P_4. This corelation between weights of CLs and serum P_4 levels was not significant in regressing CL, apparently due to delay in physical decrease in CL weight. Previous work by Assey et al., (1993) reported a non-significant low corelation between CL size and serum P_4 levels and found that at the onset of luteal regression, there is a decline in serum P_4 levels followed by loss of luteal tissue.

In conclusion, the present study indicated that LPO, NO and antioxidant play an important role in regulating luteal function during estrous cycle in buffalo-cows. The antioxidant activities of the CL which offers main way for protection against reactive oxygen species, is consequently not a static but a dynamic and regulated process. These findings are considered important not only from a physiological standpoint in illuminating the regulation of normal luteal function, but from a pathological standpoint in showing that exssive production of free oxygen radicals and/ or subnormal accumulation of antioxidant may lead to abnormal luteal function. With respect to the problem of a dysfunctional corpus luteum, the elucidation of potentially important in the levels of reactive oxygen species and antioxidant activities during estrous cycle and pregnancy can be of possible clinical importance.

REFERENCES

Anthony, F.W.; Gornall, R.J.; Lincoln, J. Richardson, M.C.; Hitchcock, A. and Stones, R.W. (1997): Expression of endothelial nitric oxide synthase in human corpus luteum. Abstract No. 168, J Reprod. Fertil. (19): 63.

Assey, R.J.; Purwantara, B.; Greve, T.; Hyttel, P. and Schmidt, M.H. (1993): Corpus luteum size and plasma progesterone levels ion cattle after cloprostenol-induced luteolysis. Theriogenology (39): 1321-1330.

Aten, R.F.; Duarte, K.M. and Behrman, H.R. (1992): Regulation of ovarian antioxidant vitamins, reduced glutathione and lipid peroxidation by lutenizing hormone and prostaglandin $F_{2\alpha}$. Biol. Repod (46): 401-407.

Atroshi, F.A; Parantaninen, J.; Sankari, S. and Osterman, T. (1986): Prostaglandins and glutathione peroxidase in bovine mastitis. Res. Vet. Sci. (40): 361-366.

Behrman, H.R. and Aten, J.D. (1991): Evidence that hydrogen peroxide blocks hormone sensitive cholesterol transport into mitochondria of rat luteal cells. Endocrinology (124): 2895-2900.

Beutler, E.; Duron, O. and Kelly, B.M. (1963): Improved method for the determination of blood glutathione. J.Lab. Clin. Med. (61): 882-888.

Bligh, E.G. and Dyer, W.J. (1939): A rapid method of total lipid extraction and purification. Cand. J. Biochem. Phys. (37): 911-917.

Ding, A.T.T.; Hathan, C.F. and Stuehr, D.J. (1988): Release of reactive nitrogen intermediates and reactive oxygen intermediates from peritoneal macrophages. J. Immunol. (41): 2407-2412.

Fields, M. J. and Fields, P.A. (1996): Morphological characteristics of the bovine corpus luteum during the estrous cycle and pregnancy. Theriogenology (45): 1295-1325.

Fridovich, I. (1986): Biological effects of the superoxide radical. Archives of Biochemistry and Biophysics (247): 1-11.

Gatzuli, E.; Alter, R.F. and Behrman, H.R. (1991): Inhibition of gonadotropin action and progesterone synthesis by xanthine oxidase in rat luteal cells. Endocrinology (128): 2253-2258.

Gospodarowicz, D. and Thakral, K.K. (1978): Production of a corpus luteum angiogenic factor responsible for proliferation of capillaries and neovasclarization of the corpus luteum. Proc. Natl. Acad. Sci. USA. (75): 847-851.

Halliwell, B. and Gutteridge, J.M.C. (1989): Free radicals in biology and medicine. 2 nd ed., pp. 237-245, Oxford:Clarendon.

Hansel, W.; Alillie, H.W.; Dowd, J.P. and Milvae, R.A. (1991): Differential origin and control mechanisms in small and large bovine luteal cells. J. Reprod. Fertil. (Suppl) (43): 77-89.

Hemler, M.E.: Cook, H.W. and Lands, W.F.M. (1979): Prostaglandin biosynthesis can triggered by lipid peroxides. Archives of Biochemistry and Biophysics. (193): 340-345.

Hesla, J.S.; Miyazai, T. Dasko, L.M.; Wallach, E.E. and Dharmarajan, A.M. (1992): Superoxide dismutase activity, lipid peroxide production and corpus luteum steriodogenesis during natural luteolysis and regression induced by estradiol deprivation of the ovary in pseudopregnant rabbits. J. Reprod. Fertil. (95): 915-924.

Hochstein, P. and Jain ,S.K. (1981): Association of lipid peroxidation and polymerization of membrane proteins with erythrocyte aging. Federation Proceedings (40): 183-188.

Juengel, J.L.; Smith, G.W.: Smith, M.F.; Youngquist, R.S. and Garverick, H.A. (1994): Pattern of protein production by bovine corpora lutea during luteolysis and characterization of expression of two major products of regressing corpora lutea. J.Reprod. Fertil. (100): 515-520.

Kerr, J.F.R. Wyllie, A.H. and Gurrie, A.R. (1972): Apoptosis: A basic Biological phenomenon with wide-ranging implication in tissue kinetics. Br. J. of Cancer. (26): 239-257.

Knight, J.A.; Anderson, S. and Jams, M.R. (1972): Chemical basis of the sulphosphvonillin reaction for estimating total lipids. J. Clin. Chem. (18): 199-204.

Laloraya, M.; Kumar, G.P. Laloraya, M.M. (1988): Changes in the levels of superoxide anion radical and superoxide dismutase during the estrous cycle of Rattus noreegicus and induction of superoxide dismutase in rat ovary by lutopin. Biochem. Biophys. Res. Comm. (157): 146-153.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with the folin phenol reagents. J. Biol. Chem. (193): 265-275.

Misra, H.P. and Fridovich, I. (1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. (247): 3170-3175.

Miyazaki, T.; Sueoka, K.; Dharmarajan, A,M.; Atlas, S.J.; Bulkley, G.B. and Wallach, E.E. (1991): Effect of inhibition of oxygen free radical on ovulation and progesterone production by in vitro perfused rabbit ovary. J. Reprod. Fertil. (91): 207-212.

Ndikum-Moffar, F.; Simmen, R.C.M.; Chang, S.M.T. and Fields, M.T. (1995): Secretary proteins of the bovine corpus luteum during estrous cycle and pregnancy. Biol. Reprod..suppl 1, (52): 110-115.

Niswender, G.D.: Juengel, J.L.; Mcguire, W.J.; Belfiore, G.J. and Wiltbank, M.C. (1994): Luteal function: the estrous cycle and early pregnancy. Biol. Reprod. (50): 239-247.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Analytical Biochem. (95): 351-358.

Okuda, K.; Kito, S.; Sumi, N. and Sato, K. (1988): A study of the central cavity in the bovine corpus luteum. Vet. Rec. (123): 180-183.

O"shea, J.D.: Nightingale, M.G. and Chamley, W.A. (1977): Changes in small blood vessels during cyclical luteal regression in sheep. Biol. Reprod. (17): 162-177.

Pauls, K.P. and Thompson, J.E. (1980): In vitro stimulation of senescence-related membrane damage by ozone-induced lipid peroxidation. Nature (383): 504-506.

Rajkumar, K.; Couture, R.L. and Murphy, B.D. (1985): Binding of high-density lipoproteins to luteal membranes. the role of protein, luteinizing hormone and circulating lipoproteins. Biol. Reprod. (32): 546-555.

Roe, J.H. (1961): Standard methods of clinical chemistry, Vol. III Seligson D (ed), p. 35, Acad press, New York.

Sawada, M. and Carlson, J.C. (1989): Superoxide radical production in plasma membrane samples from regressing rat corpora lutea. Cand. J. Physiol. and pharmac. (67): 465-471.

Sawada, M. and Carlson, J.C. (1991): Rapid plasma membrane changes in superoxide radical formation, fluidity and phospholipase A_2 activity in the corpus luteum of rat during induction of luteolysis. Endocrinology (128): 2992-2998.

Sawada, M. and Carlson, J.C. (1994): Studies on the mechanism controlling generation of superoxide radical in luteinized rat ovaries during regression. Endocrinology (135): 1645-1650.

Shimamura, K.; sugino, N.; Yoshida, Y.; Nakamura, Y.; Ogino, K. and Kate, H. (1995): Changes in lipid peroxide and antioxidant enzyme activities in corpora lutea during pseudopregnancy in rats. J. Reprod. Fertil. (105): 253-257.

Singh, J.; Pierson, R.A. and Adams, G.P. (1997): Ultrasound image attributes of the bovine corpus luteum: structural and functional correlates. J. Reprod. Fertil. 109: 35-44.

Singh, J. and Roy, K.S. (1995): Histomorphological observations on cyclic corpus luteum of Indian buffalo (Bubalus bubalis). Ind. J. Anim. Sci. (65): 305-307.

Singh, J. and Roy, K.S. (1996): Histomorphological studies on corpus luteum of early pregnancy in the buffalo. Ind. J. Anim. Sci. (66): 782-784.

Slater, T.F. (1984): Free radical mechanisms in tissue injury. Biochemical J. (222): 1-15.

Soodak, L.K.; MacDonald, G.J. and Behrman, H.R. (1988): Luteolysis is linked to luteinizing hormone induced depletion of adenosine triphosphate in vivo. Endocrinology (122): 187-193.

Sugino, N. and Kato, H. (1994): The role of ischemia-reperfusion injuries in generating reactive oxygen species during luteolysis. Advance in contraceptive delivery system. (10): 95-106.

Sugino, N.; Nakamura, Y.; Takeda, O.; Ishimatsu, M. and Kato, H. (1993): Changes in activity of superoxide dismutase and lipid peroxide in corpus luteum during pregnancy in rats. J. Reprod. Fertil. (97): 347-351.

Ward, R.J. and Peters, T.J. (1995): Free radicals. In Marshall, W.J. and Bangent, S.K. 1st Ed. pp. 765-777., Clinical Biochemistry: Metabolic and clinical aspects. Churchill Livingstone.

Weiss, S.J. (1986): Oxygen ischemia and inflammation. Acta Physiologica Scand. suppl (548): 9-37.

Young, F.M.; Illingworth, P.J. Lunn, S.F. Harrison, D.J. and Fraser, H.M. (1997): Cell death during luteal regression in the Marmoset monkey (Callithrix Jacchus). J.Reprod. fertil. (111): 109-119.

Table (1): Mean concentrations and stander deviation (S.D) of some bio-chemical constituents of corpora lutea (CLs) in buffalo-cows.

	Cyclic corpora lutea		
	Developing CL (n=13)	*Fully developed CL* (n= 18)	*Regressing CL* (n= 17)
- Lipid peroxide (nmol/mg)[1)]	1.86± 0.62[a]	3.31± 0.55[b]	6.80± 2.64[c]
- Nitric oxide (nmol/mg)[1)]	5.07± 0.81[a]	3.85± 1.38[b]	1.37± 0.30[c]
- Superoxide dismutase (ng/mg)[1)]	3.16± 0.89[a]	4.48± 0.62[b]	2.40± 0.67[a]
- Glutathione (µg/mg)[1)]	12.42± 2.39[a]	26.99± 4.86[b]	16.78± 2.73[a]
- Vit. E (µg/mg)[1)]	1.55± 0.43[a]	1.64± 0.37[a]	2.13± 0.24[a]
- Total lipids (mg/g. wet tissue)	13.75± 1.42[a]	16.15± 2.52[a]	24.63± 2.10[b]
- Cholesterol (mg/g. wet tissue)	2.95± 0.85[a]	3.28± 1.02[a]	2.67± 1.04[a]

a,b,c: values with different superscripts within the same row are significantly different (P< 0.05).
1): mg protein.

Table (2): Correlation coefficient between lipid peroxide (LPO) production, superoxide dismutase (SOD) activity in corpora lutea and serum progesterone concentrations (P_4) in buffalo-cows.

	LPO / SOD	LPO/ P_4	SOD / P_4
- Developing CL	- 0.2088 [ns]	- 0.2625 [ns]	0.3328 [*]
- Fully developed CL	- 0.4115 [**]	- 0.5244 [**]	0.7710 [**]
- Regressing CL	- 0.8325 [**]	- 0.7644 [**]	0.5812 [ns]

** P< 0.01 * P< 0.05 ns : non significant

Table (3): Serum progesterone concentrations (P_4) and weight of corpora lutea (CLs) in buffalo-cows (mean ± S.D.).

	N	*Weight of corpora lutea (CLs) (gm)*	*Serum P_4 Conc. (ng/ml)*	*Correlation coefficient*
- *Developing CL*	13	1.07± 0.18[a]	0.61± 0.23[a]	0.9852 [**]
- *Fully developed CL*	18	2.77± 0.40[b]	3.68± 0.83[b]	0.9394 [**]
- *Regressing CL*	17	1.30± 0.21[a]	0.38± 0.17[a]	0.4662 [ns]