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Total anti-oxidant capacity and oxidative stress in dairy cattle and their associations with dystocia

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Summary

The aim of study was to investigate pre-partum and postpartum changes in the total anti-oxidant capacity (TAC), total peroxide (TPX) and oxidative stress index (OSI) of cows with dystocia compared with healthy animals. The possible relationships between these markers and relevant blood parameters were also investigated.

Examinations were performed in a herd of 200 Holstein cows, aged 3-6 years. Blood samples were taken both during the 7th month of pregnancy and within the first 15 minutes postpartum. After calving, the cows were divided into two groups according to the type of birth: Group 1 dystocia (n=16) and (Group 2) normal partum (n=21). Additionally, cows in the dystocia group were divided into three subgroups, according to the reason for the dystocia: absolute birth weight (the high birth weight of calf), twin pregnancy and presentation.

The results indicated that there were no changes in oxidative stress either in dystocia or during normal parturition eutoci. Oxidative stress may be decreased using an antioxidant supplement during the pre-partum period. The study also showed that oxidative stress does not affect dystocia. It may also be concluded that additional antioxidant supplementation may be required in absolute birth weight and twin pregnancy to reduce TPX levels. Analyses of urea, uric acid, and creatinine, as well as the enzyme activities of lactate dehydrogenase, alanine and aspartate transferase pre-partum do not appear to be useful for predicting dystocia. However, decreasing serum total protein may be a parameter to be used in diagnosing pathological conditions in pregnancy – particularly during late pregnancy.

Keywords: total anti-oxidant capacity (TAC), total peroxide (TPX), oxidative stress index (OSI), dystocia, cattle

Difficult birth, termed dystocia, occurs in 3 to 25% of cattle pregnancies, and is the major cause of decline in total performance and consequently economic loss. It is one of the most serious complications of pregnancy in cattle (18, 23), and numerous factors such as pelvic area, calf's birth weight, age of dam, twin pregnancy, presentation disposition, hormonal control, nutrition of dam and other unknown factors are believed to contribute to it.

The reactive oxygen species are produced continuously by normal metabolic processes, but the rate of production may be increased markedly by various conditions (4, 10, 21). In many studies it was showed that oxidative stress increased in normal pregnancy (4, 15, 19, 21, 24). However, the nature of this mechanism is not yet known, although it is thought that it may be related to the increased metabolic activity and it is also thought, that oxidative stress is an important contributing factor to the pathology of pregnancy.

Concentrations of antioxidants can be measured individually in plasma or serum, however measurement of each antioxidant component requires an intensive labor and time. As a single measure, total anti-oxidant capacity (TAC) provides more biologically relevant information, thus it may be more useful and practical to evaluate the antioxidant status of plasma, only. Results of this method, together with measurement of total peroxide (TPX), have agreed with results by other oxidant stress markers in various disease states. Also, oxidative stress index measurement has been used in various increased oxidant stress conditions with reliable results (8, 16). The knowledge about plasma TAC and TPX in veterinary medicine is still scarce. Especially since there was no relationship found between oxidative stress and dystocia in cattle, both prepartum and postpartum.

The aim of this study was to investigate changes of the TAC, TPX and oxidative stress index (OSI) prepartum and postpartum in healthy cows and in cows with dystocia. In order to determine whether changes originated from a specific etiopathogenesis cows with dystocia were divided by clinical signs of dystocia into three subgroups: those with absolut birth weight (the high birth weight of calf), those with twin pregnancy and those with presentation disposition. We also examined relationships between the clinical markers and relevant biochemical markers (serum urea, creatinine, uric acid, total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehidrogenase (LDH)).

Materials and methods

Animal and sample collection. The study was carried out using aged 3-6 years Holstein cows from herd of 200 pregnant animals, maintained in Ceylanpınar dairy farm affiliated with Turkish Agriculture Ministry (TÝGEM) (Urfa, Turkey). During the study all animals were kept under identical conditions. None of the cows had diseases, and had received no medication. All cows received a mineral and vitamin supplement, composed of 600 mg of vitamin E, 400 mg of vitamin B_1 and 10 mg of sodium selenite (Esevil, Vilsan®), given as an intra muscular injection 45-60 days before calving (corresponding to onset of dry period). Blood samples were taken in the 7th month of pregnancy and within the first 15 minutes after delivery. Sixteen of the cows had clinical dystocia as defined earlier (18, 23) (group 1) (n = 16) and the others had normal labor. We selected only twenty one cows for control group that unassisted calving (group 2) (n = 21). Additionally, cows in the dystocia group were divided into three subgroups, by reason of kind of dystocia (1 – High absolute birth weight, 2 – twin pregnancy, 3 – presentation disposition).

Blood samples with EDTA (for TAC, TPX determinations) and without EDTA (for other relevant blood parameters determination) were obtained by jugular venapuncture, using evacuated tubes (Vacutainer®), and immediately stored in ice and then plasma and serum were separated from cells by centrifugation at 1800 × g for 10 min., and aliquots were stored at –80°C until analysis.

Measurement of the total antioxidant capacity of plasma. The total antioxidant capacity was measured using a novel automated colorimetric measurement method for the total antioxidant capacity (TAC) developed by Erel (8). In this method, the hydroxyl radical reacts with the colourless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in colour. Upon the addition of sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the colour change and thereby providing an effective measure of the total antioxidant capacity of the plasma. The assay results are expressed as mmol Trolox eq./L, and the precision of this assay is lower than 3%.

Measurement of the plasma total peroxide concentration. The total peroxide concentrations were determined using the FOX2 method (16) with minor modifications. The FOX2 test system is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides within the plasma samples, in the presence of xylenol orange which produces a coloured ferric-xylenol orange complex whose absorbance can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mM H_2SO_4 (10 ml) to give a final concentration of 250 mM ferrous iron in acid. This solution was then added to 90 ml of high-performance liquid chromatography (HPLC) – grade methanol containing 79.2 mg butylated hydroxytoluene (BHT). Finally, 7.6 mg xylenol orange was added, with stirring, to make the working reagent (250 mM ammonium ferrous sulphate, 100 mM xylenol orange, 25 mM H_2SO_4, and 4 nM BHT, in 90% (v/v) methanol in a final volume of 100 ml). Aliquots (200 mL) of plasma were mixed with 1.8 ml FOX2 reagent. After incubation at room temparature for 30 min., the vials were centrifuged at 12,000 g for 10 min. Absorbance of the supernatant was then determined at 560 nm. The total peroxide content of the plasma samples was determined as a function of the difference in absorbance between the test and blank samples using a solution of H_2O_2 as standard. The results were expressed as micromoles of H_2O_2 per litre. The coefficient of variation for individual plasma samples was less than 5%.

Oxidative stress index. To perform the calculation, the result unit of TAC, mmol Trolox equivalent/l, was changed to mmol Trolox equivalent/l and the OSI value was calculated as formula below; OSI = [(Total peroxide, mmol/L)/(TAC, mmol Trolox equivalent/l) – 100].

Measurement of the other relevant blood parameters. Serum urea, creatinin, uric acid, TP, ALT, AST, LDH activities were measured colorimetrically with auto analyzer (Olympus, AU 2700, Germany) and commercially available kits. The coefficients of variation for all variables were < 5%.

Statistical analysis. The following statistical procedures were used: analysis of differences between prepartum and postpartum stages for each group, paired-samples t test was used. The independent-samples t test was performed in order to compare two groups for each evaluated parameters. Sub-groups values of dystocia were compared by Kruskal-Wallis analysis H followed by Mann-Whitney U-test. To determine the significance of interactions between various variables in each group, Pearson's and Spearman's correlation analysis was performed. A difference with $P < 0.05$ was considered to be significant. All statistical analyses were performed with statistics package SPSS version 10.0 (SPSS Inc., Illinois, USA).

Results and discussion

The measured variables in dystocia (group 1) and normal delivery (group 2) at prepartum and postpartum stages are summarized in table 1. In addition, the concentration of the all measured variables described as dystocia sub-groups and normal parturition, were given as table 2. The differences were shown according to periods and groups in the same tables.

Increased oxidative stress has been reported in numerous studies in healthy pregnancy of both animals and humans (3, 4, 21). It is suggested that the reason of the increased oxidative stress in pregnant individuals probably due to the increased free radicals caused by increased metabolic activity (8, 15), raised insulin resistance (14) and negative energy balance (NEB) (19). Another factor for the oxidative stress in pregnancy probably was reduction of an antioxidant reserve in the pregnancy and metabolic adaptation for lactation (15). These variations were related to the physiological adaptations of cattle for their energy needs during pregnancy.

A few studies assessed the oxidant/anti-oxidant balance using TPX and TAC values in the healthy pregnant cattle. The TAC values peaked arround the parturation in pregnancy (2, 15, 22). Recently it was found that TAC values peaked 1 week after calving in parallel with MDA concentrations (3, 4). Smilarly these results, in our study identify that the plasma TAC values taken postpartum period tended to be slightly increased comparing to prepartum period in both groups, however; it was not statistically significant ($p > 0.05$). But, in this study the cows were supplemented with a vitamin mineral at onset of the dry period. On the conrary, TPX values in postpartum stage tended to decrease compared to the 7th month pregnancy in both groups ($p > 0.05$).

Although, Castillo et al. was not evaluated OSI, it can be said that this parameter were not changed because of both lipid peroxidaiton and TAC increased. In this

Tab. 1. Comparison of the mean values of various factors pre- and post-partum im cows with dystocia and with normal parturition (mean ± S.D.)

	Dystocia (group 1) (n = 16)		Normal partum (group 2) (n = 21)	
	Prepartum	Postpartum	Prepartum	Postpartum
Urea (mg/dl)	80.56 ± 29.81	68.62 ± 43.88	89,81 ± 27.09	67.14 ± 49.64
Creatinin (mg/dl)	0.65 ± 0.6	0.71 ± 0.42	0.67 ± 0.46	0.55 ± 0.36
Urea/Creat. ratio	245.66 ± 183.1	139.11 ± 109.29	223.35 ± 172.2	152.27 ± 105.42
ALT (U/l)	14.50 ± 8.75	12.00 ± 11.58	11.73 ± 8.61	10.57 ± 10.68
AST (U/l)	42.00 ± 40.82	21.25 ± 31.31	34.52 ± 23.37	18.90 ± 27.94
Urac acid (mg/dl)	0.87 ± 1.08	1.30 ± 1.11	0.70 ± 1.10	0.82 ± 0.85
LDH (U/l)	1125.5 ± 264.2[B]	1477.7 ± 425.5[B]	1133.5 ± 355.4[B]	1494.6 ± 392.3[B]
T. protein (g/dl)	6.77 ± 0.73[B]	7.73 ± 0.82[a,B]	6.91 ± 0.42[C]	8.26 ± 0.67[a,C]
TAC (mmol Trolox equiv./l)	1.87 ± 0.34	1.90 ± 0.25	1.72 ± 0.23	1.80 ± 0.27
Total peroxide (µmol H_2O_2/l)	7.90 ± 1.86	7.83 ± 2.07	9.00 ± 2.13	7.90 ± 3.82
Oxidative stress index	0.48 ± 0.11	0.42 ± 0.13	0.52 ± 0.14	0.44 ± 0.18

Explanations: The measured variables were compared within and between groups; a, b: when this period was compared with corresponding period in the other group, differences were significant ($p < 0.05$); A, B, C – differences between mean values having same number in each group is significant ($p < 0.05$, $p < 0.01$, $p < 0.001$ respectively).

Tab. 2. The concentration of all variables according to dystocia sub-groups and normal pregnancy group in pre-partum period (mean ± S.D.)

	Dystocia (Group 1) (n=16)			Normal partum (Group 2) (n = 21)
	Absolut birth weight (n = 8)	Twin pregnancy (n = 4)	Presentation disposition (n = 4)	
Urea (mg/dl)	76.37 ± 27.89	64.25 ± 20.82	105.25 ± 31.3	89.81 ± 27.09
Creatinin (mg/dl)	0.45 ± 0.35	0.66 ± 0.55	1.03 ± 0.95	0.67 ± 0.46
Urea/Creat. ratio	194.78 ± 120.55	217.22 ± 205.04	260.87 ± 230.06	223.35 ± 172.2
ALT (U/l)	16.75 ± 9.51	7.00 ± 6.22	17.50 ± 5.69	11.73 ± 8.61
AST (U/l)	44.25 ± 25.52	18.50 ± 11.27	61.00 ± 173.86	34.52 ± 23.37
Uric acid (mg/dl)	0.62 ± 0.99	0.78 ± 1.02	1.47 ± 1.37	0.70 ± 1.10
LDH (U/l)	1168 ± 317	1215 ± 164	950 ± 173	1133.48 ± 355.36
T. Protein (g/dl)	6.47 ± 0.66	7.05 ± 0.76	7.07 ± 0.76	6.91 ± 0.42
TAC (mmol Trolox equiv./l)	1.90 ± 0.29	1.65 ± 0.22	2.02 ± 0.48	1.72 ± 0.23
Total peroxide (µmol H_2O_2/l)	8.59 ± 1.08	7.60 ± 0.78	6.82 ± 3.33	9.00 ± 2.13
Oxidative stress index	0.46 ± 0.08	0.47 ± 0.09	0.55 ± 0.18	0.52 ± 0.14

Explanations: The measured variables were compared with both among the sub-groups and the other group

respect our results are partly consistent with their studies (3, 4). Howerever, our results cannot be compared with previous studies for several reasons: most of the studies used different analytical techniques, the blood samples were taken at different periods, none of the studies were the OSI values assesed to show that the elevated TPX/TAC ratio (OSI) is an important indicator of the increased oxidative stress (12).

If cattle are not supplemented with additional vitamin E and Se in 45-60 day before calving, it can be possible to increased lipid peroxidaiton and TPX values around the parturition that similar to results of the other studies in healthy cows (2-4, 22). In this respect, our results indicated that the important of antioxidant supplementation in this critical period.

The majority of the studies recognised the increaesed oxidative stress in pathological pregnancy. Human studies have shown plasma TAC activity to be lower in preeclampsia (12) and there is an imbalance between lipid peroxidase and antioxidants in preeclampsia (1, 7, 10, 21). Furthermore, OSI values were found to be significantly higher in women with preeclampsia (6). Normally, increased peroxidaition in dystocia is expected due to physical effort of calving. Also, oxidative stress may be elevated due to increased requirements of the antioxidants and elevated the free radicals production, as a result of higher metabolic activity caused by enormous fetal weight in dystocia. However, there is lack of information concerning the role of oxidative stress in the etiology of the pathological pregnancy in cattle. In these studies mainly serum antioxidant enzyme activities and lipid peroxidation were investigated in postpartum disorders such as retained fetal membranes (RFM). These investigators observed higher MDA concentrations in RFM cases than in control animals (11, 13). Also, Miller et. al. (15). have shown plasma TAC activity to be lower in RFM.

Our findings demostrate that measured oxidative stress variables were not altered in either group ($p > 0.05$). This observation point out that oxidative stress could not contrtibute in pathogenesis of dystocia. When the dystocia was evaluated by subgroups, the highest concentration of plasma TAC was found in the case of presentation disposition. This data emphasize that there is a higher demand for the antioxidants by the growing fetus.

Uric acid serves as a potent antioxidant by radical scavenging (8). However there was no statistical correlations, that low levels of serum uric acid might be responsible for increased TPX and decreased TAC values. The increased serum total protein levels observed after calving reflect increased mobilization of tissue protein in both groups ($p < 0.01$ and $p < 0.001$ respectively), in accordance with other reports in dairy cattle (4, 26). The reason of the decreased TP and uric acid levels in the 7th month of pregnancy in both groups may be associated with increased requirements of the protein, as a result of transport to the fetus via the placenta (5, 26). The lower TP concentration in cows with dystocia at postpartum stage compared to corresponding period in normal pregnant cattle ($p < 0.05$) presumably is due to high demand of protein by fetus. Furthermore, the observed tendency of the decrease in TP concentration in the case of calving of absolute birth weight calf and twin when compared with cattles having presentation dispsoition, may be support this hypothesis.

Decreasing urea, uric acid and urea/creatinine ratio is associated with low protein uptake and high protein requirements (9, 20). According to our results, tendency of the decreased urea, uric acid and urea/creatinine ratio confirms the increased protein requirements in above mentioned subgroups ($p > 0.05$). Furhermore, decreased creatinine levels also proves the increased protein requirements in these subgroups (9). Additionally, the elevated urea/creatinine ratio and lowered creatinin concentrations are important indicators of the higher GFR that increases especially in late gestation due to the increased total blood volume (9). The observed increases in creatinine levels in the presentation disposition compared to other subgroups probably reflect the increase in fetal musculature and muscle damage.

The tendency of the increased serum creatinine level postpartum in dystocia cows could be due to higher muscular work for the delivery and bearing the growing fetus. Although the not statistically significant reason of the higher urea/creatinine ratio and lower creatinin levels in prepartum than postpartum in both groups may be a consequence of increased GFR. Moreover, our results suggest that dystocia was not related with GFR, contrary to toxemia (9).

Increased mobilization of tissue protein causes the muscular damage/destruction (MD) and then increases muscular enzymes activities (9). In this respect, high level of serum LDH ($p < 0.01$) is evidence supporting an increased mobilization of tissue protein MD in postpartum period in both groups.

In results similar to ours, Fischbach (9) and Yokus et al. (26) reported that transaminase activities were not affected by the reproductive status ($p > 0.05$). Increased ALT and AST levels are found in the hepatocellular disease, viral infectious and traumatic injuries. Since we did not observe a noteworthy increase in ALT and AST levels, we conclude that biochemically cattle can be considered as having any clinical disease.

It can be concluded, oxidative stress could not contribute to the occurrrence of dystocia. Oxidative stress can be decreased with antioxidant supplementation during last months of pregnancy. Especially, antioxidant supplementation is required in the cases of absolut birth weight and twin pregnancy. Evaluation of urea, uric acid, creatinine, lactate dehydrogenase, alanine and aspartate transferase enzyme activities in prepartum period does not appear to be useful to predict the dystocia. Decreasing serum total protein concentration might be a useful parameter of in diagnosis of pathological conditions of the late pregnancy. Further studies with larger number of animals are needed to verify these results.

References

1. *Atamer Y., Kocyigit Y., Yokus B., Atamer A., Erden A. C.*: Lipid Peroxidation, Antioxidant Defense, status of Trace Metals and Leptin levels in Preeclampsia. Europ. J. Obstet. Gynecol. Reprod. Biol. 2005, 119, 60-66.
2. *Brzezinska-Slebodzinska E., Miller J. K., Quigley J. D., Moore J. R., Madsen F. C.*: Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. J. Dairy Sci. 1994, 77, 3087-3095.
3. *Castillo C., Hernandez J., Bravo A., Lopez-Alonso M., Pereira V., Benedito J. L.*: Oxidative status during late pregnancy and early lactation in dairy cows. Veterinary J. 2005, 169, 286-292.
4. *Castillo C., Hernandez J., Valverde I., Pereira V., Sotillo J., Lopez A. M., Benedito J. L.*: Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet. Sci. 2006, 80, 133-139.
5. *Church D. C., Fontenot J. P.*: Nitrogen metabolism and requirements, [in:] Church D. C.: Digestive Physiology and Nutrition of Ruminants, Vol. 2. Oregon, Corvallis, OR 1971.
6. *Davidge S. T., Hubel C. A., Brayden R. D., Capeless E. C., McLaughlin M. K.*: Sera antioxidant activity in uncomplicated and preeclamptic pregnancies. Obstet. Gynecol. 1992, 79, 897-901.
7. *El-Salahy E. M., Ahmet M. I., El-Gharieb A., Tawfik H.*: New scope in angiogenesis: role of vascular endothelial growth factor (VEGF), NO, lipid peroxidation, and vitamin E in the pathophysiology of preeclampsia among Egyptian females. Clin. Biochem. 2001, 34, 323-329.
8. *Erel O.*: A novel automated method to measure total antioxidant response against potent free radical reactions. Clin. Biochem. 2004, 37, 112-119.
9. *Fischbach F. A.*: Manual of Laboratory&Diagnostic Tests. Lippincott 2000.
10. *Gupta S., Agarwal A., Sharma R. K.*: The Role of Placental Oxidative Stres and Lipid Peroxidation in Preeclampsia. Obstet. Gynecol. Survey 2005, 60, 807-815.
11. *Gupta S., Gupta H. K., Soni J.*: Effect of Vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. Theriogenology 2005, 64, 1273-1286.
12. *Harma M., Harma M., Erel O.*: Measurement of the total antioxidant response in preeclampsia with a novel automated method. Europ. J. Obstet. Gynecol. Reprod. Biol. 2005, 118, 47-51.
13. *Kankofer M.*: The levels of lipid peroxidation products in bovine retained and not retained placenta. Prostagl. Leukotr. Ess. Fatty Acids 2001, 64, 33-36.
14. *Kocyigit Y., Bayhan G., Atamer A., Atamer Y.*: Serum levels of leptin, insulin-like growth factor-I and insulin-like growth factor binding protein-3 in women with pre-eclampsia, and their relationship to insulin resistance. Gynecol. Endocrinol. 2004, 18, 341-348.
15. *Miller J. K., Brzezinska-Slebodzinska E.*: Oxidative Stress, Antioxidants and Animal Function. J. Dairy Sci. 1993, 76, 2812-2823.
16. *Miyazawa T.*: Determination of phospholipids hydroperoxides in human blood plasma by a chemiluminesence's- HPLC assay. Free Radic. Biol. Med. 1989, 7, 209-217.
17. *Moran P., Baylis P. H., Lindheimer M. D., Davison J. M.*: Glomerular Ultrafiltration in Norm and Preeclamptic Pregnancy. J. Am. Soc. Nephrol. 2003, 14, 648-652.
18. *Oakes D. E., Parkinson T. J., England G. C. W.*: Dystocia and other disorders associated with parturition, [in:] Oakes D. E. (Ed.): Arthur's Veterinary Reproduction and Obstetrics. Saunders W. B., London 2001, 205-333.
19. *Roche J. F., Mackey D., Diskin M. D.*: Reproductive management of postpartum cows. Anim. Reprod. Sci. 2000, 60-61, 703-712.
20. *Schroder B., Schoneberger M., Rodehutscord M. Pfeffer E., Breves G.*: Dietary protein reduction in sheep and goats: different effects on L-alanine and L-leucine transport across the brush-border membrane of jejunal enterocytes. J. Comp. Physiol. B. 2003, 173, 511-518.
21. *Serdar Z., Gur E., Develioglu O., Colakogullari M., Dirican M.*: Placental and decidual lipid peroxidation and antioxidant defenses in preeclampsia lipid peroxidation in preeclampsia. Pathophysiology 2002, 9, 21-25.
22. *Stec A., Mitler J. K., Finkelstein E., Stec J., Mueller F. J., Thomas D. G., Keltner D. G.*: Plasma total antioxidant status of periparturient cow with udder edema or retained placenta. J. Dairy Sci. 1991, 74, 240-245.
23. *Stephan J. R.*: Veterinary Obstetrics and Genital Diseases (Theriogenology). chapter 7. The cause of Dystocia. Published by author, Woodstock, Vermount 1986, 277-283.
24. *Toescu V., Nuttall S. L., Martin U., Kendall M. J., Dunne F.*: Oxidative stress and normal pregnancy. Clin. Endocrinol. (Ox.) 2002, 57, 609-613.
25. *Yokus B., Cakir D. U., Kanay Z., Toprak G., Uysal E.*: Effects of reproductive status and seasonal variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. J. Vet. Med. A. 2006, 53, 275-281.
26. *Yokus B., Cakir D. U.*: Seasonal and Physiological Variations in the Serum Chemistry and Mineral Concentrations in Cattle. Biol. Trace Element Res. 2006, 109, 255-266.

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