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Evaluation of oxidative stress in maternal dystocia affected cows (*Bos indicus*): A preliminary study

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Abstract
The present preliminary study undertaken to assess the status of oxidative stress by estimation of superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and total protein (TP) in blood plasma of maternal dystocia affected Ongole and Punganur breeds of cattle. The results showed that blood plasma of maternal dystocia affected animals MDA production was increased and antioxidant enzymes levels were decreased. Increased level of oxidative stress in fetomaternal disproportion affected cattle was recorded in the present study. In conclusion, decreased levels of anti-oxidant enzymes and elevated levels of lipid peroxidation metabolites indicated progression towards deteriorative action of various reactive oxygen metabolites and acutely severe oxidative stress which have been produced during the time of calving.

Keywords: Anti-oxidant enzymes, dystocia, ongole, oxidative stress, punganur

Introduction
Ongole and Punganur cattle are precious native breed of Andhra Pradesh, used for milk production followed by agricultural work and considered as back bone of animal production in farming community in next to buffaloes, especially calves which are the future of animal wealth. Difficulty in parturition is termed as dystocia [1]. Assisted delivery in bovine pregnancies is the major cause of decline in total production and performance culminate economic losses to the farmer community due to failure of harvesting viable calf [2]. Reactive oxygen metabolites (ROM) are unavoidable intermediate compounds which are produced during normal physiological metabolism and pathological condition. ROM concentration and balance between their production and degradation are maintained by cellular enzymatic and non-enzymatic defence mechanisms [3]. Oxidative stress can be occur when imbalance between generation of reactive oxygen metabolites and scavenging capacity of anti-oxidant in reproductive organ [3]. Stress from any origin is capable of reducing the body's enzymatic and non-enzymatic anti-oxidant resources [3].
Oxidative stress in cattle is a contributory factor to increase disease susceptibility moreover parturition and lactation would be increase the production of reactive oxygen metabolites [4]. Nakao and Grunet, (1990) [5] opined that although the parturition process (calving) is physiological event it induce stress to the animal and assisted delivery or abnormal parturition add to normal stress to of calving. The common enzymatic anti-oxidant enzymes for scavenging the free radicals includes superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase [3]. The activities of antioxidant enzymes and lipid peroxidation alter significantly during oxidative stress therefore they can be used as markers of oxidative stress [6]. Large number of investigations was carried out on oxidative stress in buffaloes affected with dystocia (maternal and fetal dystocia). Bansal *et al*., (2011) [4] recently evaluated regarding oxidative stress and anti-oxidant enzymes in dystocia affected buffaloes. The information available on oxidative stress and anti-oxidative enzymatic activities are lack in dystocia affected Ongole and Punganur cattle. Therefore the current preliminary study was undertaken to ascertain the level of oxidative stress and anti-oxidant enzymes activity in blood plasma of maternal dystocia affected Ongole and Punganur cattle.

Materials and methods
Selection of animals: The oxidative stress was assessed in Ongole and Punganur cattle affected with maternal dystocia. The dystocia affected Ongole and Punganur cattle were brought to Department of Veterinary Gynaecology and Obstetrics, for the treatment with in 12 to 24 hours of onset of parturition or straining.

Complete history with regards to their gestational age, duration of straining and previous handling or medical intervention of the cattle, if any, was recorded (Table.1). All the dystocia affected animals had single dead foetuses except, live foetus relived in one an incomplete cervical dilation (ICD) and 2 uterine torsion cases.

Blood Collection: Blood was collected from jugular vein by jugular venepuncture using EDTA vials. All the blood samples centrifuged at 3000 rpm for 15 min. Plasma harvested and stored at -20^0 C for estimation of superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and total protein (TP).

Quantification of anti-oxidant enzymes: We analysed superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and total protein (TP) by standard analytical procedure and calculation in blood plasma of all cattle affected with maternal cause of dystocia.

Results and discussion

Alteration in anti-oxidant enzymatic activity (SOD and GSH), Lipid peroxidation metabolites (MDA) and Total protein (TP) profile in blood plasma of maternal dystocia affected cattle were recorded (Table 2 and Plate 1). Any alteration in the anti-oxidant values seemed to indicate the oxidative damage occurring due to difficult in parturition (Dystocia).

Malondialdehyde (MDA): Lipid peroxidation is one of the most non-enzymatic chain reaction depend on oxidation of mainly unsaturated fatty acids (lipid derivatives) and is associated with the presence of reactive oxygen metabolites. It culminate to formation of lipid peroxides molecules and other intermediate compounds. These intermediate compounds may affect the properties of cell membranes and their physiological role during stress (Buege and Steven, 1985) [7]. Comparatively MDA production was higher in fetomaternal disproportion affected Punganur cattle followed by uterine torsion in Ongole cattle. Increased level of MDA in FMD cases might be due to vigorous obstetrical manipulation by Paravet. Low level of MDA production in uterine inertia case was recorded in this study that might be due to reduced contraction of uterus and quiescence/absence of straining of the animal. The problem of dystocia and obstetrical operation like mutation and fetotomy are highly induce the stress condition in animal [1], because of elevated levels of glucocorticoids that culminate excessive production of reactive oxygen metabolites [8]. Higher level of MDA could be used as indicator for oxidative stress due to lipid peroxidation event occurs in the reproductive tract during difficult calving recorded in the present study.

Total protein (TP): Comparatively reduced level of protein in FMD affected Punganur cattle when compared to other maternal cause of dystocia in the present study. Castillo *et al*., (2005) [9] opined that slight alteration or decrease in the protein content indicates the status of oxidative changes in late pregnant animals. Decreased level of total protein occurs during stress condition (dystocia) due to high level of glucocorticoids generation [8]. Increased oxidative stress disturb the protein metabolism followed by impair the protein transport mechanism [2].

Superoxide dismutase (SOD): SOD activity was very low in FMD affected cattle than other form of maternal dystocia in the present study that may be due to excessive handling like traction and increased inflammation of reproductive tract [10]. Moreover dystocia affected animals have lower SOD activity than normally calved animal [11]. Activity of SOD progressively increased in the last 3 weeks of pregnancy, and reached the maximum, 4 days before parturition, followed by the SOD activity rapidly declined after calving to reach the levels registered before calving [12], moreover assisted delivery further decrease the SOD activity.

Reduced glutathione (GSH): GSH activity was very low in FMD affected cattle than other form of maternal cause of dystocia in the present preliminary study. Glutathione type of anti-oxidant enzyme contains Selenium (Se) as a co-factor. During assisted delivery, reactive oxygen metabolites causes reduction in selenium intake by the red blood cells that result in decreased levels of GSH concentration thereby culminate to occurrence of oxidative stress [13, 14].

Conclusion

The order of increased level of oxidative stress uterine inertia, incomplete cervical dilation, uterine torsion and Fetomaternal disproportion (FMD) recorded in the preliminary study. Increased level of oxidative stress in Fetomaternal disproportion affected cattle attributed by excessive handling and any injury to the birth canal or uterus. Moreover evaluation of oxidative stress and anti-oxidant enzymes must remain the keystone of veterinary obstetrical research because it is quantitative information gives idea and better understanding about the status of animal health during oxidative stress. Decreased levels of anti-oxidant enzymes and elevated levels of lipid peroxidation metabolites indicated progression towards deteriorative action of various reactive oxygen metabolites and acutely severe oxidative stress which have been produced during the time of calving. However, it is not a completely reflects any specific type of changes due to oxidative stress. Further studies are required to monitor the oxidative stress in depth. It is therefore recommended to evaluate the antioxidant enzymes and lipid peroxidation metabolites as a matter of critical care and attempt the dystocia with obstetrical treatment as early as possible when presentation of cases to avoid oxidative stress and further obstetrical complication.

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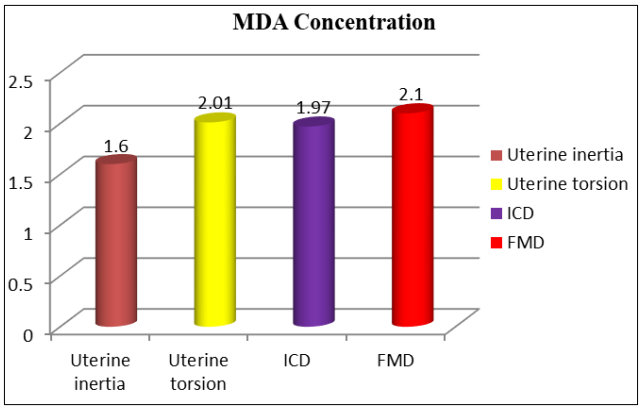
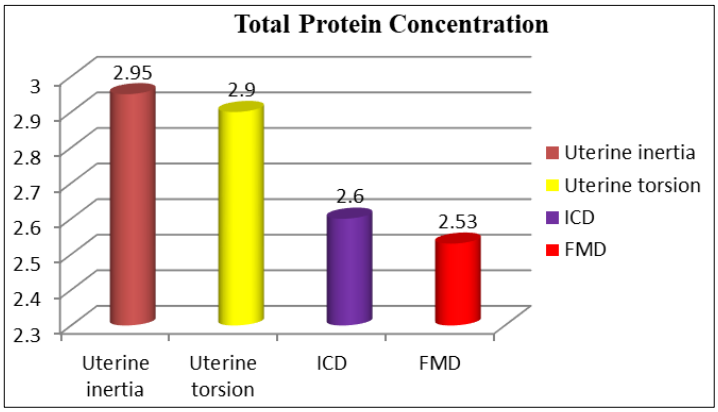
Table 1: Complete history and treatment of maternal dystocia

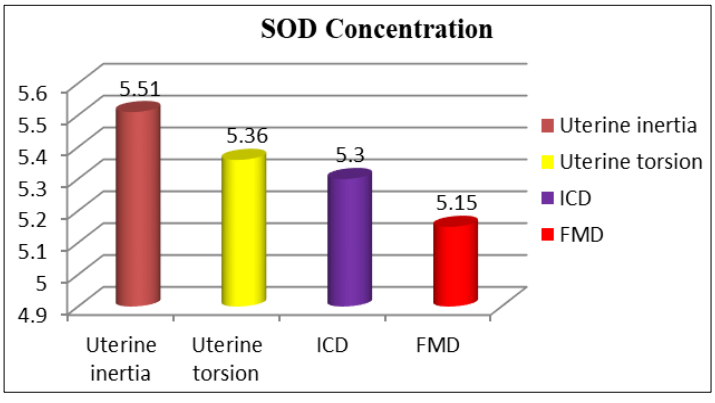
Breed	Type of maternal dystocia	Parity	Hours of straining	Handling/medical intervention	Treatment
Ongole (n=7)	Uterine inertia (n=2)	Primiparous, 2nd calving (Gestation completed)	Water bag ruptured 7 to 8 hours back	Not handled	Dextrose-3 lit (i/v), Fetal malposition corrected, oxytocin 20 IU (i/v). Dead foetuses each.
	Uterine torsion (n=2)	Primiparous, 3rd calving (Gestation completed)	18 hours before onwards colic sign started	Not handled, Treated for digestive problem	Dextrose-3 lit (i/v), Detorsion done by modified Schaffer's method with 2-rolling, live foetuses from each.
	ICD (n=1)	2nd calving (Gestation completed- 9 month and 4 days).	Abnormal straining since-24 hours	Not handled, Treated for digestive problem.	Hormonal intervention (MTP) with Pragma 500mcg-i/m, Dexamethasone 30 mg i/m. Live foetus relieved after 52 hours of initiation of treatment.
	FMD (n=2)	Both are Primiparous	6 to 8 hours of straining.	Handled by obstetrical mutation operation	Dead foetus relived by fetotomy and forced traction
Punganur (n=2)	FMD (n=2)	Both are Primiparous	8 hours of straining	Handled vigorously by obstetrical mutation operation	Dead foetuses each by C-section. 2nd dam died before attempting.

Table 2: Anti-oxidant profiles in the blood plasma of maternal dystocia affected cattle

Parameters and Units	**Ongole** (n=7)				**Punganur** (n=2)
	UI (n=2)	**UT (n=2)**	**ICD (n=1)**	**FMD (n=2)**	**FMD (n=2)**
MDA (µmoles MDA/mg protein ml-1)	1.68	2.021	1.97	1.91	1.98
	1.51	2.003	-	2.094	2.42
Total Protein (mg/ml)	3.01	2.97	2.60	2.70	2.50
	2.90	2.82	-	2.91	2.02
SOD (unit/mg protein/min)	5.62	5.32	5.29	5.22	5.30
	5.40	5.41	-	5.07	5.01
GSH (mM/mg)	0.701	0.421	0.50	0.482	0.511
	0.790	0.609	-	0.524	0.412







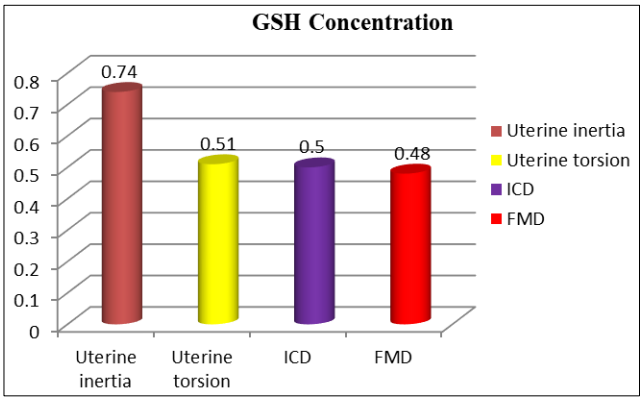


Plate 1: Anti-oxidant profiles in the blood plasma of maternal dystocia affected cattle

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