Explor Anim Med Res,
Vol.11, Issue - 1, 2021, p. 80-88
DOI : 10.52635/EAMR/11.1.80-88

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

COMPARATIVE EVALUATION OF OXIDATIVE STRESS ENZYMES ACTIVITY, THYROID HORMONES AND ELECTROLYTES AT DIFFERENT REPRODUCTIVE STAGES OF RED SOKOTO GOAT IN SOKOTO, NIGERIA

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Received 22 September 2020, revised 09 December 2020

ABSTRACT: The physiological changes in various body systems associated with different reproductive status are significantly important to support fetus, neonate and the dam as well. These adjustments affect oxidative stress enzymes, hormones and electrolytes. The objective of the study was to evaluate the changes in blood levels of oxidative stress enzymes activity, thyroid hormones and some electrolytes at different reproductive stages of Red Sokoto goat. The study was conducted using 20 Red Sokoto goats (RSG); the goats were synchronized using CIDR(R) and were naturally mated. Vet image 201 ultrasonographic machine was used to establish pregnancy. Serum thyroid stimulating hormone (TSH), total triiodothyronine (tT3) and total thyroxin (tT4) concentrations were assayed. Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were monitored. Serum levels of electrolytes (sodium (Na+), potassium (K+) and calcium (Ca2+) were determined. Analysis of variance was used for comparison between the reproductive stages. The activities of SOD and GPx varied significantly ($p<0.05$) at different reproductive stages while CAT activity was not affected by reproductive processes. Significant ($p<0.05$) changes in TSH, tT3 and tT4 concentrations were also recorded at different reproductive stages. Serum levels of Na+, K+ and Ca2+ differed significantly ($p<0.05$) at different reproductive stages. Higher postpartum changes in electrolyte levels, thyroid hormone concentrations and oxidative stress enzyme activities were discovered in Red Sokoto goat.

Key words: Reproductive stage, Oxidative stress, Thyroid hormones, Electrolytes, Red Sokoto goat.

INTRODUCTION

Goats are among the oldest domesticated animal species and they are greatly adaptable to the tropical regions of the world (Hooda and Upadhyay 2014). The northern region of Nigeria plays an important role in goat production in the African continent and the world in general (Njidda *et al*. 2013). The Red Sokoto goat is the predominant and most important breed of goat found mainly in the Sudan and Sahel savanna zones of Nigeria (Obua *et al.* 2012). They had acquired some important biological characteristics such as short generation interval, twinning, short growth periods, and do not require much space for rearing. This breed is found in almost all households and plays an important role in the economic life of many families (Abdalla *et al.* 2009). They are managed through extensive system of management (Abdalla *et al*. 2009), which coupled with different changes associated with reproductive activities predisposes them to stress and various changes in physiologic compositions that could have adverse effect

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on reproductive potential. Pregnancy is a physiological process characterized by a significant increase in energy demand to promote fetal development and growth. This suggests that both the dam and fetus are likely to experience oxidative stress during pregnancy (Mutinati *et al*. 2013). In late pregnancy, negative energy balance may be the reason for the development of oxidative stress, increased lipid peroxidation and reduced antioxidant activity that contribute to the development of complications in pregnancy (Rejitha and Karthiayini 2014). Fassah *et al*. (2015) mentioned that the high metabolic demand in lactation and late pregnancy period also induced oxidative stress. The reproductive cycle is associated with several metabolic and hormonal changes in farm animals (Browne *et al*. 2008). These changes predispose the animals to increased free radicals and oxidative stress; that has not been evaluated in the Red Sokoto goat. The study aimed at establishing thyroid hormone, electrolytes and oxidative stress changes at different reproductive stages in the Red Sokoto goat. This will provide information on the appropriate time to intervene and provide support to promote and ensure successful reproductive processes.

MATERIALS AND METHODS

Ethical approval for the study was obtained from the Faculty Animal Research Ethics Committee, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/2017/AUP-RO-06).

A total number of 23 animals comprising of 20 does and 3 bucks with the mean weight of 29 ± 0.5 kg and mean age of 2 ±0.17 years were used for the research. The animals were conditioned for two weeks and managed intensively. They were fed on bean husk; wheat bran and hay and water were supplied *ad libitum* during the period and throughout the experiment. The mean ambient temperature, relative humidity and temperature humidity index within the pen were 35.22 ± 3.47 °C, 22.33 ± 1.0 % and 79.79 ± 2.68 respectively. Meteorological values were obtained using Mason's type hygrometer (GH Zeal Ltd, London, England).

Oestrous synchronisation

Oestrous synchronisation was conducted using controlled internal drug release CIDR(R) as described previously by Islam (2011). The animal was restrained in a standing position with the tail raised and the perineum wiped with a paper towel. The CIDR(R) was lubricated using a KY jelly (a normal cellulose lubricant). The lateral commissures of the vulva were gaped, the mounted applicator was inserted into the vagina and the plunger of the applicator was pressed to release the CIDR(R) into the vagina. The CIDR(R) was removed after 17 days and the animals were monitored for signs of oestrous. Rams were introduced and natural mating was allowed and after four weeks the does were assessed for pregnancy using ultrasonography.

Ultrasonography

Ultrasonography was carried out as previously described by Streeter and Step (2007). The animals were fasted overnight by withdrawing feed as a preparation for ultrasonography. The inguinal region was shaved and cleaned with chlorhexidine. The animal was placed on standing position and acoustic gel was applied on the shaved area. Bright (B) mode real time ultrasound scanning was conducted using 2.5 MHz sector transcutaneous probe of Vet image 201(R) ultrasonographic machine of Recorders and Medicare Systems (RMS) (P) Ltd, Panchkula, Haryana, India.

Stress enzymes activity measurement

Blood samples (5ml) were collected biweekly from each goat throughout the period of the study (from the period of conditioning to three months after kidding). Blood sample (n = 120) were collected for three months (from a week after conditioning until the day of oestrous synchronisation), throughout the period of pregnancy (n = 200) (after ultrasonographic establishment of pregnancy until the day of kidding) and for three months after kidding (n = 120) (starting from a week postpartum). Blood sample collection was carried out biweekly in all the three stages. A total of 20 samples were collected at every sampling moment throughout the stages. Blood samples were collected aseptically from the jugular vein, dispensed in plane sample bottles, allowed to clot at room temperature and centrifuged at 4000 revolutions per minute for ten minutes to obtain serum.

Glutathione peroxidase activity was determined using Cayman's Glutathione Peroxidase Assay Kit (Cayman Chemicals, Michigan, USA) following manufacturer's instructions. Superoxide dismutase was assayed using Cayman's Superoxide Dismutase Assay Kit (Cayman Pharmaceuticals, Neratovice, Czech Republic) while catalase activity was determined using Cayman's Catalase Assay Kit (Cayman Chemicals, Michigan, USA) following manufacturer's instructions.

Hormonal assay

Serum total triiodothyronine (tT3) concentration and total thyroxine (tT4) were determined by competitive enzyme immunoassay (Type 5) technique as described

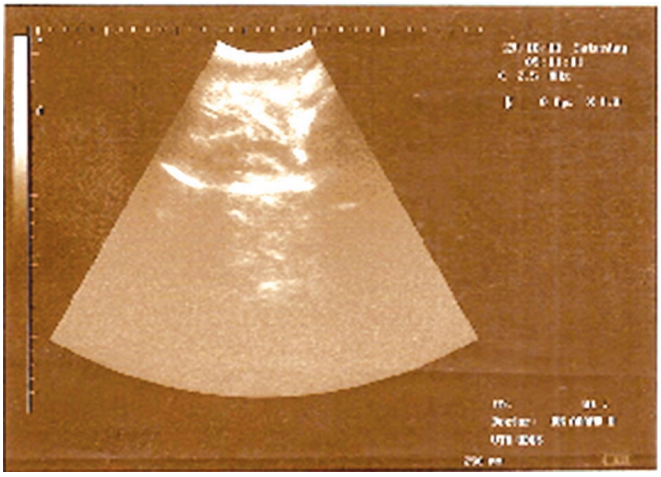

Fig. 1. Isogenic appearance of the uterine lumen indicating open uterus.

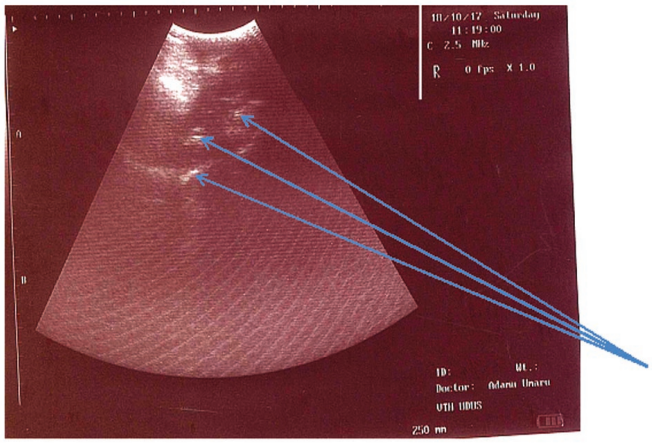

Fig. 2. Hypoechoic appearance with hyperechoic structures indicating fetal structures as pointed by the arrows.

previously by Kozwich *et al*. (1991) using tT3 AccuBind™ ELISA test kit and by Chopra *et al*. (1971) using tT4 AccuBind™ ELISA test kit manufactured by Monobind Inc. Lake Forest, California, USA respectively. The tT3 AccuBind™ ELISA test kit has a sensitivity of 95% and a specificity of 99.9%. The intra- assay coefficient of variation (CV %) at low, normal and high levels were 7.9%, 5.4% and 3.9% respectively while the respective inter -assay coefficient of variation (CV %) at low, normal and high levels were 8.9%, 6.7%, and 4.5%. The tT4 AccuBind™ ELISA test kit has a sensitivity of 95% and a specificity of 98%. The intra -assay coefficient of variation (CV %) at low, normal and high levels were 2.3%, 1.6% and 1.3% respectively while the respective inter -assay coefficient of variation (CV %) at low, normal and high levels were 6.3%, 6.1%, and 7.5%. Serum thyroid stimulating hormone concentration was determined by immunoenzymometric assay technique as described previously by Hopton and Harrap (1986) using TSH AccuBind™ ELISA test kit manufactured by Monobind Inc. Lake Forest, California, USA. The kit has a sensitivity of 95% and a specificity of 99.9%. The intra -assay coefficient of variation (CV %) in three different pools were 8.1%, 6.4% and 6.6% respectively while the inter -assay coefficient of variation (CV%) were 9.3%, 7.7%, and 5.9%. These sensitivities and specificities were obtained from the manufacturer's evaluation.

Sodium, potassium and calcium estimation

This was carried out using ion selective electrode method as described previously by Fogh-Anderson *et al*. (1984) using GE 500 Genrui Electrolyte Analyzer (Genrui Biotech Shenzhan, China). Estimation of calcium was carried out using O-cresolphthalein complexone method as described previously by Cohen and Sideman (1979) and red at 575 nm using UNICO 1201 spectrophotometer.

Data analysis

Statistical package for social sciences (SPSS) version 22 was used to analyze the data obtained. ANOVA was used to compare between the reproductive periods with Tukey post hoc test; where $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The cutaneous ultrasound scan revealed isogenic appearance of the uterine lumen indicating an open uterus in goats before pregnancy and after kidding as presented in Fig. 1. Circumscribed hypoechoic region (fluid accumulation) with a central hyperechoic structure (foetus) was recorded in pregnant goats as presented in Fig. 2.

The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and the comparison between the periods before pregnancy, during pregnancy and after pregnancy are presented in Fig. 3, 4 and 5 respectively.

The SOD activity decreased significantly ($p<0.05$) during pregnancy compared to the period before pregnancy. There was a significant ($p<0.05$) increase in SOD activity after pregnancy compared to the periods before pregnancy and during pregnancy. Activity of GPx was significantly ($p<0.05$) higher during pregnancy compared to the period before pregnancy. There was also a significant ($p<0.05$) rise in GPx activity after pregnancy compared to the period before pregnancy. Catalase activity did not show any significant difference between the three periods.

Serum levels of thyroid stimulating hormone (TSH), total triiodothyronine (tT3) and total thyroxin (tT4) and their comparison between the periods before pregnancy, during pregnancy and after pregnancy are presented in Fig. 6, 7 and 8 respectively. The TSH was significantly ($p<0.05$) higher during pregnancy compared to the periods before pregnancy and after pregnancy. Similarly, there was a significant ($p<0.05$) increase in TSH after pregnancy compared to the period before pregnancy. Serum T3 concentration rose significantly ($p<0.05$) during pregnancy compared to the period before pregnancy. In addition, there was also a significant ($p<0.05$) increase in serum T3 after pregnancy compared to the periods before pregnancy and during pregnancy. Serum T4 concentration was significantly ($p<0.05$) higher during pregnancy compared to the period before pregnancy and after pregnancy. However, it was significantly ($p<0.05$) lower after pregnancy compared to the periods before pregnancy and during pregnancy.

Values of serum Na^+, K^+ and Ca^{2+} concentrations and their comparison between the periods before pregnancy, during pregnancy and after pregnancy are presented in Fig. 9, 10 and 11 respectively. Serum levels of Na^+ decreased significantly ($p<0.05$) during pregnancy compared to the period before pregnancy. In addition, Na+ increased significantly ($p<0.05$) after pregnancy. Serum levels of K^+ also decreased significantly ($p<0.05$) during pregnancy compared to the period before pregnancy. In addition, serum K^+ level increased significantly ($p<0.05$) after pregnancy compared to the period before pregnancy. There was a significant ($p<0.05$) decrease in serum Ca^{2+} level during pregnancy compared

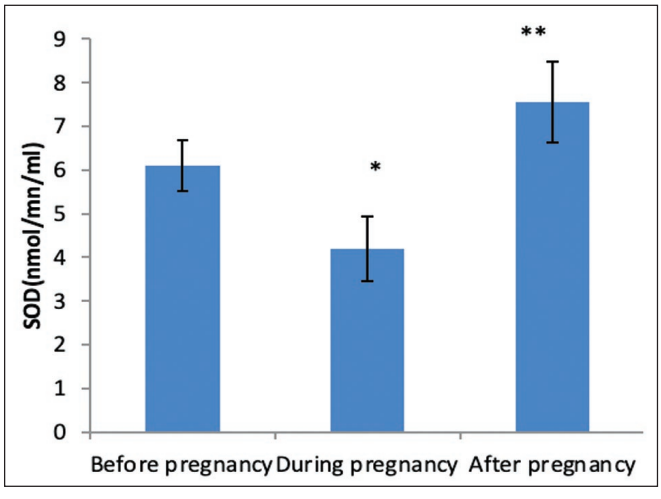

Fig. 3. Activity of Superoxide dismutase (SOD) of Red Sokoto goat before, during and after pregnancy.
(*= $p<0.05$ during pregnancy vs before pregnancy, ****** = $p<0.05$ after pregnancy vs before pregnancy).

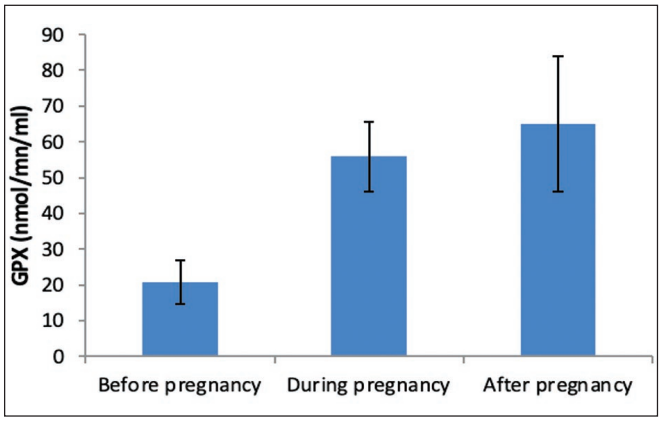

Fig. 4. Activity of Glutathione peroxidase (GPx) of Red Sokoto goat before, during and after pregnancy.
(* = $p<0.05$ after pregnancy vs before pregnancy).

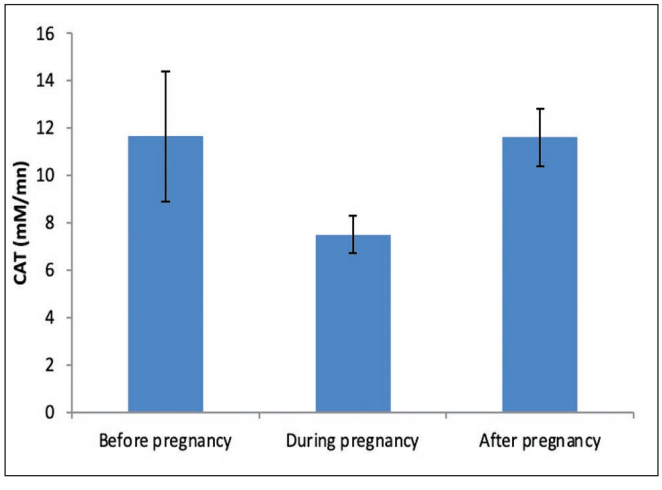

Fig. 5. Activity of Catalase (CAT) of Red Sokoto goat before, during and after pregnancy.

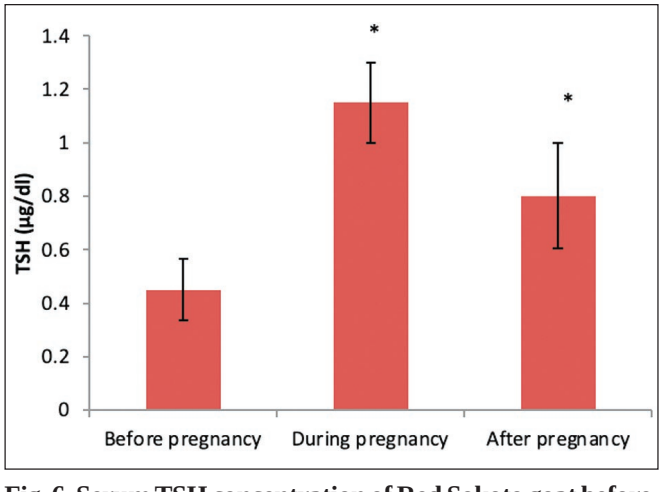

Fig. 6. Serum TSH concentration of Red Sokoto goat before, during and after pregnancy.
(*= $p<0.05$ during pregnancy and after pregnancy vs before pregnancy).

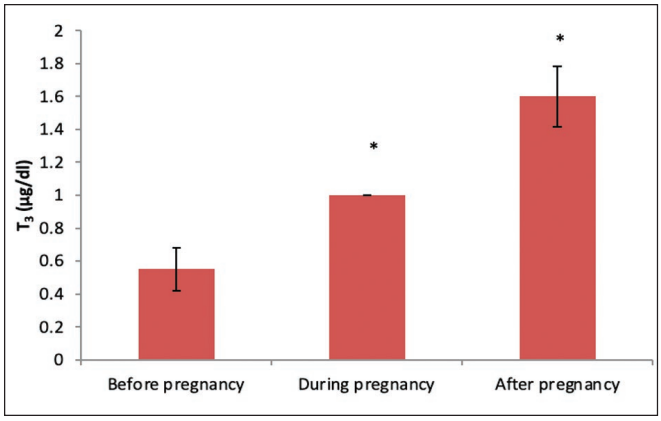

Fig. 7. Serum T_3 concentration of Red Sokoto goat before, during and after pregnancy.
(*= p<0.05 during pregnancy and after pregnancy vs before pregnancy).

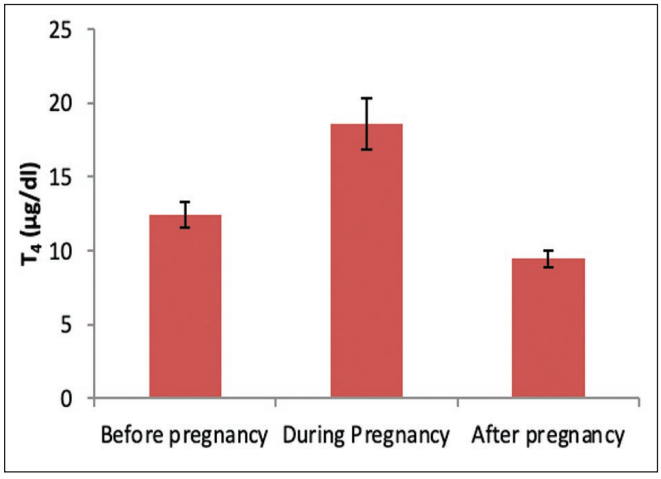

Fig. 8. Serum T_4 concentration of Red Sokoto goat before, during and after pregnancy.
(*= p<0.05 during pregnancy vs before pregnancy, ** = p<0.05 after pregnancy vs before pregnancy).

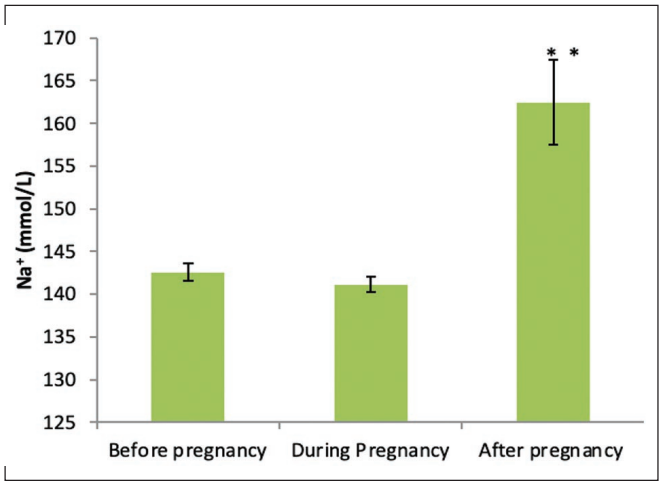

Fig. 9. Serum Na^+ concentration of Red Sokoto goat before, during and after pregnancy.
(*= p<0.05 during pregnancy vs before pregnancy, ** = p<0.05 after pregnancy vs before pregnancy).

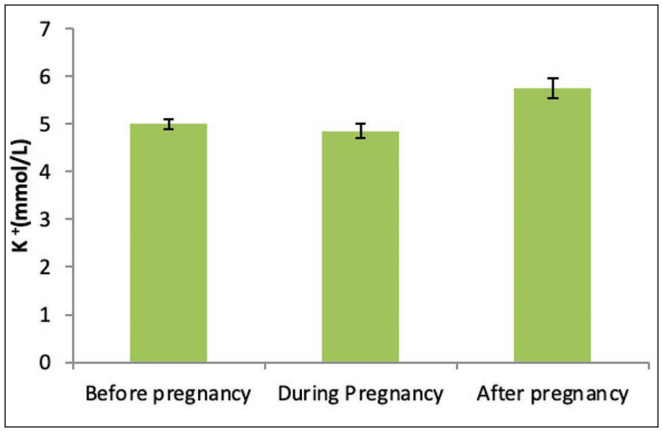

Fig. 10. Serum K^+ concentration of Red Sokoto goat before, during and after pregnancy.
(*= p<0.05 during pregnancy vs before pregnancy, ** = p<0.05 after pregnancy vs before pregnancy).

to the period before pregnancy. So also, there was a significant (p<0.05) decrease in serum Ca^{2+} level after pregnancy compared to the periods before pregnancy and during pregnancy

The study showed variable fluctuation of oxidative stress enzymes activities, thyroid hormones and electrolytes at different reproductive stages. These fluctuations are more pronounced after pregnancy. The significant increase in SOD and GPx activities after pregnancy compared to the period before pregnancy could be due to increase in nutritional and oxygen demand for lactogenesis and maternal sustenance (Bernabucci *et al*. 2002). This causes an increase in circulating levels of metabolic hormones and increased carbohydrate and lipid metabolism (Bernabucci *et al*. 2005, Boustra *et al*. 2008), which results in generation of reactive oxygen species and other free radicals leading to lipid peroxidation and increase in stress enzymes activities (Celi *et al.* 2010, Ognik *et al.* 2010). This contradicts the report of Carmen *et al*. (2020) in Mediterranean breeds, which could be due to difference in environmental condition. Sokoto is in the tropical region and heat stress could influence oxidative stress enzymes activities. It also contradicts the findings of Al-Hassan *et al*. (2016) in Aradi goats, which could be due to the difference in the duration of sampling. Al-Hassan *et al.* (2016) sampled for a month after parturition which could not give clear information on prolonged changes after pregnancy. The significant decrease in SOD activity during pregnancy compared to before and after pregnancy

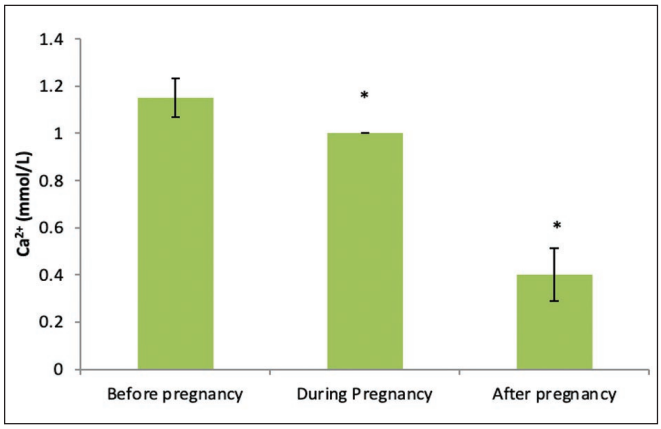

Fig. 11. Serum Ca^{2+} concentration of Red Sokoto goat before, during and after pregnancy.
(*= $p<0.05$ during pregnancy and after pregnancy vs before pregnancy).

could be attributed to increased superoxide anions generation by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during protein folding throughout pregnancy (Raijmakers *et al.* 2006). This agreed with the findings of Ghneim *et al*. (2016). CAT activity was not affected by reproductive status. This is in disagreement with the findings of Nawito *et al*. (2016) in Sinai goats, which could be attributed to the type of feed given to the animals. Nawito *et al*. (2016) fed Sinai goats with concentrate which had higher nutritive value than wheat bran and cellular utilisation of nutrients enhances the release of free radicals that could affect CAT activity.

The significant increase in TSH, T3 and T4 during pregnancy compared to the period before pregnancy could be attributed to the effect of oestrogen during pregnancy; oestrogen causes a rise in glycosylation of thyroid binding globulin (TBG) by stimulating the expression of TBG in the liver. High level of TBG causes a decrease in the concentration of free T4 in circulation, this triggers the release of TSH by the pituitary gland which stimulates the thyroid gland to release T3 and T4 thereby elevating the blood level of these hormones (Pittas and Lee 2003). This agrees with the findings of (Santin and Furlanetto 2011, Brent 2012). Increase in T3 and T4 could also be as a result of foetal demand for iodide which is obtained predominantly from maternal thyroid hormone through degradation of T3 and T4 by placental deiodinase (Lazarus *et al.* 2012). It could also be attributed to the stimulating effect of human chorionic gonadotropin on TSH receptors on the thyroid gland which also causes an increase in T3 and T4 release by the thyroid gland (Lazarus *et al*. 2012). This increase in T3 and T4 could also be associated with increased maternal glomerular filtration because iodine is passively excreted during pregnancy (Brent 2012). The increase in T3 concentration during pregnancy could also be as a result of increased energy demand for foetal development because T3 directly stimulates feed intake at the hypothalamus (Todini 2007). The elevated level of TSH and T3 after pregnancy compared to the period before pregnancy could be due to continuous supply of neonate iodine requirement by maternal source through milk by the action of sodium iodide symporter (NIS) in the myoepithelial cells (Pearce *et al*. 2007). This agrees with the findings of Stuebe *et al*. (2015) and Kurioka *et al*. (2005) but contradicts the finding of Raoofi *et al*. (2017) which could be due to genetic modification in Beetal-cross that predisposes the breed to genetic goitre. It also contradicted the finding of Teleb *et al.* (2019) which could be due to species variation and the interval between the periods of sample collection. Samples collected a month before parturition and a month after may not give the true reflection of the physiologic changes of the entire periods during pregnancy and lactation. The decrease in T4 concentration after pregnancy compared to the period before pregnancy could be due to the breakdown of T4 to obtain iodine to meet neonatal demand. This agrees with the findings of Stuebe *et al.* (2015) and Kurioka *et al.* (2005).

The significant increase in Na^+ and K^+ after pregnancy compared to the period before pregnancy and during pregnancy could be attributed to the demand for Na^+ during parturition as Na^+ and K^+ play significant role in enhancing myometrial and skeletal muscle contraction during parturition (Moen *et al*. 2009). This contradicts the finding of Mohammed *et al*. (2017) which could be attributed to the difference in the type of feed source of the animals. Mohammed *et al.* (2017) sampled animals at different seasons. Seasonal variation affects vegetation and determines the availability of feed that could affect electrolyte levels. The increase in Na+ after pregnancy compared to the period before pregnancy and during pregnancy could also be due to hypovolemia as a result of blood loss during parturition. Na+ is an important electrolyte in the extracellular environment that moves along with water to increase the vascular fluid volume (Saroja *et al*. 2013). It could also be attributed to the regulatory function of the kidney. Following blood loss during parturition, the kidney conserves water thereby retaining Na^+ (Cheung and Lafayette 2013). This contradicts the finding of Mbassa and Poulsen (1991) which could be attributed to breed difference and the parity status of the animals used. Mbassa and Poulsen

(1991) studied Danish landrace dairy goats at different parity status that could have developed adaptive electrolytes regulatory mechanisms. It also contradicts the report of Kumar and Kaur (2017) which could be due to species variation and age of the animals used. The significant decrease in Ca^{2+} concentration during pregnancy compared to the period before pregnancy could be due to foetal demand of Ca^{2+} for skeletal development (Kumar and Kaur 2017, Almaghamsi *et al*. 2018). It could also be associated with decreased serum albumin which also causes decrease in Ca2+ level (Hussain *et al.* 2001, Bjournerem *et al*. 2011, Goyal 2020). It could also be due to increased Ca2+ excretion during pregnancy (Kovacs and Fuleihan 2006, Kumar and Kaur 2017). This coincides with the findings of Mohammad *et al.* (2017). The decrease in the level of calcium after pregnancy compared to the period before pregnancy could be associated with Ca^{2+} depletion as it is mobilized during lactogenesis (Canal- Macias *et al*. 2013, Kovacs 2017, Almaghamsi *et al*. 2018). This contradicts the finding of Carmen *et al.* (2020) which could be ascribed to the type of ecosystem where the research was carried out. Carmen *et al*. (2020) studied in the Mediterranean region where herbages have higher mineral contents that could result in increased serum levels of electrolytes.

CONCLUSION

The study aimed at establishing changes in thyroid hormone levels, electrolyte levels and oxidative stress enzymes activities at different reproductive stages. It was discovered that these changes are prominent after parturition. Attention should be given to nutritional adjustment and supplementation to red Sokoto goat after parturition.

ACKNOWLEDGEMENT

Authors acknowledge the contribution of Yusuf Yakubu of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

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*__Cite this article as:__ Saidu B, Mojiminiyi FBO, Alawa CBI, Onwuchekwa C, Ja'afaru AI, Dahiru A, Abdulazeez N, Garaba B, Erebioga AS, Adamu U, Abdullahi AM (2021) Comparative evaluation of oxidative stress enzymes, thyroid hormones and electrolytes at different reproductive stages of Red Sokoto goat in Sokoto, Nigeria. Explor Anim Med Res 11(1): 80-88. DOI : 10.52635/EAMR/11.1.80-88