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ARTICLE

Quantified Effects of Late Pregnancy and Lactation on the Osmotic Stability of Sahel Goat Erythrocytes

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ABSTRACT

Pregnancy and lactation are physiological states mediated by metabolic and endocrine factors capable of affecting erythrocyte osmotic stability. Alteration in erythrocyte osmotic fragility (EOF) during late pregnancy and lactation was investigated in 46 apparently healthy adult Sahel goats weighing 18-30 kg consisting of 16 non-pregnant dry (NPD), 15 pregnant (PRE) and 15 lactating (LAC) animals. The PRE and LAC were in third trimester and nursing periods, respectively. Packed cell volume, erythrocyte count, mean corpuscular volume and EOF were determined with heparinised venous blood using standard methods. Erythrocyte parameters of NPD, PRE and LAC did not vary significantly ($p > 0.05$). The mean fragilities of NPD, PRE and LAC varied significantly ($p < 0.05$) at saline concentration of 8 g/L, with left and right shifts of PRE and LAC fragility curves from NPD curve at saline concentrations of 7-8 g/L, respectively. At 10-70% haemolysis, mean saline concentrations increased ($p < 0.05$) by 0.31 ± 0.07 (0.2-0.4) g/L for LAC and decreased ($p < 0.05$) by 0.19 ± 0.09 (0.1-0.3) for PRE from mean values for NPD, so that the aggregate shift of mean saline concentrations between PRE and LAC was 0.50 ± 0.10 (0.40-0.70) g/L. EOF decreased in late pregnancy and increased during lactation, perhaps, due to changes in the composition of erythrocyte membranes associated withthe physiologic states.

Keywords: Erythrocyte osmotic stability, osmotic fragility, late pregnancy, lactation, Sahel goat.

INTRODUCTION

Erythrocyte osmotic fragility (EOF) is evaluated by estimating haemolysis when erythrocytes are exposed to various hypotonic concentrations of buffered saline and comparative studies involving EOF is more robust with estimates of saline concentration at regular endpoints of haemolysis (Igbokwe and Igbokwe, 2015). EOF is affected by erythrocyte membrane fluidity (Moretti *et al*., 2002) and deformability (Chung *et al*., 1998). The cell membrane consists of a lipid bilayer with the peripheral proteins being responsible for its plasticity and deformability (Tanner, 1983). Length of fatty acid chains (Mineo and Hara, 2005), class of phospholipids, type and quantity of fatty acids, ratio of cholesterol to phospholipids and degree of saturation of phospholipids with fatty acids in erythrocyte

membrane can affect its fluidity (Hagve *et al*., 1993; Olver *et al*., 2010). Alterations in concentrations and interactions of lipids and proteins in erythrocyte and activities of ATPases, anion transport protein, protein kinases, glyceraldehyde-3-phosphate dehydrogenase and acetylcholinesterase membrane could influence the deformability of the membrane (Delaunay, 1977; Blasiak *et al*., 1991). A negative correlation has been reported between haemolytic end points of EOF and ratio of free cholesterol to phospholipids (Horii *et al*., 1981). Cholesterol is a neutral lipid in cell membranes that stabilizes membrane structure and reduces membrane permeability (Brukdorfer *et al*., 1969; Kroes and Ostwald, 1971; Bloom and Mouritsen, 1988).

Changes in many biochemical parameters occurring during pregnancy may influence erythrocyte stability (Lockitch and Gamer, 1997). Progesterone, produced by corpus lutuem and placenta, is the major hormone that maintains pregnancy, but estrogen, prolactin, lactogen and relaxin are also produced by the dam and fetal gonads during late pregnancy (Davidson and Stabenfeldt, 2007). Progesterone interacts with soluble protein components of the membrane to improve the stability of erythrocyte membrane (Devenuto *et al*., 1969) and the hormone has been reported to reduce EOF of human erythrocytes (Kaya and Saito 1985; Yoong *et al*., 2003). Women in the third trimester of pregnancy had increased erythrocyte fragility by incubated glycerol lysis time when compared with non-pregnant ones (Magid *etal*., 1982), but no significant difference in EOF was reported between pregnant and non-pregnant women (Suhail *et al*., 2010). EOF had been assessed in studies of pregnant women (Nakamura, 1983; Arora *et al*., 1994; Emembolu and Mba, 1994). Erythrocyte membrane deformability progressively decreases in pregnant women, but increases after delivery (Lukacin *et al*., 1996). Pregnant West African dwarf (WAD) sheep seemed to have higher osmotic resistance than dry and lactating ones (Durotoye, 1987). No significant variations in EOF were attributed to pregnancy and lactation in Red Sokoto goats (Habibu *et al*., 2014).

After parturition, lactation and nursing periods are commenced with endocrine and metabolic changes occurring in the animals which may affect erythrocyte membrane composition and stability (Torres *et al*., 2002). Prolactin, growth hormone and thyroid hormone are required for initiation and sustenance of lactation, while progesterone is suppressed (Forsyth, 1986) and essential nutrients taken by the dam are used for milk synthesis (Torres and Trugo, 2009).

The present research was designed to investigate whether the physiological states of late pregnancy and lactation in Sahel does would influence the osmotic stability (fragility or resistance) of the erythrocytes.

MATERIALS and METHODS

Animals:

Apparently healthy Sahel goats that were managed semi-intensively in the university livestock farm, consisting of 16 non-pregnant dry (NPD), 15 pregnant (PRE) and 15 lactating (LAC) does, were selected for the study. Physiological states of no pregnancy, late (third trimester) pregnancy and lactation were ascertained by physical examination of the animals' abdomen and mammary glands, checking farm records and interview of farm staff. The animals were offered water and salt lick, fed with cereal offal, grass and legume hays within the pens, and allowed to graze and browse for up to 6 h daily in the surrounding sahelian bushes outside the fence perimeter. They had estimated age of 2.6 ± 0.6 (1-3½) years by dental examination (Chibuzo and Silvalchevan, 1994) and weighed 26.8 ± 5.3 (18-30) kg.

Blood sample collection:

Blood sample (5ml) was collected early in the morning from the external jugular vein of each doe using syringe and needle and put into plastic tubes containing lithium heparin (Silver Health

Diagnostics, Nigeria). The samples were transported to the laboratory in ice packs and analysed within four hours.

Determination of erythrocyte parameters:
Packed cell volume (PCV) and erythrocyte count (EC) were determined using microhaematocrit and haemocytometric methods, respectively; while mean corpuscular volume (MCV) was calculated from PCV and RBC values using standard formula (Schalm *etal*., 1975).

Determination of erythrocyte osmotic fragility (EOF):
The technique for determining EOF was described by Parpart *et al*. (1947)asupdated by Ochei and Kolhatkar (2007). A stock solution of 10% buffered saline was prepared as follows: 90.0g sodium chloride (NaCl), 13.65g disodium hydrogen phosphate (Na_2HPO) (BDH, England), 2.34g sodium dihydrogen phosphate (NaH_2PO_4) (BDH, England), were dissolved and made up to 1 L with deionized distilled water. The working solution of 1% NaCl was prepared by dilution of the stock solution from which other lower concentrations of saline were prepared. The dilutions were made using the formula reported by Igbokwe and Igbokwe (2015). Each test tube contained 5 mL of isotonic or series of hypotonic saline or deionised distilled water (5 mL),had an aliquot of blood sample (5 µL) from each doe added to it, mixed by inversion and allowed to stand for 30 min under room temperature (35-38°C). After centrifugation of the tubes at 3000 × *g* for 15 min, the supernatant of the hemolysate in each tube was harvested with suction pipette into a curvette and the haemoglobin colour was estimated as absorbance units with a spectrophotometer (ALL PRO, Shibei, Qingdao, China) set at 540 nm, with the supernatants of the tubes containing isotonic saline and deionized distilled water serving as blank (0%) and complete (100%) haemolysis, respectively. The degree of haemolysis (%) at each level of dilution was calculated (Ochei and Kolhatkar, 2007; Igbokwe and Igbokwe, 2015). The haemolytic endpoints (% haemolysis) obtained for each blood sample of an animal were plotted on a coordinate graph against saline concentrations. From the EOF curve (fragiligram), saline concentrations at 10% intervals of haemolytic endpoints (10-90%) were obtained.

Data analysis:
Data were summarized as means ± standard deviations. Means were compared by one-way ANOVA with Turkey post-hoc test using computer software (GraphPad Instat, version 3.05, 1992-2000, GraphPad Software Inc, USA).

RESULTS
Effects of late pregnancy and lactation on mean body weight, PCV, RBC and MCV of Sahel does are in Table I. PRE had significantly ($p<0.05$) higher mean body weight than NPD and LAC; and mean body weight of LAC was higher ($p<0.05$) than that of NPD. Mean PCV, RBC and MCV of NPD, PRE and LAC did not vary significantly ($p > 0.05$).

TABLE I: EFFECTS OF LATE PREGNANCY AND LACTATION ON BODY WEIGHT, PACKED CELL VOLUME, ERYTHROCYTE COUNT AND MEAN CORPUSCULAR VOLUME OF SAHEL DOES

	Non pregnant dry (n=16)	Pregnant (late)(n=15)	Lactating (n=15)
Age (Year)	2.37±0.79[a]	2.80±0.75[a]	2.78±0.37[a]
Body weight (kg)	22.94±3.39[a]	32.21±4.57[b]	26.80±4.54[c]
Packed cell volume (%)	33.86±4.11[a]	32.07±2.40[a]	31.47±3.11[a]
Erythrocyte count ($x10^{12}$/L)	13.86±2.68[a]	12.21±1.68[a]	12.60±1.88[a]
Mean corpuscular volume (fL)	24.44±3.81[a]	26.52±2.36[a]	25.14±1.49[a]

[a,b,c]Means ± standard deviations with different superscripts are significantly ($p<0.05$) different

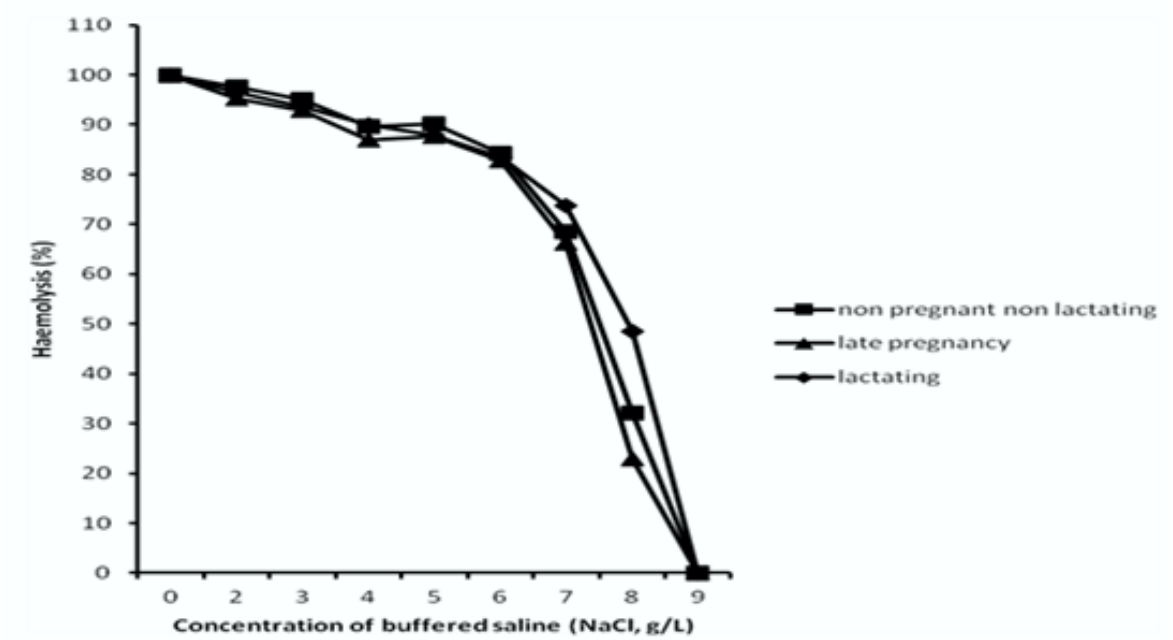

Fig. 1: Fragiligram of erythrocytes of Sahel does at various physiological states in buffered saline media.

The EOF curves of NPD, PRE and LAC in Fig. 1 showed that they were hyperbolic, without remarkable shift of PRE and LAC curves from NPD curve at saline concentrations of 2-6 g/L, but with left and right shifts of PRE and LAC curves, respectively, from NPD curve at saline concentrations of 7-8 g/L. The mean fragilities of NPD, PRE and LAC varied significantly ($p < 0.05$) at saline concentration of 8 g/L, with values of 48.5 ± 15.2%, 32.1 ± 19.4% and 22.8 ± 21.0% for LAC, NPD and PRE, respectively.

The effects of late pregnancy and lactation on saline concentrations at which 10-90% haemolysis occurred are summarized in Table II. PRE and LAC had comparable and equally lower ($p < 0.05$) saline concentrations than NPD at haemolytic endpoint of 90%, but the saline concentrations did not vary significantly ($p > 0.05$) at 80% haemolysis. The saline concentrations at which the haemolysis of 10-70% occurred were significantly ($p < 0.05$) higher for LAC than NPD and lower ($p < 0.05$) in PRE than NPD. Mean saline concentrations responsible for the 10-70% haemolysis increased by 0.31 ± 0.07 (0.2-0.4) g/L for LAC

and decreased by 0.19 ± 0.09 (0.1-0.3)g/L for PRE from those of NPD and these quantitative shift was significantly (p = 0.0003) higher in LAC than PRE. The aggregate shift of mean saline concentrations causing various levels of haemolysis between PRE and LAC was 0.50 ± 0.10 (0.40-0.70) g/L.

DISCUSSION

PRE weighed more than NPD and LAC apparently because of the pregnancy and LAC weighed more than NPD probably due to delay in uterine involution and expansion of the mammary glands. Erythrocyte parameters (PCV, RBC, MCV) of does were within the normal range for the species (Jain, 1993) and were not affected by changes in physiological states indicating that hormonal changes associated with these states did not interfere with erythropoietic processes. Similarly, PCV was not affected by pregnancy in Sahel (Waziri *et al*., 2010) and Red Sokoto goats (Habibu *et al.,* 2014). The PCV in pregnant WAD ewes was higher when compared to that of non-pregnant ones (Durotoye and Oyewale, 2000). Also, PCV was higher in lactating than pregnant Red Sokoto does (Habibu *et al.,* 2014). Higher MCV was reported in Baladi does during late pregnancy (Azab and Abdel-Maksoud, 1999) and Red Sokoto does during lactation period (Habibu *et al*., 2014). It was observed that the alterations in EOF of PRE and LAC were not associated with any variation in erythrocyte parameters pointing to other variables related to the physiological state as probably responsible for the changes in membrane stability.

TABLE II: EFFECTS OF LATE PREGNANCY AND LACTATION ON CONCENTRATIONS OF BUFFERED SALINE CAUSING VARIOUS LEVELS OF HAEMOLYSIS OF SAHEL GOAT ERYTHROCYTES

	Concentration of buffered saline (NaCl, g/L) at various physiological states of does		
Haemolysis (%)	**Non pregnant dry(n=16)**	**Pregnant (late)(n=15)**	**Lactating(n=15)**
90	4.80±0.15[a]	4.60±0.15[b]	4.50±0.17[b]
80	6.20±1.00[a]	6.00±0.13[a]	6.00±0.15[a]
70	6.80±0.07[a]	6.50±0.08[b]	7.00±0.06[c]
60	7.20±0.06[a]	6.90±0.07[b]	7.60±0.04[c]
50	7.50±0.04[a]	7.40±0.04[b]	7.90±0.03[c]
40	7.80±0.04[a]	7.70±0.04[b]	8.10±0.03[c]
30	8.00±0.04[a]	7.90±0.04[b]	8.30±0.02[c]
20	8.30±0.03[a]	8.10±0.04[b]	8.50±0.01[c]
10	8.50±0.03[a]	8.30±0.04[b]	8.80±0.01[c]

[a,b,c]Means ± standard deviations with different superscripts are significantly ($p<0.05$) different

The increase in erythrocyte osmotic stability during late pregnancy in Sahel goats compared to non-pregnant ones was considered a physiological adjustment to the plasma environment charged with elevated reproductive hormones (Davidson and Stabenfeldt, 2007) and lipids (Sandabe *et al*., 2004; Qureshi *et al*., 1999; Nazifi *et al*., 2002; Waziri *et al.,* 2010; Hafid *et al*., 2013). Progesterone may increase osmotic resistance

of erythrocytes (Kaya and Saito, 1985; Yoong *et al*., 2003) by affecting the function of erythrocyte membrane proteins (Devenuto *et al*., 1969). Estrogen has not been reported to have any effect on EOF in female Sahel goats, but it can potentially reduce erythrocyte stability (March *et al*., 1966) and therefore may not have played a role in the decrease in EOF of PRE. Increased plasma cholesterol during late pregnancy in Sahel does (Sandabe *et al*., 2004; Waziri *et al*., 2010) due to lipolysis (Diderholm *et al*., 2005) is expected to increase the cholesterol content of erythrocyte membrane which would produce a stabilizing effect on the membrane by inhibition of transmembrane cation transport mediated through decrease in activities of cation pumps and Na^+ K^+ cotransport (Lijnen and Petrov, 1995).

During lactation, EOF was increased to a greater extent than it was decreased in PRE. The role of hormones that sustain lactation was pertinent in the reducing stability of the erythrocyte membrane. Prolactin alters erythrocyte function by enhancing erythrocyte sodium content (Gopalakrishnan *et al*., 1980) which may enhance erythrocyte swelling by obligatory water diffusion into the cell. Thyroid hormone reduces plasma cholesterol concentration (Greco and Stabenfeldt, 2007) which would cause a depletion of membrane free cholesterol. Growth hormone decreases the cholesterol-phospholipid molar ratio (Leese *et al*., 2000). After parturition and transition into lactation period, plasma concentration of cholesterol and trigycerides decrease from the prepartum levels (Qureshi *et al*., 1999; Nazifi *et al*., 2002). EOF was reported to be increased after depletion of erythrocyte membrane cholesterol (Bruckdorfer *et al*., 1969). A decrease in erythrocyte cholesterol corresponded with an increase in EOF; while serum free cholesterol and cholesterol-phospholipid molar ratio were inversely correlated with EOF (Sagawa and Shiraki, 1980; Horri *et al*., 1981).

CONCLUSION

Late pregnancy and lactation did not affect PCV, MCV and RBC values of Sahel goats. Osmotic stability of erythrocyte membrane increased in late pregnancy and decreased during lactation in comparison to non-pregnant dry does, suggesting that EOF of Sahel goats in hypotonic buffered saline solutions was affected by pregnancy and lactation, probably, due to changes in erythrocyte membrane composition associated with these physiological states. Therefore, the susceptibility of erythrocytes to lysis in disease conditions of the animals affecting membrane stability may be influenced by these physiological states.

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