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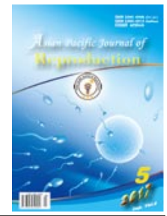

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Anion gap as bio-diagnostic index in prediction of dystocia, retained placenta and delayed uterine involution of Baladi goat (Field study)

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ABSTRACT

Objective: To assess the anion gap (AG) validity in prediction the goat's fertility before parturition. **Methods:** This study was carried out on two groups (20/20, aged 4–6 years and weighted 30–43 kg) of pluriparous pregnant Baladi goats. Animals were subjected to clinical examination, blood sampling to assess serum biochemical parameters in correlation with utrasonographic assessment of uterine horn involution (UTI) and placental dropping time after kidding. Unpaired student *t*–test was used to detect the significance of differences (P<0.05). Correlation coefficient was used to study the relationships among the different assessed parameters, analysis (r) was assigned at P<0.05 using the same statistics program. **Results:** Results revealed that there were highly significant differences (P<0.05) between normally and abnormally parturated goats concerning Cl^-, K^+, Na^+, P, Mg^{2+}, Total/Ca^{2+}, albumin, glucose and AG. Goats suffered from dystocia having high levels of HCO_3^-, cortisol and lactate. Additionally, there were significant differences (P<0.05) between normally and abnormally parturated goat does regarding the process of UTI starting from 3rd to 10th wk after parturition. Furthermore, there was a highly significant difference (P<0.05) between normally and abnormally parturated goat does concerning their placental dropping time; where, distressed/abnormally parturated goat having the longest time. Furthermore, there were highly strong correlation coefficient between Mg^{2+}, K^+, Na^+, HCO_3^-, glucose, albumin, AG and corrected AG concentrations during goat's prepartum period and UTI, placental dropping time. **Conclusions:** Reporting serum bio-chemical changes and AG during goat's prepartum period could be used as diagnostic tool to judge the normality of parturition process, placental dropping, UTI and genital health, in other word, could be used as predictor for goat's fertility prior to parturition.

1. Introduction

Goats are considered one of the important pillars of the Egyptian national security strategies, because they are a building block in their animal production that intended for meat and milk production. Moreover, goats could be raised in small or medium sized herds and play an important role in animal agriculture eco-system balance. So, all goats producing countries shall intend to increase this animal population by all possible means to improve the standard of living of the rural people.

The transition from pregnancy to motherhood (3 wk before and after parturition) includes metabolic, physiological, and physical changes to accommodate pregnancy, parturition and the onset of

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lactation[1,2]. During this period of gestation-lactation cycle, there are clear changes in the hematological and biochemical status of the animal and these changes are much more dramatic and stressful. Consequently, suboptimal transition during this period can cause economic losses as it impairs reproductive and production performances[3]. Thus, optimal transition during this period requires comprehensive studying for biochemical changes occurring during the periparturient period[4].

The anion gap (AG) is closely related to the acid-base balance and widely used as diagnostic tool in the evaluation of animal metabolic disorders[5,6]. Where, AG equal to the difference between unmeasured anions and unmeasured cations concentrations in serum[7]. So, AG could be defined as the difference between primary measured cations likes Na^+ and K^+, and the primary measured anions as Cl^- and HCO_3^- in serum[8]; and this expressed by the following equation: Serum AG = $(Na^+ + K^+) - (Cl^- + HCO_3^-)$ by Kraut *et al*[9].

The mammalian body is electrically neutral. Electrolytes are present in different body fluids like Na^+, HCO_3^- and Cl^- are mainly in extracellular fluid; while, K^+, Ca^{2+}, Mg^{2+} and PO_4^{3-} are predominately present in the intracellular fluids[10].

Na^+, K^+ and Cl^- "fixed ions" are not metabolized and their balance plays a crucial role in determining acid-base balance in biological fluids[11]. The importance of these ions lies in their indirect roles in maintaining osmotic pressure, acid base homeostasis, pumping mechanisms and integrity cell membranes; as well as in enzyme and nerve function[12]. Calcium is used for nerve conduction, cell signaling and muscle contraction[13]. Cows with hypocalcemia are more likely to develop difficult parturition[14], and uterine prolapse due to loss of uterine muscles tone[15]; additionally, cows suffering from hypocalcemia had an increased concentration of plasma cortisol[16], reduced neutrophils proportion with lowered phagocytic activity[17]. This reduction in immune cell function had linked to periparturient diseases such as retained placenta[18]; and delay uterine involution resulting in increased metritis incidence[19]. These conditions and diseases during the postpartum period have dramatic negative impacts on production profitability and fertility rate of dairy farms[20]. Where, retained placenta affecting production profitability since it delays uterine involution, prolonged calving interval, predisposes ovarian cystic degeneration endometritis, metritis, finally reduces fertility[21-25]. The relationship between haematological and biochemical indices and reproduction is complex and often quite variable. Where, chronic small reductions in pH affect wide range of physiologic processes and may have a substantial impact on development of many diseases[26]. For example, elevated blood pressure with increased insulin resistance were associated with little increases in serum AG and little decreases in bicarbonate levels independent of body size and kidney function even in healthy individuals whose acid-base parameters were within normal limits[27-29].

Therefore, the present study was designed to gain detailed information on commonly measured biochemical parameters in goats during their periparturient period; to ensure that laboratory profiling, not only can detect sick animals, but also those herds at higher risks of developing metabolic or reproductive diseases could be identified. Therefore, extensive pre- and post-parturient hazardous could be captured. Moreover, the study could provide valuable bio-diagnostic tool or index to judge the future goat's fertility before their parturition.

2. Materials and methods

2.1. Animals

This study was carried out on two groups (20/20) of pluriparous pregnant goats (with at least three previous parturition and two or three fetuses at time of check) - Baladi goats (field cases). The selected goats aged 4-6 years and weighted 30-43 kg.

2.2. Clinical examination of pregnant goats

Clinical examination of pregnant goats was done according to Radostits *et al*[30]. Depending on clinical examination of the pregnant during post-parturient period and at time of parturition, animals were classified into two groups. The first group included those goats that did not show any clinical abnormality at time of parturition. The goats in second group showed signs of restlessness, anorexia and dullness (first stage); then weakness, inability to stand, lateral recumbency, with or without muscular tremors with delayed kidding (second stage)[31].

2.3. Blood sampling and analysis

Pregnant goats were bloody sampled from 14 d before anticipated kidding time. Blood samples (10 mL) were collected by jugular vein puncture into anticoagulant free clean vacuum tubes (JieRui Vaccu System, Shandong Weigao, China). Each tube was inverted two or three times to ensure thorough mixing. Blood samples for biochemical analyses were allowed to clot at room temperature within 3 h of collection. The serum samples were stored at -20 ℃ for biochemical studies. Where, serum biochemistry profile was analyzed by Hitachi-917 multichannel analyzer (ROCHE-HITACHI-917) except magnesium level was detected using a commercially available kit (abcam, ab102506)® according the manufacture manual. Cortisol with detected by ELISA procedures using EIA1887, DRG International, Inc. kits.

2.4. Examination of the postpartum goat uterine horn involution (UTI)

Uterine involution was checked weekly by trans-rectal ultrasound examination starting from one week after delivery through complete uterine involution using B-mode ultrasound machine (Eickemeyer, Magic 2200, MN-08101509). The scanner was provided with multi frequency trans-rectal linear transducer 4 and 6 MHz which provided with special design to be suitable for examination of goats per rectum. Examination with adequate restraining before endo-rectal scanning was necessary. Ultrasonic examination was done in the standing position. Feces and air were removed via manual palpation with the aid of lubricated coupling gel. Lubricated transducer (fixed to modified extension rod) was passed into the rectum then moved medially and laterally on the uterine horn to get the maximum diameter that recorded. Complete uterine involution was stated when there was no further reduction in the uterine diameter for two successive examinations[32].

Normally goat's placenta dropped out within 3-5 h after parturition. If the placenta not dropped until 12 h after parturition it considers retained[33].

2.5. Statistical analysis

Results were expressed as means±SE. The analysis of variance between means was analyzed using unpaired student *t*-test to detect the significance of differences. Significance was assigned at $P<0.05$ using Graph Pad Prism software 2007 version 5.03 (Graph Pad Prism, San Diego, CA). Correlation coefficient was used to study the relationships among the different assessed parameters, analysis (*r*) was assigned at $P<0.05$ using the same statistics program.

3. Results

Table 1 revealed that there were highly significant differences ($P<0.05$) between normally and abnormally parturated goats considering Cl^-, K^+, Na^+, P, Mg^{2+}, total/Ca^{2+}, albumin, glucose and AG; reflecting that these serum biochemical parameters were essential for normal parturition. Moreover, low serum AG in Table 1 (14.49±3.29) was associated with dystocia, delayed uterine involution (Table 2) and high incidence of placental retention (11.64±1.64). Furthermore, Table 1 illustrated that female goats suffering from dystocia at parturition had high levels of HCO_3^-, cortisol and lactate compared to normally parturated goat does, and they were significantly different at $P<0.05$. While there was no significant difference between normally and abnormally parturated female goats regarding serum cholesterol, which indicated that cholesterol was not vital for proceeding of normal parturition process in goats.

Table 1
Serum biochemical analysis of pregnant goat before anticipated parturition by 14 d with reference to AG (mean ± SE).

Items of hematological analysis	Healthy/normally parturated goat does	Distressed/abnormally parturated goat does
Cl^- (mmol/L)	106.20±2.13*	99.15±2.40
K^+ (mmol/L)	4.83±0.16***	3.76±0.17
Na^+ (mmol/L)	141.90±2.96**	129.40±2.53
P (mmol/L)	6.98±0.96*	4.21±0.51
Mg^{2+} (mmol/L)	1.35±0.58*	0.96±0.10
Total/Ca^{2+} (mmol/L)	8.46±0.79*	6.21±0.40
HCO_3^- (mmol/L)	19.93±0.72	22.40±0.59*
Cortisol (ng/dL)	16.98±0.92	26.32±1.23***
Albumin (g %)	3.41±0.17***	2.44±0.16
Glucose (mg/dL)	59.05±1.44***	49.82±1.50
Cholesterol (mg/dL)	61.58±4.50	58.55±1.72
Lactate (mmol/L)	1.51±0.15	2.16±0.19*
AG (mmol/L)	24.59±2.77*	14.49±3.29
AGCAPL (mmol/L)	12.39±3.31	5.11±3.53

AGCAPL: corrected anion gap for albumin and phosphate. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ significant differences between groups by student unpaired *t*-test.

Table 2
Trans-rectal utrasonographic assessment of goat uterine horn involution (mean±SE) (cm).

Days after parturition	Healthy/normally parturated goat does	Distressed/abnormally parturated goat does
7	5.38±0.12	5.59±0.10
14	4.06±0.17	4.42±0.14
21	3.11±0.16	4.21±0.12**
28	2.34±0.20	3.97±0.09**
35	1.97±0.09	3.58±0.08***
42	1.81±0.04	3.45±0.06***
49	1.75±0.02	3.06±0.08***
56	1.75±0.02	2.69±0.09***
63	1.73±0.02	2.31±0.10**
70	1.69±0.00	1.86±0.04*
77	1.68±0.00	1.72±0.04
85	1.68±0.00	1.67±0.03

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ significant differences between groups by student unpaired *t*-test.

3.1. Trans-rectal utrasonographic assessment of goat UTI

Table 2 of trans-rectal utrasonographic assessment of goat's uterine horn involution showed that there were significant differences between healthy/normally and distressed/abnormally parturated goats does from 3rd to 10th week after parturition ($P<0.05$). On the other hand, there was no significant difference between the two groups concerning the same item on the 1st and last 2 wk.

3.2. Goat's placental dropping time (h)

Goat's placental dropping time (h) after kidding showed that there was a highly significant difference ($P<0.05$) between healthy/

normally parturated goats does (5.06±1.43) and distressed/ abnormally parturated goats (11.64±1.64).

3.3. Correlation coefficient: (r)

P value of correlation coefficient (*r*) between serum biochemical parameters, uterine involution (cm) and placental dropping time (h) for healthy/normally parturated female goats does showed that there were strong correlations (P<0.05) between Mg^{2+} and UTI at 4th week (r=0.03). HCO_3^- was highly correlated with UTI during the 1st and 2nd wk (r=0.01 for each). Furthermore, albumin was highly correlated with UTI at 3rd and 10th wk (r=0.03 and r=0.00, respectively), which reflected that Mg^{2+}, HCO_3^- and albumin concentrations during the prepartum period of goats were determining factors for completion of UTI in a normal process. Moreover, *P* value of correlation coefficient (*r*) denots that sufficient glucose concentration prior to parturition of female goat was essential for the placental dropping where glucose was highly correlated with placental dropping (r=0.00) in the healthy animals.

Additionally, *P* value of correlation coefficient (*r*) between serum biochemical parameters and uterine involution (cm) for distressed/ abnormally parturated female goats demonstrated the highly significant correlation between K^+, Na^+ and UTI at 11th wk (r=0.01 and r=0.04, respectively). HCO_3^- was correlated with UTI at 2nd and 6th wk (r=0.02 and r=0.01, respectively). Furthermore, cortisol was highly correlated with UTI at 3rd wk (r=0.03). Glucose and lactate were also correlated with UTI at 10th wk after parturition (r=0.01 and r=0.03, respectively). Also, both AG and corrected AG for albumin and phosphate were strongly correlated with UTI at the end (12th wk post-partum) of the involution period (r=0.01 and r=0.01, respectively).

4. Discussion

Serum biochemical profiles had been used extensively by veterinarians to evaluate and prevent many of nutritional and metabolic disorders of ruminants[34]. Even though, little is known about the direct link between serum biochemical profiles and reproductive disorders especially in goats. So, for the first time the results of the current study revealed that any changes in the concentrations of K^+, Na^+, HCO_3^-, cortisol, glucose and lactate out of normal level during the prepartum period drastically affecting the whole puerperium of goats and their future fertility. Moreover, most of these parameters were used for AG calculation and AG is strongly correlated with UTI. Therefore, AG could be used as bio-diagnostic index for judging future goat fertility prior to their parturition.

The present data also demonstrated that goats that are suffering from dystocia at parturition have high levels of HCO_3^- if compared with normally parturated goats, which reflect metabolic alkalosis. While, there was a highly significant decrease serum of Cl^-, K^+, Na^+, Total/Ca^{2+}, albumin, glucose and AG. These results came in harmony with findings of Azab *et al*, Yildiz *et al*, Ruckebusch *et al* and Peek *et al*[35-38]. They found that there was a significant decrease in the plasma Na^+ and K^+ concentrations near and on the day of parturition. They interpreted these findings on bases of ions loss in colostrum or due to hard trials of the animal body to maintain a constant ratio of Na^+ and K^+ in the extracellular fluid during this period.

Electro-neutrality of extracellular fluid must be maintained by reabsorbing an equivalent charge of cations and anions; the reabsorption of chloride and of bicarbonate in the kidneys are contrariwise proportional to each other[30]. Thus, with decreased serum chloride, the kidneys will compensate for the hypochloremia by increasing bicarbonate reabsorption, which may proceed until metabolic alkalosis develops[30]. Moreover, physiologically hypokalemia can also be the consequence of the intracellular movement of K^+ due to metabolic alkalosis[38]. Hypokalemia causes muscle weakness by reducing resting the membranes potential leading to low excitability of neuromuscular tissue[30]. In addition, alkalosis induces functional hypocalcaemia by increasing the binding ability of calcium to albumin as pH increases[39]. In the current study, serum Ca level was highly significant deceased in distressed abnormally parturated goats compared with normally parturated goats. Similar results were stated by Heppelmann *et al*[40] who cited that cows suffering from subclinical hypocalcemia characterized by delayed diminishing of uterine length mainly due to reduction of myometrial contractility compared with cows having normal calcium level. Additionally, Martinez *et al.*[41] found that hypocalcemic cows had an increased plasma cortisol concentration[16], a lowered neutrophils proportion with low phagocytic activity[17], with lowered cytosolic Ca^{2+} concentrations in mononuclear cells[42]. This decrease in immune cell function measures together with hypocalcemia during periparturient period had interrelated with many of post-parturient diseases such as retained placenta[18].

Our data showed that correlation coefficient for distressed abnormally parturated goats revealed that highly significant correlation between K^+, Na^+, HCO_3^-, Ca^{2+} and uterine involution suggested that these parameters are good indicators for early prediction of uterine involution and uterine diseases of pregnant goat during periparturient period.

Magnesium is an important mineral involved in Ca homeostasis, and a decrease in Mg^{2+} concentrations could induce hypocalcemia[43]. Our results showed highly significant decreases in serum Mg^{2+} between normally and abnormally parturated goats. This result was similar to that obtained by previous studies that there was significant decrease in serum magnesium of single and twin pregnant ewes than the control during the last 2 weeks of pregnancy and in the first week of postpartum period[35,36]. The observed decrease in Mg^{2+} level during pregnancy could be related to the physiochemical changes that took place in the blood during pregnancy, which favored the passage of Mg^{2+} from the mother to the fetus[36]. Furthermore, this study clarified that there are highly strong correlation between Mg^{2+} and uterine horn involution. The current results coincided with Rayssiguier *et al*[44] that reported a moderate deficiency of magnesium interfered with normal parturition process and uterine involution as magnesium deficiency impairing oxytocin functions on the uterine contraction during parturition causing dystocia[45].

Phosphorous is a component of phospholipids which are important in lipid transport and skeletal and dent formation[46]. A statistically significant decrease for serum phosphorous levels during pregnancy on day 100 and day 150 of pregnancy was observed by Yildiz *et al*[36]. This reduction in the inorganic P level during the periparturient period could be due to increased demand for P for mineralization of the fetal skeleton. Although, Azab *et al*[35] observed that there were no significant differences in plasma P concentrations between different physiological stages. In the current study, there was significant decreased serum P level in distressed goats compared with normally parturated goats that suggested the important role of P during normal parturition.

Glucose is the major energy substrate for the developing fetus and is also utilized by uterus and placenta[47]. Our data showed that serum glucose concentration was significantly decreased during late pregnancy in goats and this could be attributed to glucose utilization by the growing fetus[47-49].

During involution, the uterus decrease in size and immune cells infiltrate the uterus to clear residual placental tissue along with infectious microorganisms[50]. Glucose is stored as glycogen within polymorphonuclear neutrophils and used as the primary metabolic fuel to generate the oxidative burst that leads to killing activity. Galvão *et al*[51] observed that cows developing uterine disease had lesser concentrations of glycogen in their PMN. Their conclusion was that lesser glycogen reserve led to a reduced capacity of oxidative burst in PMN and uterine disease. Our data demonstrated that glucose was significantly correlated with UTI after parturition and placenta dropping time. These findings came in harmony with the result of Moyes *et al*[52] who concluded that glucose level could be used as predictive indicator for the postpartum uterine disease.

Cortisol is a potent immune suppressive hormone during the periparturient period[53,54]. Our findings demonstrated highly significant increased serum cortisol in goat suffering with dystocia during the parturition compared with normal parturated goat during late pregnancy. These finding came in harmony with Tharwat *et al*[55]. Although, Firat and Ozpinar[56] reported that the cortisol level decreased during late pregnancy as result of metabolic disorder.

Our results also illustrated that goat suffering from dystocia at parturition having high levels of cortisol and lactate compared with normally parturated goat. This was supported by findings of Vaughan *et al*[57] who recorded an increase in maternal cortisol concentrations within the physiological ranges during sheep pregnancy that alter utero-placental metabolism. Concomitantly, lactate was produced at a higher rate by the utero-placental tissues of cortisol-treated ewes and distributed into both the fetal and maternal circulation. Furthermore, the present data revealed that highly significant correlation between cortisol and uterine involution 6th wk after parturition that could be attributed to immune suppression effect of the cortisol that delay the uterine involution and increase susceptibility to uterine diseases.

Highly significant reduction in serum albumin in pregnant distressed goats compared with normal goats was recorded in this study, and this could be explained by the findings of Batavani *et al* and Karapehlivan *et al*[58,59], which reported an increased nutrient requirements of the placenta, growing fetus and mother's basal metabolic rate, together with the transfer of immune globulins, serum albumin, and amino acids from the blood stream to the mammary gland for colostrum synthesis.

In the pregnant goat little is known about AG and corrected AG. Our results firstly reported highly significant change regarding the AG in distressed pregnant goat compared with healthy ones. It had been documented that AG decreased in the presence of hypoalbuminaemia[39]. Moreover, Figge *et al*[60] found a direct correlation between serum albumin concentration and the serum AG during the prepartum period. Thus, serum albumin might be considered the most important circulating protein during the prepartum period that could alter the serum AG[9].

Based on the results obtained from this study, it appears that biochemical parameters and serum electrolyte status during periparturient period could be used as an early predictor for metabolic and reproductive problem that may confront the animal post parturient.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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