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Changes in urinary dipstick parameters in cyclic and early pregnant Murrah buffaloes

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Abstract
Sixteen apparently healthy Murrah buffaloes aged 5 – 6 years and divided into two groups: Cyclic/Non pregnant and Pregnant, comprising of eight animals each and maintained at private farms in Mumbai were selected. Urine samples were collected from both the groups at an interval of seven days *i.e* in Control/Non-pregnant group on day 0 (day of estrus),7, 14, 21, 28, 35 and 42 (up to two estrous cycles) and in Pregnant group on day 0/day of estrus/AI, day 7, 14, 21, 28, 35, 42, 49 and 56. The analysis of urine protein, pH, specific gravity, ketone and glucose were done by reagent strips of Yercon Company. The average values of specific gravity ranged from (1.009 ± 0.011) to (1.010 ± 0.006) and that of pH ranged from (7.43 ± 0.14) to (7.62 ± 0.14) in both the groups, showing pH in the alkaline side. Protein, ketone and glucose were absent in the urine samples. No significant difference was observed in specific gravity and pH between the groups and within the group. Correlation between urine pH and SG was found positive (r = 0.11) although non-significant. However, no association between either pH or SG was observed with protein, ketone or glucose.

Keywords: Urine, specific gravity, pH, protein, ketone, glucose, correlation

1. Introduction
India has total buffalo population 108.7 million[1].On an average, buffalo is considered to be nearly four times as productive as an average female indigenous cow in India[2]. Buffalo is reputed as an efficient converter of low grade, fibrous feed into high value milk containing 7% fat that is almost twice that of cow's milk. Buffalo meat production accounts for about 30% of the total 4.9 million tonnes of meat production of the country. In India, nearly 55% of the milk is produced by buffaloes, despite the fact that they constitute 30% of the total animal population [2]. However, the reproductive efficiency and productivity of these animals is low [3]. Therefore in recent years, to augment the productivity of bovine species different scientific interventions have been adopted [4]. Infectious diseases due to unscientific management of buffaloes during pregnancy can lead to detrimental effects on general health status and productivity of the animals and hence urine analysis is a remarkable tool that can reveal many diseases that could go unnoticed and undiagnosed. Observing the colour, transparency, studying microscopic and chemical characteristics of urine and urinary sediments identification of urinary tract disorders in domestic animals can be done [5]

2. Materials and Methods
2.1 Study site:
Sixteen apparently healthy Murrah buffaloes, maintained at private farms in Aarey Colony, Goregaon, Mumbai were taken for the present study.

2.2 Animals and experimental design
The buffaloes were maintained under uniform standard conditions of feeding and management with *ad-libitum* water. These animals were then divided into two groups: Control/Non pregnant/Cyclic and Pregnant, comprising of eight animals each. All the animals were in the age group of 5 to 6 years.
Urine samples were collected from both the groups at an interval of seven days i.e in Cyclic/Control/Non-pregnant group on day 0/day of estrus, day 7, 14, 21, 28, 35 and 42 (up to two estrous cycles) and in Pregnant group on day 0/day of estrus/AI, day 7, 14, 21, 28, 35, 42, 49 and 56. Pregnancy was confirmed by rectal palpation in pregnant group on day 45.

2.3 Collection of urine
A total of 72 samples from pregnant and 56 samples from non pregnant buffaloes were collected during morning hours in clean and sterile glass vials of 30 ml capacity by midstream clean catch technique.

2.4 Analysis of urine parameters
After collection, analysis of urine protein, pH, specific gravity, ketone and glucose were done by reagent strips of Yercon Company, Yercon Diagnostic Co. Ltd.

2.5 Procedure
The strip was carefully inserted by handling it by the end which is away from the reagent area. The reagent area was immersed in fresh urine for 1-2 seconds and then removed. After the time indicated for each analyte, the strip was held close to the colour chart and matched carefully and the test areas were compared with the reference chart. The results were expressed as either negative or varying degrees of positive, indicating different amounts of the specific analyte present. Dipstick tests were done on the farm within 30 minutes of urine sample collection.

2.6 Statistical analysis
Statistical Analysis of the data was done by completely randomized design according to Snedecor and Cochran [6]. For studying relationship among pH, specific gravity, protein, ketone and glucose the correlation coefficients were calculated.

3. Results and Discussion
Urine specific gravity (non pregnant/cyclic and pregnant buffaloes) and pH (non pregnant/cyclic and pregnant buffaloes) are given in the table 1 and table 2 respectively.

Table 1: Urine specific gravity (Mean ± S.E) in non-pregnant/cyclic and pregnant Murrah buffaloes.

Days	Cyclic/Non pregnant (Mean ± S.E)	Pregnant
0	1.009 ± 0.011	1.009 ± 0.011
7	1.009 ± 0.011	1.009 ± 0.011
14	1.010 ± 0.006	1.010 ± 0.006
21	1.010 ± 0.006	1.010 ± 0.006
28	1.009 ± 0.011	1.009 ± 0.011
35	1.010 ± 0.006	1.010 ± 0.006
42	1.010 ± 0.006	1.010 ± 0.006
49	-	1.009 ± 0.011
56	-	1.010 ± 0.006

In the present study it was observed that there was no significant difference in specific gravity between non-pregnant and pregnant group. Within the group, specific gravity remained almost constant. The specific gravity ranged from (1009±0.011) to (1010±0.006). These observations were in accordance to Shahir *et al.* [7] in pregnant cows and Leendertz *et al.* [8] in wild chimpanzees. They found that specific gravity was not affected by estrus or pregnancy. The urinary specific gravity in the first trimester of pregnancy was 1.01 ± 0.0007 in pregnant cows.

According to Parrah *et al.* [5], urinary specific gravity in cattle is directly proportional to urine osmolality, measures solute concentration and urine density or ability of the kidney to concentrate or dilute the urine over that of plasma. Urinary specific gravity value less than 1.010 in humans indicated relative hydration and greater than 1.020 indicated relative dehydration [8] during any physiological stage of estrus or pregnancy.

Table 2: Urine pH (Mean ± S.E) in non-pregnant and pregnant Murrah buffaloes.

Days	Non pregnant (Mean ± S.E)	Pregnant (Mean±S.E)
0	7.50 ± 0.18	7.50 ± 0.18
7	7.50 ± 0.18	7.43 ± 0.15
14	7.50 ± 0.09	7.50 ± 0.18
21	7.43 ± 0.14	7.50 ± 0.18
28	7.50 ± 0.18	7.50 ± 0.18
35	7.50 ± 0.09	7.43 ± 0.15
42	7.56 ± 0.06	7.50 ± 0.18
49	-	7.56 ± 0.06
56	-	7.62 ± 0.14

There was no significant difference in pH between non-pregnant and pregnant groups. Within the group, pH remained almost same and was alkaline. In both the groups, from day 0 till day 42 and from day 0 to day 56, the pH ranged from 7.43 to 7.62 and was not affected by estrus or pregnancy. These results were in agreement with Leendertz *et al.* [8]. According to Parrah *et al.* [5] in cattle and Mavangira *et al.* [10] in goats the pH is alkaline since cattle and goats are herbivores and produce alkaline urine. Though urinary pH reflects internal homeostasis of acid-base balance to keep a constant body pH [11], it is not affected by estrus or pregnancy [8]. In contrast acidic pH in cows was reported in first trimester which turned subsequently to alkaline as the pregnancy advanced [7].

None of the samples were positive for urinary protein in both non pregnant and pregnant buffaloes. Since the protein was absent in urine, it was not consistent with the kidney disease. These observations were in accordance with Shahir *et al.* [7]. According to Leendertz *et al.* [8] in chimpanzee, even low level of protein in urine is normal and not a cause of concern. Non pathological causes of proteinuria include a high protein meal, exercise and stress and pathological causes of proteinuria include renal disease, where glomerular leakage of proteins occur resulting urinary tract infections and haematuria [12]. The presence of urinary protein could be an indication of predisposing factors for urolithiasis as about two thirds of the matrix of all urinary stones is composed of proteins [13] and excreted protein includes a mucoprotein called Tamm–Horsfall protein [14].

The ketone bodies in urine samples were absent in non-pregnant and pregnant animals. These findings were similar to those reported by Parrah *et al.* [5] who gave its reference range as negative to traces during early pregnancy. Similarly in cows Shahir *et al.* [7] and in chimpanzees Leendertz *et al.* [8] absence of ketone bodies was reported. These ketones occur in urine when increased fat metabolism owing to insufficient supply of energy in the form of carbohydrate occurs as in cases of starvation [11]. The absence of urinary ketones in both the non-pregnant and pregnant buffaloes in the present study indicated that the animals were well fed with sufficient supply of energy.

The urine samples were absent for glucose in both the groups and not affected by estrus or early pregnancy. This was in accordance to Shahir *et al.* [7] in cows, Leendertz *et al.* [8] in chimpanzees and Parrah *et al.* [5]. Glucose is freely filtered and then reabsorbed in proximal tubules to be used again as a source of energy [5] and thus negative to traces of urinary glucose was present during pregnancy.

In the present study, urine pH was positively correlated with

specific gravity (r =0.11). However, with protein, ketone and glucose it did not show any association. These relationships were statistically non-significant. In contrast, no association between urinary pH and SG was reported in cows [7]. The urine SG did not show any relationship with urine protein, ketone and glucose. Similarily, urine protein, ketone and glucose also did not show any correlation among each other.

There was non-significant difference between specific gravity and pH of urine in pregnant and non-pregnant groups with absence of protein, ketone and glucose in the urine samples. Similarly, a non-significant correlation was observed between urine pH and specific gravity whereas no correlation was found among urine protein, ketone and glucose.

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