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Measurements of Pregnanediol-3-glucuronide and urinary parameters in cyclic and early pregnant Murrah buffaloes

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Abstract
Sixteen healthy Murrah buffaloes aged 5 - 6 years were divided into two groups: Cyclic/Non pregnant and Pregnant, comprising of eight animals each. Urine samples were collected at an interval of seven days in non pregnant group i.e day 0/day of estrus, day 7, day 14, day 21, day 28, day 35 and day 42 as well as in pregnant group i.e day 0/day of estrus/day of AI, day 7, day 14, day 21, day 28, day 35, day 42, day 49 and day 56 respectively. The PdG concentration in both the groups increased significantly ($p < 0.01$) on day 7 and on day 14. It declined significantly ($p < 0.01$) on day 21 and day 42 i.e at the end of subsequent estruses in non pregnant animals indicating luteal regression. However, the concentrations significantly increased ($p < 0.01$) from day 42 to day 56 with a modest decrease on day 28 in the pregnant animals accounting for the presence of persistent CL. The urine samples were absent for blood, nitrites and bilirubin. Negative to traces of leucocytes were observed which could be due to contamination of urine by vaginal discharge. The concentration of urobilinogen in all the samples was within the normal range (3.2 µmol/l to 16 µmol/l). All the urine dipstick parameters were independent of estrus and pregnancy and showed no overall correlation among them.

Keywords: PdG, blood, nitrites, bilirubin, leucocytes, urobilinogen

1. Introduction
As India enters an era of economic reforms, it is recognized that dairying could play a more constructive role in promoting the rural welfare and reducing poverty. 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 production [2].
Buffalo is known for silent estrus, anestrus, lower rate of ovulation, delayed maturity etc., that ultimately hinder utilization of buffalo's full potential in terms of reproductive health and productivity. Reproduction is a major factor contributing to the efficiency of milk and meat production by farm animals [3]. Increased reproduction efficiency and increased number and quality of animals depend on the determination of an early diagnosis of gestation [4]. The maintenance of pregnancy calls for specific metabolic and functional changes between conception and gestation, mediated through the interplay of various hormones involved in the maintenance and termination of pregnancy [5]. An early pregnancy diagnosis is crucial to shortening the calving interval enabling the farmer to identify open animals so as to rebreed them at the earliest opportunity. Some of the direct methods of pregnancy diagnosis practised are per-rectal palpation and ultrasonography. Similarly indirect methods of pregnancy diagnosis are the estimation of progesterone in serum and its metabolites in metabolites in urine and faeces, PAGs, Interferon tau, early conception factors etc.
Infectious diseases due to the 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 [6]. Considering the following factors, the present study was undertaken to estimate pregnanediol-3-glucuronide (PdG) and analyze urinary parameters during the estrous cycle and early pregnancy.

2. Materials and Methods

Sixteen apparently healthy Murrah buffaloes, aged 5 to 6 years maintained at private farms in Aarey Colony, Goregaon, Mumbai were divided into two groups: Control/Non pregnant/Cyclic and Pregnant, comprising of eight animals each.The buffaloes were maintained under uniform standard conditions of feeding and management with *ad-libitum* water. 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 the pregnant group on day 45.The urinary pregnanediol-3-glucuronide was estimated by using Arbor Assay DetectX Pregnanediol-3-glucuronide (PdG) Enzyme Immunoassay kit (Species independent).

Samples were collected during morning hours in clean and sterile glass vials of 30 ml capacity by midstream clean catch technique. After collection, analysis of blood, leucocytes, bilirubin, nitrites and urobilinogen were done by reagent strips of Yercon Diagnostic Co. Ltd.

Statistical Analysis of the data was done by completely randomized design according to Snedecor and Cochran [7].

3. Results and Discussion

The of PdG profile (ng/ml) between non-pregnant and pregnant buffaloes is presented in table 1.On comparing the PdG concentrations between non-pregnant and pregnant buffaloes from day 0 to day 42, it was observed that there was no significant difference upto day 14. Later, from day 21 highly significant difference ($p<0.01$) between the concentrations of PdG was observed till day 42 which indicated the difference between the pattern of PdG in non-pregnant and pregnant Murrah buffaloes. The PdG concentration in both the groups increased significantly ($p < 0.01$) on day 7 and on day 14.It declined significantly ($p < 0.01$) on day 21 and day 42 i.e at the end of subsequent estruses in nonpregnant animals indicating luteal regression. However, the concentrations significantly increased ($p < 0.01$) from day 42 to day 56 with a modest decrease on day 28 in the pregnant animals accounting for the presence of persistent corpus luteum.

The result of PdG concentrations in pregnant and non pregnant buffaloes obtained in the present study was in accordance with Yang *et al.* [8] in Holstein cows, Kirkpatrick *et al.* [9] in North American bison, Montfort *et al.* [10] in Eld's deer, Shimizu *et al.* [11] in chimpanzee and French *et al.* [12] in Marmosets. Though the PdG levels during early pregnancy varied in different species yet the trend was similar physiologically.

Yang *et al.* [8] in Holstein cows observed that the concentration of PdG during the luteal phase was 3-4 times higher than the follicular phase and Kirkpatrick *et al.* [9] and Shimizu *et al.* [11] stated that the initial presence of PdG from day of estrus to its subsequent rise till mid luteal stage was due to ovulation. Thereafter, the temporal decrease in PdG on day 21 and day 42 i.e on subsequent estrus phases indicated that the luteal regression has been initiated [10].

Table 1: PDG concentrations (ng/ml) between control/non-pregnant and pregnant Murrah buffaloes from day 0 (day of estrus/AI) to day 42.

Days	Mean ± S.E (Control/Non-Pregnant)	Mean ± S.E (Pregnant)
0	$13.65^{e} \pm 1.15$	$14.78^{f} \pm 1.03$
7	$36.69^{c} \pm 1.94$	$35.49^{e} \pm 2.71$
14	$54.71^{a} \pm 2.66$	$57.20^{cd} \pm 4.40$
21	$28.42^{d} \pm 2.26$	$57.74^{cd} \pm 5.32$
28	$36.88^{c} \pm 2.80$	$50.52^{d} \pm 3.36$
35	$45.26^{b} \pm 3.91$	$56.27^{cd} \pm 4.22$
42	$25.65^{d} \pm 1.71$	$68.04^{bc} \pm 7.69$
49	-	$77.09^{b} \pm 5.36$
56	-	$91.40^{a} \pm 4.78$

Means with at least one common superscript do not differ significantly ($P< 0.05$).

Kirkpatrick *et al.* [9] reported that the rise in PdG levels in pregnant buffaloes could be accounted for the presence of luteinised follicles, persistent corpora lutea and extra-ovarian progestins. According to them, the non-cyclic increase in PdG levels revealed progesterone production during early pregnancy while Shimizu *et al.* [11] showed that urinary PdG increased during luteal phase when first gestational rise was observed and then subsequently decreased. Later, a second gestational rise in PdG concentration was observed from day 36.3 and modestly elevated followed by the progressive rise. Similar pattern was also observed in the present study wherein the second gestational rise in PdG concentration was observed from day 42.

The urine samples were absent for blood, nitrites and bilirubin in both the groups and were independent of estrus and pregnancy. These observations were in accordance to Shahir *et al.* [13] in pregnant cows. Leendertz *et al.* [14] in wild chimpanzees observed that although the urinary blood levels were marginally higher in females with estrus swelling, there was overall no difference in the frequency of occurrence of blood in the urine samples and the level of blood was not affected by estrus or pregnancy. They also observed that urine samples of very few females were positive for bilirubin and nitrites.

Patel [15] stated that bilirubin is a byproduct of red blood cell (RBC) breakdown in the reticuloendothelial system. Normally bilirubin and nitrite is not found in urine [6]. Presence of bilirubin in urine indicates either bile duct obstruction or intrinsic hepatic disease. While Echeverry *et al.* [16] observed urinary tract infections in the presence of nitrite.

Negative to trace levels of leucocytes were found in non pregnant and pregnant buffaloes and presence of leucocytes were not dependent on estrus or pregnancy. This was accordance to Shahir *et al.* [13] in pregnant cows where trace amount of leucocytes was found in urine samples during the second trimester. In contrast, Leendertz *et al.* [14] in chimpanzee found that of all urine parameters, leucocytes were most common positive test result and females had more leucocytes than males which were neither due to estrus or pregnancy. Leucocytes in female urine were due to contamination of urine by vaginal discharge.

Urobilinogen level ranged from 3.2 µmol/l to 16 µmol/l (0.2 to 1 mg/dl) [17] which was under normal physiological level. This was in agreement to Shahir *et al.*[13] in pregnant cow and they observed trace levels of urobilinogen ranging from 1mg/dl to 4mg/dl while in contrast, Leendertz *et al.* [14] in chimpanzee observed no urobilinogen in the urine samples. Urobilinogen is an indicator of liver pathology [18]. Gerber and

Blendler [19] in human reported small amount (~1– 4mg/day), remains in the bloodstream and is excreted by the kidneys in urine. Simerville *et al.* [20] observed that normal urine contains only small amounts of urobilinogen. Amongst the urinary parameters no correlation was observed between them and was statistically non-significant.

4. Conclusion

Thus, it can be concluded that urinary PdG can be one of the parameters to diagnose early pregnancy, although more research is required to prove it as an effective tool for early pregnancy diagnosis. In addition, urine analysis provides an empirical guidance for screening out undiagnosed diseases.

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