

1 **Salivary crystallization pattern: An unconventional tool for timing of insemination and early**
2 **pregnancy diagnosis in Zebu cows**

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8 **SUPPLEMENTARY FILE**

9 **Materials and methods**

10 Management of experimental animals

11 The cows included in the study were apparently free from reproductive disorders, and were
12 vaccinated against common diseases (Brucellosis, Foot and Mouth Diseases, Hemorrhagic
13 Septicemia and Black Quarter) as per the standard management practices of the farm. Cows were
14 maintained in a loose housing system under group management practice. Daily ration of the
15 experimental cows consisted *ad libitum* green fodder (Maize, Jowar, Cowpea, Berseem and Oat)
16 and measured quantity of concentrates as per the standards.

17 ELISA Kit sensitivity details

18 The minimal detectable concentration of estradiol using the kit was 4.75pg/mL. The intra-
19 assay variation was 5.4% and the inter-assay variation was 10.8%. The minimal detectable
20 concentration of progesterone using the kit was 0.5ng/mL. The intra-assay variation was 6.3% and
21 the inter-assay variation was 13.8%.

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23 **Figure legend**

24 **Figure S1:** Plasma concentrations of estradiol (pg/ml) during oestrous cycle and pregnancy in Zebu
25 cows. * Significant ($P < 0.05$)

26 **Figure S2:** Plasma concentrations of progesterone (ng/ml) during oestrous cycle and pregnancy in
27 Zebu cows. * Significant ($P < 0.05$)

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29 **Table legend**

30 **Table S1:** The proportion of occurrence of different crystallization patterns in the saliva of Sahiwal
31 cows on different days of estrous cycle

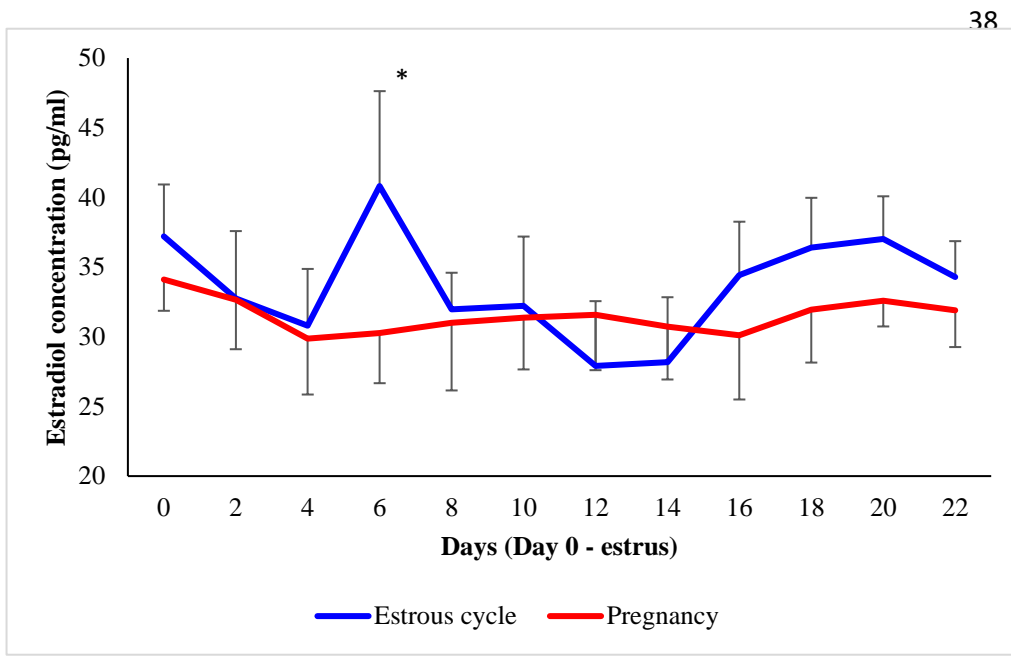
32 **Table S2:** The proportion of occurrence of different crystallization patterns in the saliva of Sahiwal
33 cows during different days of pregnancy

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37 **Figure S1**



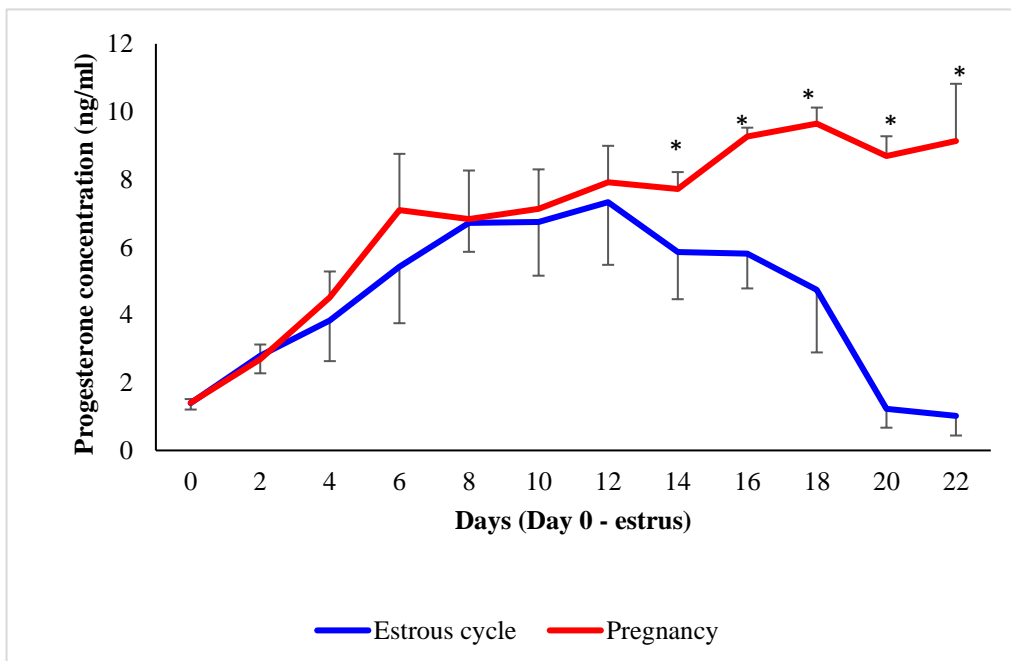
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50 **Figure S2**

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62 **Table S1**

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Pattern/Days of cycle	0	2	4	6	8	10	12	14	16	18	20	22
Branch-like Pattern	0	0.25	0	0.25	0.75*	0.25	0.5*	0.25	0	0	0	0.25
Fern-like Pattern	1*	0.25	0.25	0	0	0.25	0.25	0	0.25	0	1*	0.25
Fir-like Pattern	0	0.5*	0.5*	0.25	0	0.25	0	0.5*	0.25	0.25	0	0.25
Branch-fir pattern	0	0	0.25	0.25	0	0	0	0	0.5*	0.25	0	0
Fir-fern Pattern	0	0	0	0.25	0	0	0	0	0	0	0	0.25
Branch-fern Pattern	0	0	0	0	0	0	0	0.25	0	0	0	0
Atypical Pattern	0	0	0	0	0	0.25	0	0	0	0.5*	0	0
Nil pattern	0	0	0	0	0.25	0	0.25	0	0	0	0	0

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66 The expected proportion for each pattern was 0.125 ($1/8 = 0.125$). Statistical significance indicated
67 by *, $P \leq 0.001$. For instance, the proportion of typical Fern-like pattern at day 0 was significantly
68 different from the expected proportion as revealed by one proportion Z test.

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74 **Table S2**

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Pattern/Days of pregnancy	0	2	4	6	8	10	12	14	16	18	20	22
Branch-like Pattern	0	0	0	0	0.25	0.25	0.25	0	0	0.125	0.125	0.125
Fern-like Pattern	1*	0.75*	0.5*	0.75*	0.25	0.25	0	0	0	0.125	0.125	0.125
Fir-like Pattern	0	0	0	0	0.25	0.25	0	0	0	0.5*	0	0
Branch-fir pattern	0	0.25*	0.5*	0	0.25	0.25	0.25	0.5*	1*	0.25	0	0.25
Fir-fern Pattern	0	0	0	0	0	0	0.25	0	0	0	0.75*	0.25
Branch-fern Pattern	0	0	0	0	0	0	0.25	0	0	0	0	0
Atypical Pattern	0	0	0	0	0	0	0	0.5*	0	0	0	0
Nil pattern	0	0	0	0.25	0	0	0	0	0	0		0.25

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