

Investigation of conceptus stimulated gene expression in buffalo peripheral blood mononuclear cells as potential diagnostic markers of early pregnancy

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SUPPLEMENTARY FILE

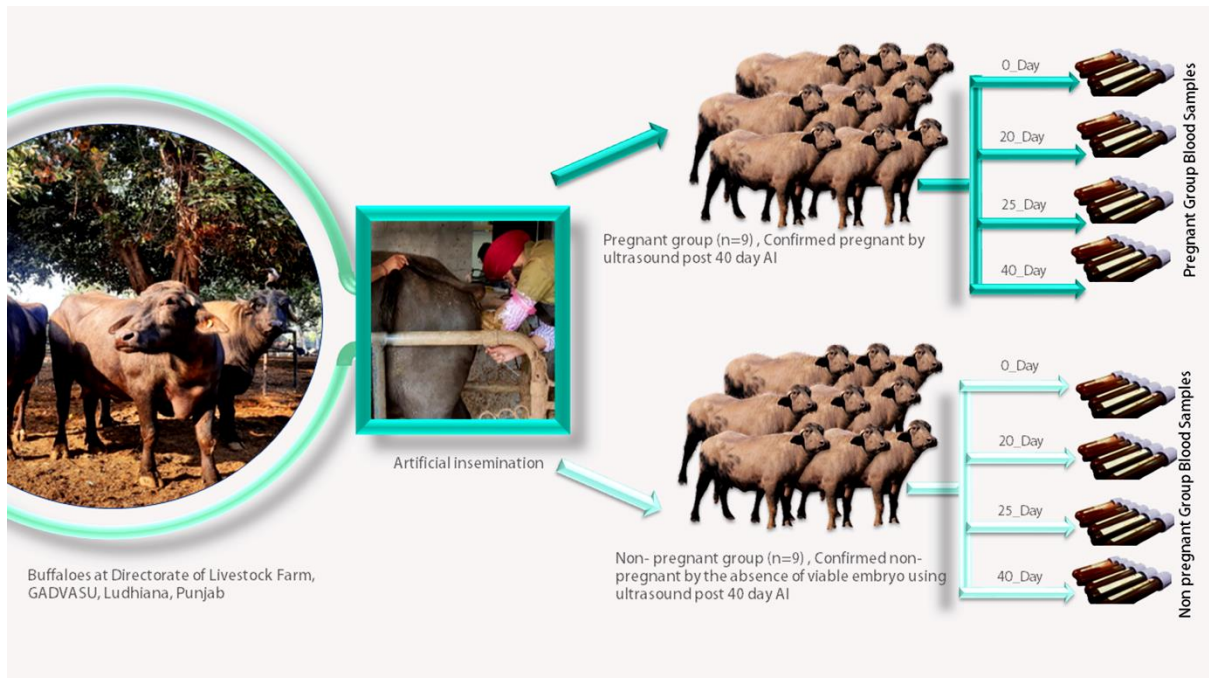
Supplementary table 1: Primer sequences, amplicon length, and annealing temperature (°C) of genes used for RT-qPCR.

Target Gene	Sequences of primers (5' → 3')	Amplicon Length (bp)	Annealing temperature (°C)
<i>ISG15</i>	F- ATCCTGGTGAGGAACGACAA R- TATTCCTCCAGCGGGTGTTC	170	60°C
<i>LGALS3BP</i>	F- CCCAGCTTCTCTTCCATGA R- GTCATCAGAGGAGCACGAGA	102	60°C
<i>CD9</i>	F- TTCCCACAAGGAGGAGGTGAT R- GTCAAACCACAGCAGTCCAG	135	60°C
<i>PPIA</i>	F- CTGAGTGGTTGGATGGCAAG R- TGTCCACAGTCAGCAATGGT	133	60°C

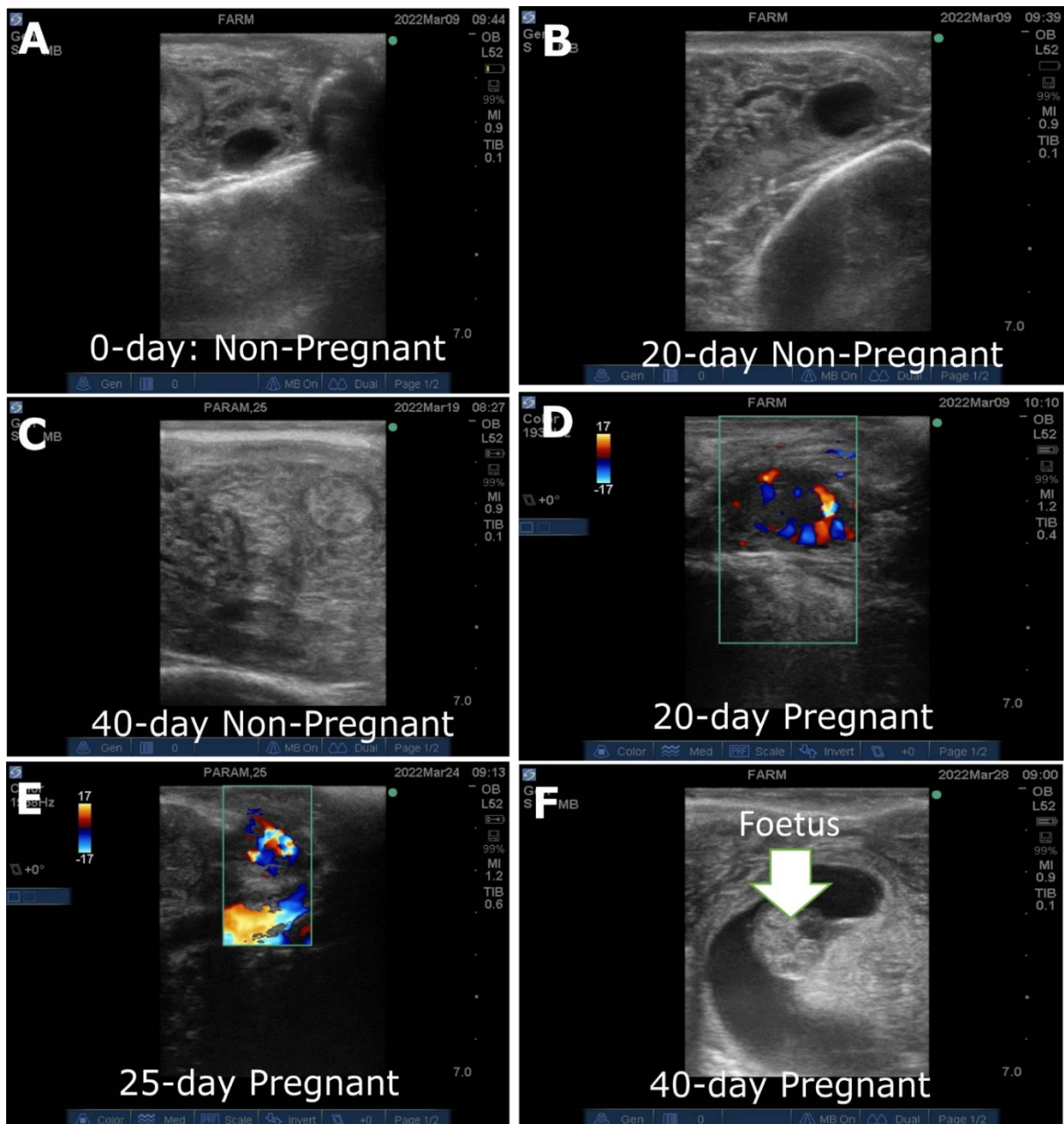
Supplementary table 2: Quantitative and qualitative analysis of total RNA isolated from buffalo PBMC.

Sample No:	Conc. (ng/μl)	OD _{260/280}	OD _{260/230}
1	1066.2	2.0	1.98
2	779.4	2.0	0.47
3	1469.5	2.09	1.33
4	2748	2.0	2.3
5	4324.2	2.04	1.29
6	8159.4	1.96	2.34
7	4093	2.05	1.97
8	4751.6	2.08	1.82
9	2000.4	2.04	2.19
10	1399.1	2.03	2.16
11	1006.4	2.02	1.85
12	1903.4	1.99	1.44
13	1460.2	2.03	0.94
14	1655.5	1.98	1.67
15	863.6	1.99	1.95
16	416.4	1.98	0.69
17	972.5	1.97	2.13

18	698	2.13	0.28
19	493.5	1.9	0.84
20	2111.3	2.01	0.92
21	934.9	1.99	1.98
22	1611.7	2.05	1.98
23	1028.7	2.02	1.97
24	1662.1	2.03	2.15
25	1070.8	2.01	2.2
26	1405.6	1.89	2.04
27	3096.1	2.04	2.18
28	1502.6	2.08	1.11
29	1643.5	1.95	1.31
30	808.6	2.18	0.58
31	851.2	1.98	2.05
32	2395.9	2.03	0.69
33	1002.9	2.0	1.57
34	2716.7	1.88	2.1
35	434.2	1.97	1.63
36	2025.6	2.01	1.85
37	1011.8	1.98	2.18
38	1363.5	1.97	2.18
39	680.3	2.02	0.95
40	7780.3	1.89	2.24
41	2297.6	1.95	1.97
42	1416.7	1.99	2.07
43	687.9	2.04	0.81
44	2544.7	2.04	2.22
45	1659.3	2.01	1.72
46	2354.2	1.84	1.33
47	1121.4	2.02	1
Average	1903.838	2.003191	1.630213
S.E.	236.2279	0.008967	0.085001



Supplementary figure 1: Layout of an animal experiment conducted on buffalo to detect early pregnancy in PBMC



Supplementary figure 2: Pregnancy examination by ultrasonography. Non-pregnant animals at 0-, 20- and 40-day no change in blood flow rate to the ovary (A-C). An active corpus luteum was indicating luteal blood perfusion (red and blue) by Doppler Ultrasonography (D, E). On 40-day, a viable fetus attached to the uterine was observed in transrectal ultrasonography (F).