Indian Journal of Animal Sciences **94** (7): 610–619, July 2024/Article
https://doi.org/10.56093/ijans.v94i7.149620

MAP3K1, *SPEF2* and *PLCZ1* genes association with fertility and semen quality traits of AI bulls in Andhra Pradesh

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Received: 14 March 2024; Accepted: 6 May 2024

Supplementary Table 1. Details of samples collected from different genetic groups

Breed	Location				Total no. of bulls
	FSBS Banavasi	FSBS Nandyal	FSBS Visakhapatnam	LRS, LAM Guntur	
Murrah	40	29	36	-	105
Ongole	-	9	5	50	64
Holstein Friesian	17	1	5	-	19
Holstein Friesian crossbred	6	1	5	-	12
Jersey	15	2	-	-	17
Jersey crossbred	16	6	6	-	28
Total	94	47	54	50	245

Supplementary Table 2. Primers and protocol for digestion of PCR products of *MAP3K1*, *SPEF2* and *PLCZ1* genes

Primer Sequence (5' - 3')	Length (bp)	Product (bp)	PCR products digestion protocol					
			RE (10 units/ µl)	10X buffer	PCR product	NFW	Total reaction volume	Incubation temperature & time
*MAP3K1/ CviQ*I (rs463712269)								
F:AGCACAGCAACTTCTGACTTA	21	256	5 units					25°C for
R:GTAAACAGGGAGATGCCTCAA	21		(0.5 µl)	1 µl	3 µl	5.5 µl	10 µl	2 h 30 min
*SPEF2/HpyCH4*V (rs722354121)								
F:CAGCTTACCCATGATGTCATTC	22	243	1 units					37°C for
R:TGGATCCTGGATGGCTTT	18		(0.1 µl)	1 µl	2 µl	3.9 µl	7 µl	6 h
*PLCZ1/Ava*II (rs208019489)								
F:GGGTTCAAGCCTAGGTCTCC	20	439	10 units	1.5 µl	3 µl	4.5 µl	10 µl	37°C for
R:ACCACCCGTTTCAACTGTTC	20		(1 µl)					12 h

Supplementary Table 3. Distribution of genotypes and allele frequencies at *MAP3K1/CviQ*I locus in cattle and buffalo bulls

Breed/Group	Total no. of animals (n)	Observed genotypic frequency			Allele frequency		Expected genotypic frequency			χ^2 value	P-value
		CC	*CT*	*TT*	*C*	*T*	*CC*	*CT*	*TT*		
Holstein Friesian	19	0.63 (12)	0.37 (7)	0 (0)	0.82	0.18	0.66 (12.57)	0.31 (5.86)	0.03 (0.57)	0.81	0.367
HF Crossbred	12	0.33 (4)	0.67 (8)	0 (0)	0.67	0.33	0.43 (5.22)	0.46 (5.56)	0.01 (1.22)	2.57	0.109
Jersey	17	0.65 (11)	0.29 (5)	0.06 (1)	0.79	0.21	0.62 (10.64)	0.34 (5.73)	0.04 (0.63)	0.31	0.576
Jersey Crossbred	28	0.57 (16)	0.36 (10)	0.07 (2)	0.75	0.25	0.56 (15.65)	0.38 (10.69)	0.06 (1.66)	0.12	0.724
Ongole	64	0.05 (3)	0.66 (42)	0.29 (19)	0.37	0.63	0.14 (8.88)	0.47 (30.24)	0.39 (24.88)	9.86**	0.002
Murrah	99	0 (0)	0.12 (12)	0.88 (87)	0.06	0.94	0.003 (0.34)	0.115 (11.33)	0.882 (87.33)	0.37	0.54

Figures in parenthesis are the number of animals. **, Highly significant ($P<0.01$)

Supplementary Table 4. Distribution of genotypes and allele frequencies at *SPEF2/ HpyCH4*V locus in cattle and buffalo bulls

Breed/Group	Total no. of animals (n)	Observed genotypic frequency			Allele frequency		Expected genotypic frequency			χ^2 value	P-value
		TT	*CT*	*CC*	*T*	*C*	*TT*	*CT*	*CC*		
Holstein Friesian	19	1 (19)	0 (0)	0 (0)	1.00	0.00	1 (19)	0 (0)	0 (0)	-	-
HF Crossbred	12	0.92 (11)	0.08 (1)	0 (0)	0.96	0.04	0.92 (11)	0.08 (1)	0 (0)	0.00	1.00
Jersey	17	1 (17)	0 (0)	0 (0)	1.00	0.00	1 (17)	0 (0)	0 (0)	-	-
Jersey Crossbred	28	0.96 (27)	0.04 (1)	0 (0)	0.98	0.02	0.96 (27)	0.04 (1)	0 (0)	0.00	1.00
Ongole	64	0.97 (62)	0.03 (2)	0 (0)	0.98	0.02	0.9690 (62.008)	0.0309 (1.98)	0.0001 (0.008)	0.008	1
Murrah	99	0.67 (66)	0.33 (33)	0 (0)	0.83	0.17	0.69 (68.68)	0.28 (27.64)	0.03 (2.68)	3.82*	0.049

Figures in parenthesis are the number of animals. *, Significant ($P<0.05$).

Supplementary Table 5. Distribution of genotypes and allele frequencies at *PLCZ1*/*Ava*II locus in cattle and buffalo bulls

Breed/Group	Total no. of animals (n)	Observed genotypic frequency			Allele frequency		Expected genotypic frequency			χ^2 value	P-value
		GG	*CG*	*CC*	*G*	*C*	*GG*	*CG*	*CC*		
Holstein Friesian	19	0.74 (14)	0.26 (5)	0 (0)	0.87	0.13	0.75 (14.27)	0.24 (4.46)	0.01 (0.27)	0.34	0.559
HF Crossbred	12	0.84 (10)	0.08 (1)	0.08 (1)	0.87	0.13	0.76 (9.13)	0.23 (2.74)	0.01 (0.13)	6.98**	0.008
Jersey	17	0.94 (16)	0.06 (1)	0 (0)	0.97	0.03	0.94 (16.00)	0.06 (1.00)	0 (0)	0.00	1.00
Jersey Crossbred	28	0.57 (16)	0.43 (12)	0 (0)	0.79	0.21	0.62 (17.2)	0.34 (9.6)	0.04 (1.2)	1.88	0.16
Ongole	64	0.64 (41)	0.22 (14)	0.14 (9)	0.75	0.25	0.56 (35.90)	0.38 (24.19)	0.06 (3.91)	11.66**	0.00
Murrah	99	0.97 (96)	0.03 (3)	0 (0)	0.98	0.02	0.97 (96.02)	0.0299 (2.97)	0.0001 (0.01)	0.02	0.90

Figures in parenthesis are the number of animals. **, Highly significant ($P<0.01$).