## **Research** Article



## Evaluation of Anti-Sperm Antibodies (ASAs) in the Serum and Seminal Plasma of Dromedary Male Camels with Infertility History

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**Abstract** | Anti-sperm antibodies (ASAs) have been shown to contribute to male infertility in multiple species but never been examined in male camels. We have developed and evaluated an ELISA assay to specifically detect and quantify ASAs in the serum and seminal plasma of 29 infertile dromedary male camels, which were of two different breeds (Waddah & Majaheem) and three different ages (> 3 to  $\leq 5$ ,  $\geq 5$  to  $\leq 7$ , and >7 years). ASAs were detected in  $\geq$  80% of the examined animals with considerable individual variation within each group. The serum and seminal plasma ASAs indexes (%) were significantly elevated in the >7 years group compared to the >3 to  $\leq 5$  and  $\geq 5$  to  $\leq 7$  years groups ( $P \leq 0.05$ ), and in the  $\geq 5$  to  $\leq 7$  years group compared to the >3 to  $\leq 5$  years and  $\geq 5$  to  $\leq 7$  years groups and seminal plasma ASAs indexes were significantly higher in the Waddah breed compared to the Najaheem breed ( $P \leq 0.05$ ). The sperm motility and viability were significantly higher in the >7 years group compared to the > 3 to  $\leq 5$  years and  $\geq 5$  to  $\leq 7$  years groups ( $P \leq 0.05$  and  $P \leq 0.01$ , respectively). Significant differences were also observed between the examined two breeds concerning sperm viability and motility with sperm viability being higher in the Majaheem breed ( $P \leq 0.05$ ) and sperm motility in the Waddah breed ( $P \leq 0.05$ ). Our data demonstrated the presence of ASAs in sera and seminal plasma plasma of infertile dromedary male camels. Dura results suggested that age and breed influenced serum and seminal plasma plasma levels of ASAs in male camels. Data presented in the current study highlight the potential role of ASAs in camel infertility; however, more work is needed to determine ASAs' contribution to reproductive challenges in camels.

Keywords | Anti-sperm antibodies, Dromedary Camels, Seminal plasma, Serum, ELISA

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**Supplementary Table 1:** Evaluated ELISA assay parameters of camel serum of infertile and control samples (individual cases)

Case-No.	ODs at first dilution (1:1)	Conc. at first dilution (1:1) ng/100µl	ODs of cut- off values *	Conc. at cut-off values ng/100µl	Antibody titer at cut-off values	Antibodies index (%)	Breeds
1	0.17	21.1	0.041	15.1	1/4	10.711**	Majaheem
2	1.05	263.0	0.041	15.1	1/128	84.351	Majaheem
3	1.08	354.8	0.039	14.8	1/128	86.861	Waddah
4	0.72	120.2	0.041	15.1	1/128	56.736	Waddah
5	0.81	134.9	0.042	14.8	1/128	64.267	Waddah
6	0.13	19.9	0.042	15.1	1/4	7.3640**	Waddah
7	0.67	85.11	0.045	16.9	1/128	52.552	Waddah
8	0.68	89.1	0.044	16.6	1/128	53.389	Waddah
9	0.81	138.0	0.046	14.1	1/256	64.267	Majaheem
10	1.11	380.2	0.041	14.8	1/32	89.372	Majaheem
11	0.92	177.8	0.041	14.4	1/64	73.472	Majaheem
12	0.39	44.7	0.037	14.1	1/8	29.121**	Majaheem
13	0.38	42.6	0.041	14.8	1/8	28.284	Majaheem
14	0.55	97.7	0.037	14.1	1/16	42.510	Majaheem
15	0.36	43.6	0.044	12.9	1/16	26.610**	Waddah
16	0.44	47.8	0.039	14.1	1/16	33.305**	Waddah
17	0.637	17.8	0.041	12.8	1/64	49.790	Waddah
18	1.037	331.0	0.044	13.5	1/128	83.263	Waddah
19	0.842	138.0	0.037	14.1	1/16	66.945	Waddah
20	1.036	223.8	0.040	12.6	1/64	83.179	Waddah
21	1.038	218.8	0.041	13.5	1/256	83.347	Waddah
22	0.937	199.5	0.037	11.7	1/32	74.895	Majaheem
23	0.836	190.5	0.039	12.9	1/32	66.443	Majaheem
24	0.844	190.5	0.039	13.21	1/32	67.112	Waddah
25	1.22	398.1	0.041	15.8	1/256	98.577	Waddah
26	1.06	257.0	0.031	20.9	1/32	85.188	Waddah
27	1.08	354.8	0.039	15.1	1/128	86.861	Waddah
28	0.842	151.3	0.037	18.6	1/16	66.945	Waddah
29	1.036	223.8	0.042	22.4	1/64	83.179	Waddah
Mean -ve	0.042 ±0.0014	13.741 ±2.19	0.041 ±0.00	13.102 ±1.054	1/2	6.876	
Mean+ve	1.221 ±0.038	406.1 ±11.71	0.0396± 0.0009	15.4 ±0.565	1/256	98.855	
Sensitivity %	93.10%						

Specificity 100% %

\*The cut-off (also known as the threshold) is the unit of activity in a serodiagnosis test above which animals are classified as positive and below which they are considered negative.

\*\* False positive

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**Supplementary Table 2:** Evaluated ELISA assay parameters of camel testicular seminal plasma of infertile and control samples (individual cases)

Case-No.	ODs at first dilution (1:1)	Conc. at first dilution (1:1) ng/100µl	ODs of cut-off values *	Conc. at cut-off values ng/100µl	Antibody titer at cut-off values	Antibodies index (%)	Breeds
1	0.132	19.9	0.041	15.1	1/64	8.298**	Majaheem
2	0.51	50.11	0.041	12.6	1/16	39.668	Majaheem
3	0.46	44.7	0.039	15.1	1/32	35.518	Waddah
4	0.68	51.3	0.037	18.6	1/32	53.775	Waddah
5	0.36	44.7	0.042	18.6	1/32	27.219	Waddah
6	0.087	15.1	0.031	13.5	1/32	4.5643**	Waddah
7	0.36	40.7	0.037	12.9	1/32	27.219	Waddah
8	0.38	41.7	0.031	13.2	1/512	28.879	Waddah
9	0.47	43.6	0.037	14.8	1/16	36.348	Majaheem
10	0.57	50.1	0.037	14.1	1/8	44.647	Majaheem
11	0.38	44.7	0.040	14.1	1/16	28.879	Majaheem
12	0.076	14.1	0.030	12.0	1/16	3.6514**	Majaheem
13	0.42	42.6	0.037	15.1	1/16	32.199	Majaheem
14	0.064	75.8	0.032	10.2	1/16	2.6556**	Majaheem
15	0.051	13.1	0.031	11.2	1/16	1.5767**	Waddah
16	0.39	41.7	0.036	12.8	1/128	29.709	Waddah
17	0.082	17.8	0.031	11.7	1/32	4.1493**	Waddah
18	0.56	44.7	0.033	13.2	1/256	43.817	Waddah
19	0.76	125.9	0.034	14.1	1/256	60.414	Waddah
20	0.87	199.5	0.033	14.1	1/16	69.543	Waddah
21	0.90	223.9	0.032	14.1	1/32	72.033	Waddah
22	0.50	64.6	0.031	15.1	1/32	38.838	Majaheem
23	0.85	162.2	0.032	15.5	1/64	67.883	Majaheem
24	0.79	186.2	0.031	14.8	1/64	62.904	Waddah
25	0.48	45.7	0.041	17.8	1/8	37.178	Waddah
26	0.52	51.3	0.037	18.6	1/8	40.497	Waddah
27	0.47	43.6	0.036	16.6	1/8	36.348	Waddah
28	0.66	125.9	0.037	16.9	1/8	52.116	Waddah
29	0.50	70.8	0.037	15.5	1/8	38.838	Waddah
Mean -ve	0.032±0.0009	14.001±2.909	0.033166 ±0.006	13.9±2.333	1/1	1.2346	
Mean+ve	1.221±0.038	406.1±11.71	0.0396 ±0.0009	15.4±0.565	1/256	98.855	
Sensitivity	79.31%						

%

Specificity 100%

%

\*The cut-off (also known as the threshold) is the unit of activity in a serodiagnosis test above which animals are classified as positive and below which they are considered negative.

\*\* False positive