Detection of Pregnancy in Horses by Breath Analysis using Differential Ion Mobility Spectrometry (DMS)

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Aim

First results of pilot studies in human medicine, regarding the analysis of volatile organic breath compounds (VOC) for diagnostic purposes by means of spectrometric techniques, i.e. detection of gravidity, specification of medications or different diseases, encouraged us to investigate, whether one of these techniques is useful in veterinary medicine.

Material and methods

A portable spectrometric system – Differential Ion Mobility Spectrometry (DMS) – was utilised in a first pilot study in equine species. The technical device was adapted to the animals using a face mask, completed by various other components (tubes, valves). Breath samples of fifteen pregnant and ten non-pregnant mares were taken in these first investigations. Clusters of peaks representing the same VOC were evaluated using a special statistical algorithm and compared to serum levels of sexual hormones.

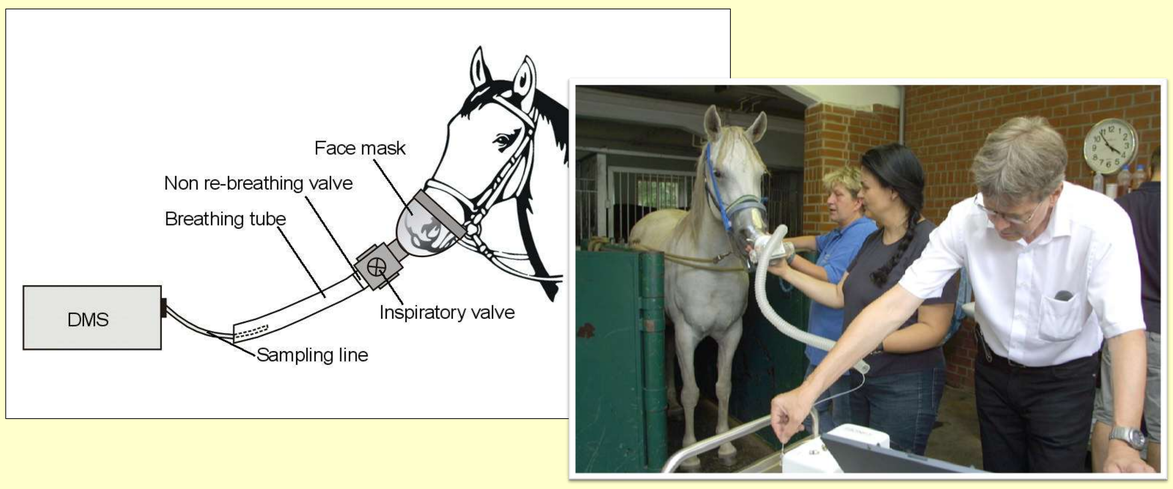

Fig. 1: Principle of adaption of the DMS to the equine species via face mask (left), performing sample collection of a horse (right)

Results

It was possible to detect significant differences in clusters (Coordinates of cluster position Table 1) of exhaled peaks between pregnant and non-pregnant mares and discriminate between both groups (Levels of significance Table 2). Calculating these clusters it is visible that certain VOC's were increased (e.g. cluster 17, 30 72; Figures 2a - c) in pregnancy, new clusters, not detectable in non-pregnant occurred in breath of pregnant animals (e.g. Cluster 24, Figure 2d). Furthermore, visible clusters with reduced peaks in pregnancy (e.g. cluster 68, Figure 2e) were found. Surprisingly it was possible to identify a cluster (cluster 3) in breath which correlates with the estrone sulphate level (Table 3) in serum of pregnant mares inversely.

Table 1 and 2: Position of selected clusters as a function of retention time and compensation voltage (left) and significance levels (p-values) of differences between the pregnant and non-pregnant group (15 and 10 mares, respectively), Mann and Whitney test

Cluster	Position	
	Retention time (s)	Compensation voltage (V)
17	236.5897	-1.1387
24	83.6897	-7.2200
30	110.0455	-7.2200
68	79.1522	-6.1226
72	92.2927	-7.2200

Cluster	Levels of significance pregnant vs. non-pregnant (w test)	
	Sample 1	Sample 2
17	0.0166	0.0002
24	0.0082	0.0018
30	0.0008	0.0003
68	0.0246	0.0134
72	0.0059	0.0019

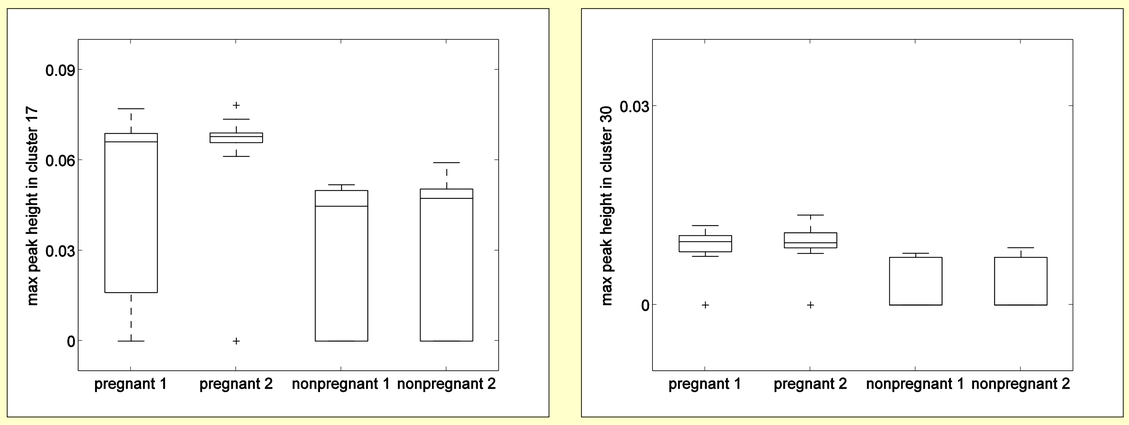

Fig. 2a - e: Maximum peak heights in selected clusters (17, 24, 30, 68, and 72) of pregnant and non-pregnant mares (15 and 10 mares, respectively), separately shown for the first and second breath analysis as Box-and-Whisker Plots

Tab. 3: Levels of eCG, progesterone, estrone sulphate in fifteen pregnant mares, n.d. = non-detectable

Animal	Age (years)	Day of pregnancy	Pro-gesterone (ng/ml)	eCG (IU/ml)	Estrone sulfate (ng/ml)
1	11	57th	15.2	91.4	8.3
2	5	62th	19.9	20.9	14.5
3	13	84th	16.2	0.9	49.6
4	6	84th	13.6	1.0	146.1
5	14	85th	25.8	59.0	43.9
6	10	87th	35.5	1.6	172.6
7	12	87th	12.8	31.8	194.1
8	20	88th	12.1	1.7	55.6
9	4	87th	27.7	4.8	160.1
10	6	92th	13.9	37.2	224.1
11	16	93th	30.1	0.8	278.2
12	16	95th	13.7	32.4	223.4
13	13	96th	8.9	11.1	196.2
14	7	96th	12.3	n.d.	163.5
15	12	97th	10.5	75.0	171.2

Conclusion

Veterinarians do a lot of work detecting pregnancy in farm animals. It would be very useful to replace this physically hard job by another detection method, like the analysis of volatile organic compounds in breath.

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