	n	ΔA (mean)	SD	Coefficient of variation
Sperm plasma bulls, 50× diluted	6	0.2488	±0.0038	1.5%
Sperm plasma boars, 4× diluted	6	0.2880	±0.0046	1.6%

The existing Boehringer coupled reaction assay[8] was split up into 2 separate reactions because of difficulties encountered in our initial experiments with sperm plasma. Some plasmas, especially when diluted with certain buffers, showed own absorption at 340 nm and/or developed turbidity when treated with Boehringer's reagent mixture. Besides, unwanted influence on ΔA determination may be caused by the presence of lactate dehydrogenase and glutamate dehydrogenase[10,11]. The described TCA treatment immediately stops the initial GOT reaction, yielding a deproteinized and clear solution. Under these conditions oxaloacetate proved not to be decomposed. After this, Tris was added to restore a favourable pH environment for the 2nd, MDH catalysed, reaction. Furthermore undiluted or slightly diluted sperm plasma appeared to be an adsorbent for oxaloacetate, thus partly inactivating this reaction component in the MDH reaction. This holds especially for bull sperm. Preliminary dilution of sperm plasma with phosphate buffer (50 times for bulls and 4 times for boars) eliminated this and other problems, connected with unwanted shifts of reaction equilibria when using undiluted or only slightly diluted plasma samples. Under these circumstances it proved to be desirable to raise the temperature and to lengthen the duration of the GOT reaction (respectively to 37 °C and 30 min) in order to produce enough oxaloacetate for a reliably sensitive measurement of the NADH decrease. For the ΔA measurements a reaction time of 5 min appeared to be sufficient for a complete conversion of oxaloacetate.

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