Use of refractometry to prevent inadequate semen extender preparation and consequent impaired quality of porcine semen doses

R Großfeld[1] , K Rüdiger[2], M Schulze[2]

[1]Landshut, Germany; [2]Institute for the Reproduction of Farm Animals Schönow Inc., Bernau, Germany

Introduction

Flaws in extender preparation can cause severe economic losses for AI centers, if claims for compensation are made due to improperly preserved boar semen causing fertility reduction on sow farms. In addition to this, there is currently no suitable and easy to use quality control system for boar semen extender preparations that allows a fast, simple and economic control to show that the extender has been prepared correctly. Measuring the osmotic pressure of the extender would show if the extender has been prepared correctly but this requires expensive equipment.

The aim of this study was therefore to validate a new tool for controlling boar semen extender preparations under practical conditions.

Refractometry (RHB-32ATC, Minitube, Germany) was tested to evaluate if it could be used as a new quality control tool for boar semen extenders and to test if this tool was capable of detecting imprecise dosage of extender before negative effects on sperm quality occur.

Methods

For this brix values and osmolality of correct and incorrect set-up BTS extender were recorded in 10%-steps from 50% to 200% of the correct amount. Twelve boar ejaculates were evaluated after semen dose preparation from these extender solutions for motility with CASA and for mitochondrial and membrane status, as well as for sperm morphological parameter.

Results

The brix value for the correct set-up extender (100%) was 4.6°±0.0°, corresponded to 316±16.0 mOsm kg^{-1} and correlated significantly with osmolality (mOsm kg^{-1} = 74.8 x °Bx+15.8; R^2=0.98; *P*<0.001; **Fig. 1**). The sperm conserving capabilities were influenced by the extender concentration (**Table 1** & **Fig. 2**).

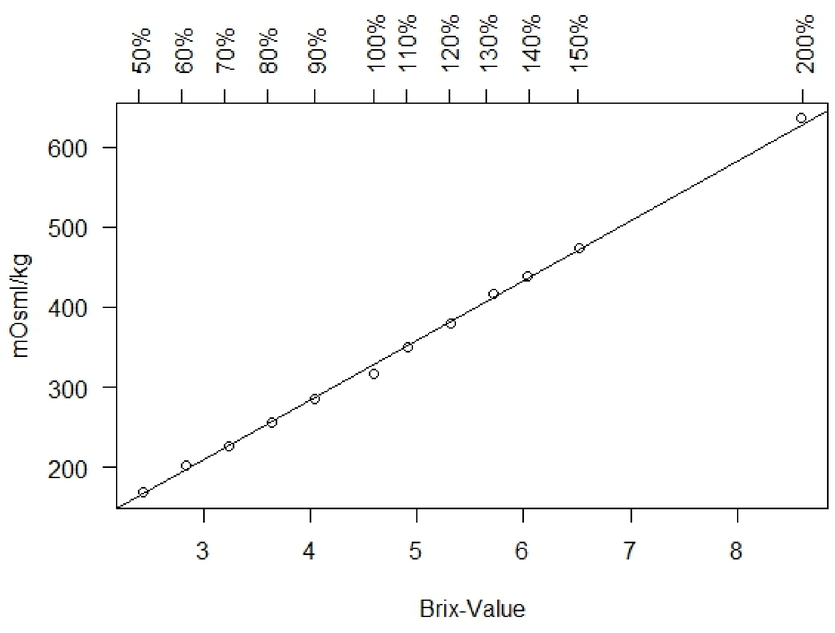

Fig. 1: Correlation of brix value (°) and Osmolality (mOsm kg^{-1}) at different extender powder concentrations

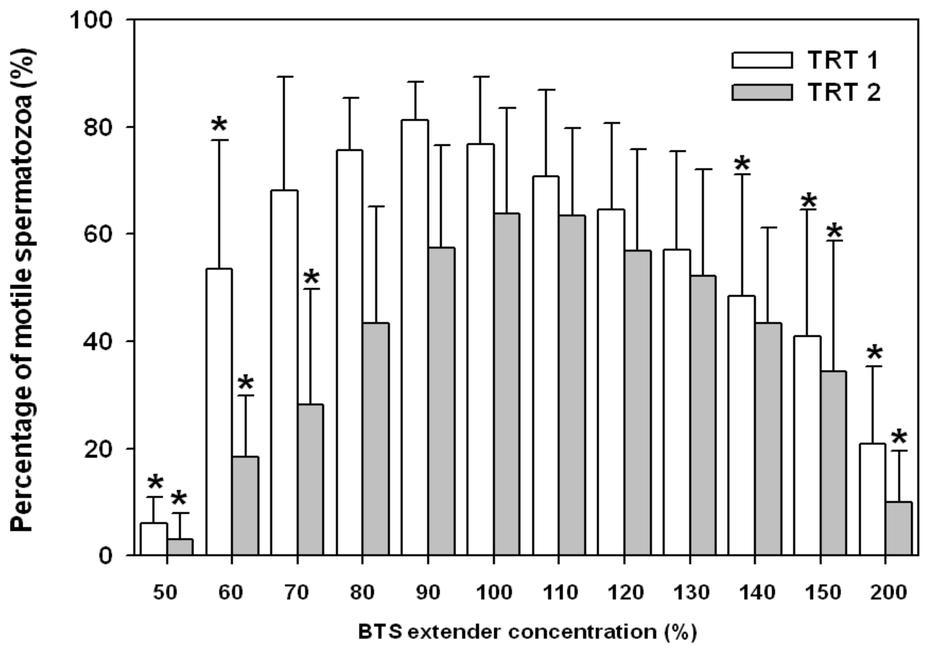

Fig. 2: Sperm motility after a thermoresistance test depending on extender powder concentration
***indicates significant differences from 100% BTS (*P*<0.05)**

Table 1: Selected sperm quality parameters depending on extender powder concentration

Parameter	BTS extender concentration											
	50%	60%	70%	80%	90%	100%	110%	120%	130%	140%	150%	200%
A	13.3±10.2[a]	69.4±21.3[a]	86.2±9.3	88.5±8.0	89.5±8.3	88.7±7.2	88.8±6.1	85.1±7.4	84.8±7.1	84.9±9.3	84.5±9.8	76.3±18.9[a]
B	12.9±3.9[a]	8.0±3.5	4.8±3.1	3.5±2.7	2.2±1.5	3.1±1.5	2.7±1.3	5.4±3.2	4.8±3.2	7.4±6.4	9.1±9.5[a]	13.8±2.7[a]
C	67.3±9.6[a]	13.8±15.0[a]	1.1±1.5	0.5±0.7	0.4±0.7	0.1±0.3	0.3±0.3	0.1±0.3	0.2±0.3	0.0±0.1	0.1±0.2	0.1±0.2
D	5.8±6.2[a]	36.3±21.9[a]	59.6±15.4[a]	73.3±5.8	77.5±6.2	82.5±5.4	80.8±4.2	79.6±3.3	77.5±3.4	76.3±5.3	73.8±9.1	32.9±25.4[a]
E	60.3±10.9[a]	76.5±8.3	84.4±6.6	85.4±4.7	87.3±2.9	89.3±3.3	87.7±5.2	84.7±5.8	78.5±12.1	75.6±15.1	71.8±16.1[a]	63.5±5.2[a]
F	64.6±10.5[a]	70.2±10.0[a]	78.5±7.4	82.8±8.6	84.3±5.4	85.2±5.5	82.1±9.7	80.9±10.2	74.6±11.1	59.9±13.8[a]	58.7±12.2[a]	54.7±8.8[a]
G	3.9±4.4[a]	40.7±20.2[a]	59.2±10.4[a]	70.8±8.7	75.4±5.4	79.2±4.7	78.8±4.8	75.8±9.0	72.1±14.4	70.0±14.3	62.9±16.0	20.8±16.9[a]

Values are expressed as mean ± SD (n=12).
[a] Significant (*P*<0.05) effect of EC (treatment *vs.* 100% EC) within rows.
A) Total percentage of morphologically normal spermatozoa (%)
B) Secondary apical ridge defects (%)
C) Bent tails (%)
D) Sperm motility on d1 (%)
E) Percentage of spermatozoa with intact acrosomes and plasma membranes (%) by staining with PI/FITC-PSA/PNA on d2 (%)
F) Percentage of PI negative spermatozoa with active mitochondria by staining with R123/PI on d2 (%)
G) Sperm motility on d4 (%)

Conclusion

Sperm motility, morphological, mitochondrial, and membrane status are affected by inexact boar semen extender preparation. The brix-index is an indicator of osmolality and may be used to verify precise extender preparation. The sensitivity is sufficient to detect deviations from correct extender preparation before negative effects on sperm quality occur.