

ISSN 1313-7050 (print) ISSN 1313-3551 (online)

**Original Contribution** 

## COMPARISON OF THREE COMMERCIAL DILUENTS FOR SHORT-TERM STORAGE OF BOAR SEMEN

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### ABSTRACT

This study compares the biological and biochemical parameters, and fertility results for boar semen diluted in BTS, MR-A 3 days<sup>®</sup> and DZNB diluents stored up to 48 hours before artificial insemination. The percentage of motile spermatozoa, the sperm viability (live/dead spermatozoa), abnormal gametes, pH, pyruvate, lactic acid, phospholipids and cholesterol were assessed in fresh and stored semen. The fertilizing ability of semen was assessed by artificial insemination of sows with doses containing 3.0 x  $10^9$  spermatozoa. After 48 hours of semen storage, the motility of the spermatozoa decreased significantly in the samples diluted with BTS and DZNB extenders. The proportion of live spermatozoa was higher in fresh semen and decreased only insignificantly in stored semen. Following storage the number of abnormal sperm cells increased significantly in the BTS diluent. The pH of the samples did not increase significantly in any of the diluents. The concentration of the pyruvate decreased insignificantly tendency for increased phospholipids and cholesterol in the seminal plasma. Higher fertility was obtained with MR-A 3 days diluent – 94.44 % used 0-12 hours after collection and 87.10 % with BTS extender after 24-48 hours of semen storage.

Key words: swine, artificial insemination, diluents, fertility.

### **INTRODUCTION**

Artificial insemination (AI) is widely used in pig production. The success of artificial insemination technology in sows is attributed to the availability of suitable diluents (1, 2). Nearly all commercial diluents available today are derived from a formulation proposed over decade ago (3).

Most Bulgarian pig farms produce their own AI doses and in this case the short-term diluents are widely used in practice. The studies of some authors (4, 5) show that stored diluted boar semen stored up to 72 hours maintain a good reproductive performance. A study with 3200 sows showed 89.9 % farrowing rate and an average of 11.1 stillborn pigs per litter after AI with semen used within 24-48 hours of storage (6).

In the current state of the market some companies produce various commercial extenders (BTS, Kiev, MR–3 days, Bio Pig, Mr. PIG 2) for short-term liquid storage of boar semen. In this case the important question is the choice of the suitable diluent as optimal combination of the protective quality and the price.

The aim of this study was to examine some diluents (BTS, MR-A 3 days<sup>®</sup> and DZNB) for short-term storage of boar semen.

### MATERIAL AND METHOD

### Animals

Danube White sows (n=100) housed on a commercial farm were used for the study. The body weight of the sows ranged from 250 to

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280 kg. The lactation length was 25 days. Sows were weaned in individual gestation stalls. The stalls were 0,61 m wide and 2,13 m long. Depending on their body conditions, the sows were fed daily 1,80 to 2,30 kg of balanced diet for gestating sows. All sows and gilts were provided *ad libitum* access to water. All animals were inspected daily for aspects concerning their health, welfare and body conditions.

### Semen collection and treatment

Semen was collected no more than twice per week from *Duroc* boars housed on the same farm by using the gloved hand method.

The ejaculates which met or exceeded the minimum spermiogram standards for fresh, non-diluted semen (7) were divided into three and diluted equal parts using three commercially available short-term diluents -BTS (Magapor, S. L., Spain), MR-A 3 days<sup>®</sup> (Kubus, S. A., Spain) and DZNB (Multidesign, Bulgaria), and packaged in plastic bottles. The dilution rate of pooled semen was 1:3 - 1:5 (v/v). The bottles of semen were placed in the semen cooler in 20 to 30 min. after dilution of semen. Diluted sperm was stored in a cooler unit at 17° C until used within 48 hours after collection.

### Semen quality tests

Volume (without gel fraction) was determined by using a graduated glass vessel with precision of 0.01 ml. The number of sperm cells was determined by using a Thoma counting chamber. The percentage of motile spermatozoa (a scale ranging from 0 to 100 %) was subjectively evaluated with a light microscope at a magnification of 100X. The viability (live/dead spermatozoa) and abnormal spermatozoa were performed using eosin-nigrosin smears under light microscope. The pH of the samples was measured by OP-21 electrode type.

### Biochemical analyses

The spermatozoa were separated from seminal plasma by centrifugation (500 x g for 10 min). The supernatant was centrifuged again under the same conditions, giving a second supernatant, which was then centrifuged at 3000 x g for 30 min. The final supernatant was considered *"seminal plasma"* and frozen (-20<sup>o</sup>C) until further analyses.

After a rapid thawing the obtained supernatants were used for the colorimetric

determination of pyruvate (8), lactic acid (9), phospholipids (10) and cholesterol (11).

# Oestrus detection and artificial insemination

Oestrus detection of weaned sows was performed twice per day (09.30 to 10.30 and 15.00 to 16.00) starting on day 3 after weaning. Eight to ten weaned sows were placed in a pen and checked for oestrus with a mature boar.

Animals that showed a standing reflex were used for the experiments. Cervical inseminations were performed in the traditional manner by inserting a Spiral type catheter through the vagina into the cervix. Animals were inseminated twice at 8 h and 24 h after the onset of standing reflex with doses containing  $3.0 \ge 10^9$  spermatozoa. Pregnancy was diagnosed at 28 to 30 days after insemination by ultrasonography (PREG-TONE<sup>®</sup>, Renco Corporation, USA). All pregnant animals were allowed to carry litters to term.

### Statistical analysis

The data from the experiments were subjected to statistical analysis by Student's *t*-test using the Statistica program package (Windows version, Stat. Soft. Inc., Tulsa, USA, 1994).

### RESULTS

The results from semen quality tests on fresh and stored boar sperm are presented on Table 1. After dilution of the semen with the diluents the motility of spermatozoa did not increase significantly in comparison with neat sperm. However, after 48 h of storage, the motility decreased significantly in BTS extender (by 8.83 relative percent, p<0.05), MR-A 3 days diluent (by 15.00 relative percent, p<0.05) and by 10.00 relative percent (p<0.05) in DZNB diluent. The percentage of live spermatozoa was higher in fresh semen and not significantly lower in stored semen. Analogous changes were observed in the percentage of dead spermatozoa. The storage of the diluted semen was not adversely affected with regard to the sperm cells but the number of dead spermatozoa was higher in stored semen samples - 26.00 % (BTS), 26.33 % (MR-A 3 days) and 25.84 (DZNB). The percentage of abnormal spermatozoa in the semen after dilution was low - 9.50 % (BTS), 9.00 % (MR-A 3 days) and 8.17 % (DZNB). After storage of the semen there was a

tendency for increase of the abnormal sperm cells, but only in BTS diluent the difference was significant (p<0.05). pH values of freshly diluted ejaculates were different depending on the pH of the extenders. During the storage period, pH of the semen samples increased by 0.06 points (0.02-0.09) for all diluents, a nonsignificant effect.

The concentration of the pyruvate as the final metabolic product on glycolytic pathway after dilution of the semen was of similar levels, but was higher in DZNB diluent - 57.00 nmol/ml (p<0.05) (Table 2). The storage of the semen had no significant effect on the pyruvate concentration in all extenders. This dynamic of the pyruvate concentration was connected with the changes in lactic acid concentration in the sperm. The lactic acid level in the freshly diluted ejaculates did not show any significant differences in all the diluents. After storage of the semen increase of the lactic acid concentration - 11.06 µmol/ml (p<0.05) in BTS diluent, 16.56 µmol/ml (p<0.05) in MR-A 3 days extender and 2.58 µmol/ml in DZNB diluent was observed.

In fact, during of storage the concentration of the phospholipids and cholesterol in the seminal plasma increased in all diluents - 1.12 µmol/ml (BTS), 0.82 µmol/ml (MR-A 3 days), 0.51 (DZNB) and 0.32 mmol/l (BTS), 0.07 mmol/l (MR-A 3 days), 0.51 mmol/l (DZNB) respectively. The highest levels of the phospholipids and cholesterol were observed in DZNB extender after storage of the semen. The better farrowing rate was obtained with MR-A 3 days diluent - 94.44 % (p<0.05) used 0-12 hours after collection (Table 3). The BTS extender had 87.10 % farrowing rate after 24-48 hours of semen storage. The number of pigs born and the number pigs born alive were similar after artificial insemination with fresh and stored semen in all diluents.

### DISCUSSION

By using semen quality tests and biochemical analyses, we evaluated the biological ability of boar spermatozoa under short-term liquid storage conditions. The results showed that the extenders ensured the good conditions of the gametes which were connected with increasing of the motility. The high motility of the spermatozoa in the diluted semen compared to neat semen was logical and it was connected with the "*dilution effect*" in mammalian sperm (12). After 24-48 hours of storage the motility was >60 %, but it was the optimal conditions to ensure the optimal fertilizing ability (13). During the storage period, pH value increased with no significant differences in all extenders which was an indicator of pH stability of the diluted and stored semen determined by the buffer components in the diluents. Similar results were obtained by other authors (14).

The dynamics of pyruvate and lactic acid during storage of the semen resulted from accelerated anaerobic metabolism of the spermatozoa, since there was shortage of oxygen in the semen. On the other hand, the total oxygen consumption was more/higher (i.e. presence of "dilution effect") and the gametes were in the partial hypoxemia. In this case, H<sup>+</sup> from glyceraldehyde-3-phosphate dehydrogenase reaction was transformed through LDH at the pyruvate and was accumulated in the lactic acid. The accelerated anaerobic metabolism was compensational mechanism for the energy requirements of the spermatozoa in the diluted semen. This tendency was clearer in MR-A 3 days diluent and less in DZNB extender. Probably, these effects were connected with the different carbohydrate levels in the diluents which ensured the source of the energy metabolism of the spermatozoa.

After storage of the semen there was the tendency for increased phospholipids and cholesterol in the seminal plasma. In this aspect, the high concentrations of these plasmatic components demonstrated the destructive changes in sperm membranes. These biochemical effects were synchronized with the dynamic of the semen quality parameters in the stored sperm.

The reproductive parameters (farrowing rate, number of pigs born and number of pigs born alive) demonstrated, that all extenders had high protective quality, regarding the preserved biological ability of the spermatozoa.

## CONCLUSION

We therefore conclude that BTS and MR-A 3 days<sup>®</sup> extenders are an effective diluents for short-term liquid storage of boar semen.

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*Table 1.* Biological parameters of fresh and short-term liquid storage (+15-17 %/48 hours) boar semen (*Mean*  $\pm$  *S.E.*, *n=6*)

	0 h of storage			48 h of storage			
Sperm parameters	neat semen	BTS	MR-A 3 days	DZNB	BTS	MR-A 3 days	DZNB
Motility (%)	$76.66\pm5.2$	$79.16\pm2.0^{\rm A}$	$77.50\pm7.6^{\rm B}$	$78.33 \pm 5.2^{\circ}$	$70.33 \pm 0.8$ <sup>A</sup>	$62.50 \pm 8.8^{B}$	$68.33 \pm 5.2^{\circ}$
Live spermatozoa (%)	$78.80 \pm 15.8$	$74.00\pm4.12$	$73.67\pm2.9$	$74.16\pm6.5$	$71.60 \pm 1.2$	$70.80\pm9.7$	$72.00\pm9.6$
Dead spermatozoa (%)	$21.20\pm5.8$	$26.00 \pm 4.12$	$26.33 \pm 2.9$	$25.84 \pm 6.5$	$28.40 \pm 1.2$	$29.20\pm9.2$	$22.00\pm9.7$
Abnormal spermatozoa (%)	$10.00\pm3.4$	$9.50\pm5.5^{\rm \ A}$	$9.00 \pm 2.1$	$8.17 \pm 4.5$	$14.00 \pm 4.5$ <sup>A</sup>	$9.00 \pm 1.0$	$11.00 \pm 1.4$
pH	$7.43\pm0.4$	$7.08\pm0.3$	$7.23 \pm 0.2$	$6.71\pm0.4$	$7.17\pm0.2$	$7.25\pm0.2$	$6.80\pm0.5$

<sup>ABC</sup> same superscripts within row indicate differences between means (p<0.05)

pH of BTS =7.04 pH of MR-A 3 DAYS = 7.10 pH of DZNB = 6.86

Table 2. Biochemical	parameters of fresh and short-term l	iquid storage (+15-17°C/48	hours) boar semen (Mean $\pm S.E. n=6$ )
	1		

	0 h of storage			48 h of storage			
<b>Biochemical parameters</b>	neat semen	BTS	MR-A 3 days	DZNB	BTS	MR-A 3 days	DZNB
Pyruvate (nmol/ml)	$88.58\pm37.9$	$45.50\pm27.1^{\rm A}$	$42.17\pm27.3^{\rm A}$	$57.00\pm44.4^{\rm A}$	$37.80 \pm 25.2$	$40.20\pm29.9$	$52.00 \pm 31.3$
Lactic acid (µmol/ml)	$58.28 \pm 9.1$	$31.45\pm13.4^{\rm A}$	$33.39 \pm 13.4^{\mathrm{B}}$	$32.38\pm10.8^{\rm C}$	$42.51\pm6.3^{\rm A}$	$49.95\pm26.8^{\mathrm{B}}$	$34.96 \pm 12.5^{\circ}$
Phospholipids (µmol/ml)	$8.55\pm5.8$	$1.17\pm0.9$	$1.17 \pm 0.1$	$2.41 \pm 1.2$	$2.29\pm2.1$	$1.99\pm0.9$	$2.95\pm2.1$
Cholesterol (mmol/l)	$1.93\pm0.9$	$1.65\pm0.5$	$1.89\pm0.8$	$1.73\pm0.9$	$1.97\pm0.8$	$1.96 \pm 1.3$	$2.24\pm0.4$

<sup>ABC</sup> same superscripts within row indicate differences between means (p<0.05)

*Table 3.* Reproductive performance of sows inseminated with short-term liquid storage (+15-17  $^{\circ}C/48$  hours) boar semen (*Mean*  $\pm$  *S.E.*)

		0-12 h of storage			24-48 h of storage		
Reproductive parameters	BTS	MR-A 3 days	DZNB	BTS	MR-A 3 days	DZNB	
Farrowing rate (%)	88.24 <sup>A</sup>	94.44 <sup>AB</sup>	78.71 <sup>B</sup>	87.10 <sup>C</sup>	84.62 <sup>D</sup>	71.43 <sup>CD</sup>	
Number pigs born (nrs.)	$11.04 \pm 1.8$	$10.00\pm3.7$	$10.27\pm2.5$	$11.92\pm2.1$	$10.30\pm4.05$	$12.00\pm0.8$	
Number born alive (nrs.)	$10.70\pm1.6$	$9.93\pm3.6$	$10.18\pm2.48$	$11.61\pm2.3$	$9.80\pm4.3$	$10.00\pm0.1$	

<sup>ABCD</sup> same superscripts within row indicate differences between means (p<0,05)

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