

## Effect of antioxidants added to boar semen extender on the semen survival time and sperm chromatin structure

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### SUMMARY

The aim of the experiment was to determine the effect of potential antioxidants (adenosine, L-cysteine hydrochloride, ascorbic acid, magnesium fumarate and prolactin) supplementing the Biosolwens extender on semen survival time and sperm chromatin structure. The semen motility was examined every day and the susceptibility of sperm chromatin to denaturation was evaluated on collection day and day 15 of storage. The addition of magnesium fumarate to Biosolwens extender increased sperm survival but resulted in the highest increment in the proportion of sperm with damaged chromatin. Biosolwens supplemented with 200 mg of L-cysteine hydrochloride brought the best results. It is possible that lower concentrations of this component would act in a more protective manner. The examination of the chromatin structure appears to be an useful tool for investigation of semen preservation. *Reproductive Biology* 2003, 3(1): 81-87 .

**Key words:** boar, semen, diluent, survival time, chromatin structure

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## INTRODUCTION

Long-term efforts to develop a simple and efficient method for cryopreservation of boar semen have been unsuccessful. A small number of insemination doses continues to be obtained from one ejaculate and frozen-thawed boar spermatozoa are still of inferior biological value. This means that insemination with frozen semen generally results in low fertility and fecundity. Although this line of research has not been abandoned, great emphasis is placed on the improvement of extenders used for storage of boar semen in above-zero temperatures (about 15°C). Attempts have been made to extend the time of semen storage without reducing fertility indices [9, 10].

Every extender provides the preserved cells with components which ensure a source of energy, proper pH and osmotic pressure. It also prevents thermal shock and inhibits bacterial growth. Other substances are being sought to improve the preserving properties of the extender. It was discovered, however, that some of the extender components may cause an increase in chromatin damages [8] thus reducing semen fertility.

The aim of the present experiment was to determine the effect of potential antioxidants i.e. adenosine [1], L-cysteine hydrochloride [1, 3], ascorbic acid [1], magnesium fumarate [1] and prolactin [5] as supplemented to the Biosolwens extender on survival time of semen and sperm chromatin structure.

## MATERIAL AND METHODS

Fresh semen from three crossbred boars aged less than two years was used in the experiment. After collection, separation of the gel and assessment of motility and concentration, the semen was divided into several portions. Depending on the experiment, semen was diluted with PBS, Biosolwens extender (glucose, sodium citrate, EDTA, sodium acid carbonate, PVP (polyvinylpyrrolidone), KCl, gentamycin) or one of its modifications (for details see table 1) and kept at 15°C.

**Sperm motility.** Samples of semen were kept at 38°C and motility was assessed at 30 and 60 min of incubation. The procedure was repeated every

Table 1. List of semen extender supplements

Supplement	Modification no.	Concentration (per 1 l)
adenosine	1	150 mg
	2	750 mg
	3	1500 mg
cysteine	4	200 mg
	5	500 mg
	6	2500 mg
ascorbic acid	7	250 mg
	8	500 mg
	9	2500 mg
magnesium fumarate	10	250 mg
	11	500 mg
	12	2500 mg
prolactin	13	100 i.u.

day until the motility reached 30% of normally motile sperm which signifies the end of survival period.

**Chromatin damage.** Chromatin damage was examined on the sampling day (day 0) and day 15 of the storage. The increase of chromatin abnormalities was calculated according to the following formula: [value of examined parameter on day 15] – [value of examined parameter on day 0]. Sperm chromatin abnormalities were analysed using the SCSA (Sperm Chromatin Structure Assay) method [6]. Briefly, partial denaturation of sperm chromatin was induced by decreasing the semen pH. Next, spermatozoa were stained with metachromatic fluorochrome - acridine orange. The green (normal DNA) and red (denatured DNA of abnormal chromatin) fluorescence was analyzed using Coulter Elite flow cytometer.

Spermatozoa with structurally abnormal chromatin were analyzed on the basis of an artificial parameter -  $\alpha t$  calculated for each spermatozoon, where

$\alpha t = \text{red} / (\text{green} + \text{red})$  fluorescence. The following calculations were performed for each sample: 1/ percentage of spermatozoa outside the main population of  $\alpha t$  parameter (COMPalpha-t) – reflecting percentage of spermatozoa with abnormal chromatin, and 2/ standard deviation of  $\alpha t$  parameter (SDalpha-t) – reflecting progress of chromatin destruction (higher SDalpha-t means higher chromatin damages).

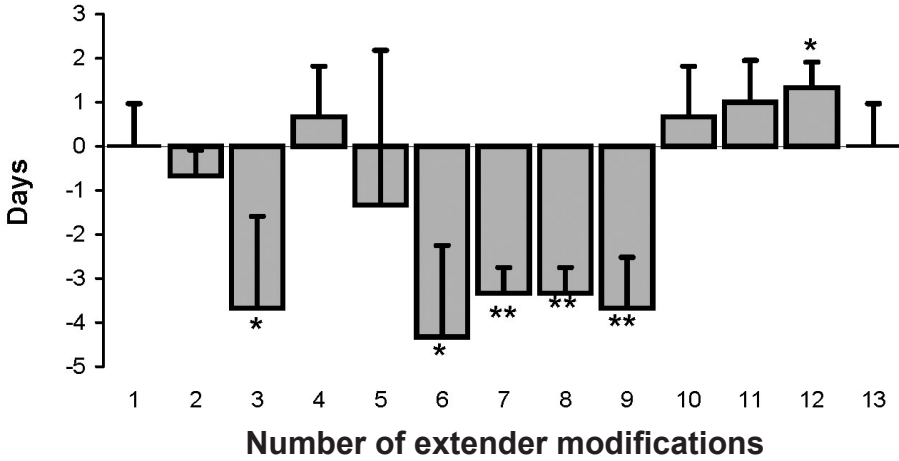
To illustrate the effect of adding antioxidant to Biosolwens as the basic extender on each of the traits, values for each modification were calculated according to the formula: [value of trait for a given modification] - [value of trait for Biosolwens]. The result were analysed statistically with the T test.

## **RESULTS AND DISCUSSION**

Changes in semen survival time after the application of different modifications of Biosolwens extender are shown in fig. 1. The mean survival time of the semen diluted with Biosolwens extender was 4.4 days (2.3 – 5.5 days for different boars).

The enrichment of Biosolwens extender with magnesium fumarate (modifications no. 10, 11 and 12) increased the time of semen survival above that observed for Biosolwens alone. The effect was dose dependent (0.67 - 1.33 days). All ascorbic acid modifications (no. 7, 8 and 9) shortened semen survival time (-3.33 to -3.67 days). The shortest survival time was noted for semen preserved in the extender containing 2500 mg of L-cysteine hydrochloride (-4.33 days, no. 6) and 1500 mg of adenosine (-3.67 days, no. 3).

Changes in the proportion of sperm with damaged chromatin in different extenders and PBS are shown in figure 2. The highest increment in the proportion of sperm with damaged chromatin in relation to Biosolwens extender was observed in the group treated with two doses of magnesium fumarate (500 and 2500 mg) and PBS: 6.27%, 15.4% and 6.04%, respectively. The best results were obtained for 500 mg L-cysteine hydrochloride (no. 5) where the number of damaged spermatozoa decreased by 1%.

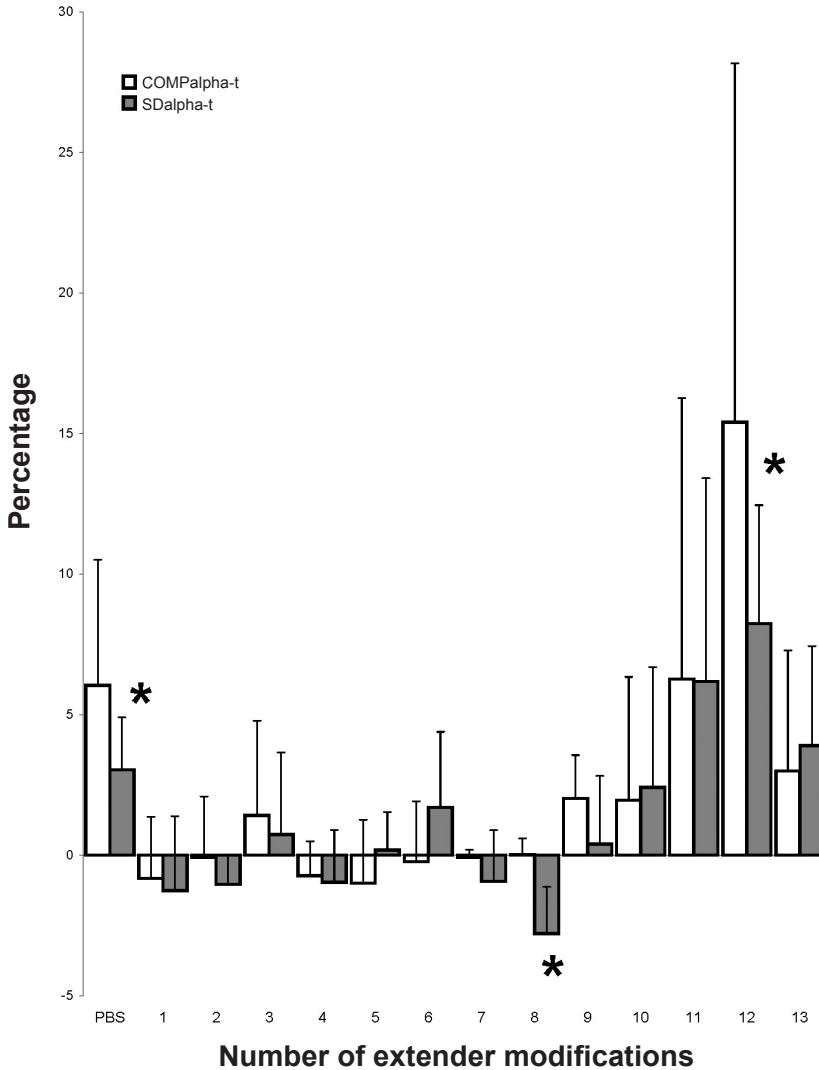


*Fig. 1.* Relative survival time (days) of boar ( $n=3$ ) semen (mean $\pm$ SD) stored in 13 different modifications of Biosolwens extender. Bars represent differences between semen survival time achieved by using Biosolwens extender and its particular modification. List of the modifications is presented in tab. 1. Level 0 describes survival time of semen diluted with Biosolwens extender alone; bars above the level 0 designate survival time longer than that of Biosolwens extender alone; bars below the level 0 designate survival time shorter than that of Biosolwens alone; \* $p<0.05$ ; \*\* $p<0.01$ .

It is of interest that semen samples enriched with magnesium fumarate (no. 10, 11, 12) showed increases in both survival time and sperm chromatin abnormality. This strongly supports hypothesis that chromatin abnormalities are independent on other sperm quality parameters [2, 4, 7].

The present study is of a preliminary nature. The beneficial effect of adding 200 mg L-cysteine hydrochloride to the extender on its preserving capacity suggests that the protective action of this amino acid may be greater in lower concentrations. Possibly the use of lower amounts of the other compounds would give a desired effect.

The use of flow cytometry for evaluating chromatin structure appears to ensure a quick estimation of various media and substances for semen preservation.



*Fig. 2.* The percentage of boar spermatozoa with structurally abnormal chromatin (COMPalpha-t) and chromatin destruction progress (SDalpha-t) in semen (n=3) diluted in PBS and 13 different modifications of Biosolwens extender. Data are presented as means $\pm$ SD. Bars represent differences between COMPalpha-t or SDalpha-t achieved by using Biosolwens extender and its particular modification. List of the modifications is presented in tab. 1. Level 0 describes parameters of chromatin abnormalities in semen diluted with Biosolwens extender alone; bars above the level 0 designate higher values than that of Biosolwens alone; bars below the level 0 designate lower values than that of Biosolwens alone; \*p<0.05.

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**REFERENCES**

1. Balch JF **1998** The Super Antioxidants: Why They Change the Face of Healthcare in the 21st Century. M Evans & Co., New York.
2. Bochenek M Smorag Z Pilch J **2001** Sperm chromatin structure assay of bulls qualified for artificial insemination. *Theriogenology* **56** 557-567.
3. Bounous G **2000** Whey protein concentrate (WPC) and glutathione modulation in cancer treatment. *Anticancer Research* **20** 4785-4792.
4. Darzynkiewicz Z Juan G Li X Gorczyca W Murakami T Traganos F **1997** Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis). *Cytometry* **27** 1-20.
5. Drózdź M Ryszka F Pardela M **1998** Prolactin (PRL) – a review of current knowledge and new perspectives of clinical use. *Medical Science Monitor* **4** 1991-1994.
6. Evenson DP **1990** Flow cytometric analysis of male germ cell quality. In *Methods in cell Biology*, vol **33** *Flow Cytometry*, pp 401-410. Eds Darzynkiewicz Z Crissman HA. Academic Press, Inc., San Diego, California, USA.
7. Gorczyca W Traganos F Jesionowska H Darzynkiewicz Z **1993** Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells. Analogy to apoptosis of somatic cells. *Experimental Cell Research* **207** 202-205.
8. Karabinus DS Vogler CJ Saacke RG Evenson DP **1997** Chromatin structural changes in sperm after scrotal insulation of Holstein bulls. *Journal of Andrology* **18** 549-555.
9. Kuster CE Althouse GC **1999** The fecundity of porcine semen stored for 2 to 6 days in Androhep and X-CELL extenders. *Theriogenology* **52** 365-376.
10. Weitze KF **1990** The use of “long-term extender” in pig AI – a view of the international situation. *Pig News and Information* **11** 23-26.