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# Comparison of different extenders on the quality characteristics of turkey semen during storage

N. Iaffaldano<sup>1</sup>, M.P. Rosato<sup>1</sup>, A. Manchisi<sup>1</sup>,  
G. Centoducati<sup>2</sup>, A. Meluzzi<sup>3</sup>

<sup>1</sup> Dipartimento Scienze Vegetali e dell'Ambiente, Università del Molise, Italy

<sup>2</sup> Dipartimento Sanità e Benessere degli Animali, Università di Bari, Italy

<sup>3</sup> Dipartimento Scienze degli Alimenti, Università di Bologna, Italy

*Corresponding author:* Nicolaia Iaffaldano. Dipartimento Scienze Vegetali e dell'Ambiente. Via De Sanctis, 86100 Campobasso, Italy – Tel: +39 0874 404697 – Fax: +39 0874 404855 – Email: nicolaia@unimol.it

**RIASSUNTO** – Confronto di differenti diluenti sulle caratteristiche qualitative del seme conservato di tacchino. È stato valutato l'effetto di tre differenti diluenti: BPSE, Lake e IGGKPh sulla qualità del seme di tacchino durante la conservazione per 48 h a 5°C. Ogni pool di seme è stato diviso in 3 aliquote le quali sono state diluite rispettivamente con i tre medium e determinate in vitro la motilità (procedura Accudenz®), la vitalità (SyBr-PI) e l'integrità di membrana previo stress iposmotico. La qualità del seme di tacchino peggiora ( $P < 0,01$ ) durante la conservazione per 48 h, tuttavia i valori di vitalità, mobilità ed integrità di membrana risultano più alti ( $P < 0,01$ ) dopo 24 h e 48 h con BPSE rispetto ai diluenti IGGKPh e Lake.

**Key words:** turkey, extender, semen quality, semen storage.

**INTRODUCTION** – Semen of the domestic turkey cannot be stored longer than 6 h without a loss of fertilizing capability. The improvement of long-term liquid storage procedures of semen is important since the commercial production of turkey relies almost entirely on artificial insemination. Therefore, studies improving storage regimens would allow longer storage and consequently hen fertility (Iaffaldano and Meluzzi, 2003). Since the search for an optimal extender composition for semen storage is still in progress, the aim of this paper has been to study the effects of different extenders on the quality of turkey semen during the storage for 48 h at 5°C.

**MATERIAL AND METHODS** – BUT turkey toms 42 weeks of age were used. Six pools of semen, each pool originating from 4 toms, were collected by abdominal massage. The spermatozoa concentration was determined by a Thoma-Zeiss chamber. Each pool was divided into three aliquots and diluted to obtain a concentration of  $2 \times 10^9$  spermatozoa/ml with different extenders: BPSE (Sexton and Fewlass, 1978), Lake (Lake and Ravie, 1984) and IGGKPh (Voronina *et al.*, 1986). The semen aliquots were kept at 5°C for 48 h. The composition (mM) of diluents was for BPSE: fructose 27.7, K citrate 2.1, Na acetate 46.3, Na glutamate 46.3,  $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  1.8,  $\text{K}_2\text{HPO}_4 \times 3 \text{H}_2\text{O}$  62,  $\text{KH}_2\text{PO}_4$  5.2, pH 7.5, osmotic pressure (mOsm/l) 366; for Lake diluent: Na glutamate 58.78, glucose 18.16, K citrate 3.94, BES 143, Na acetate 17.80, Mg acetate  $\times 4 \text{H}_2\text{O}$  3.73, NaOH 56  $\text{Na}_2\text{HPO}_4$  9.57, pH 7.1, mOsm/l 390; for IGGKPh: glucose 49.9, Na glutamate 82.77, K citrate 4.36,  $\text{Na}_2\text{HPO}_4$  69,  $\text{NaH}_2\text{PO}_4$  17.5, inositol 50, pH 6.95, mOsm/l 380. Each semen aliquot was evaluated for sperm viability, membrane integrity and motility after 3, 24 and 48 h of in vitro storage. The sperm mobility was assessed using a Accudenz® solution and was expressed as O.D. (Donoghue *et al.*, 1998) while a dual associa-

tion of stains was used to assess sperm viability according to Donoghue *et al.*, (1995). To determine membrane integrity, a hypo-osmotic H<sub>2</sub>O test was used (Donoghue *et al.*, 1996). Data were submitted to ANOVA (SPSS, 2002) and percentages were transformed into arc sine before analysis.

**RESULTS AND CONCLUSIONS** – The results of SMT, viability and membrane test are shown in Figures 1, 2, 3 respectively.

Cold storage of turkey semen for 48 h worsened ( $P < 0.01$ ) the mobility, viability and membrane integrity of the spermatozoa diluted with the different extenders although the semen diluted with BPSE was less deteriorated during storage. After 3 h of *in vitro* storage, all the parameters were better with BPSE compared to Lake and IGGKPh extender respectively, but no significant differences emerged.

Figure 1. Mobility (SMT) of sperm diluted with BPSE (●) IGGKPh (■) and Lake (▲) extender at 3, 24, 48 h of *in vitro* storage at 5°C. Different letters indicate significant differences ( $P < 0.01$ ).

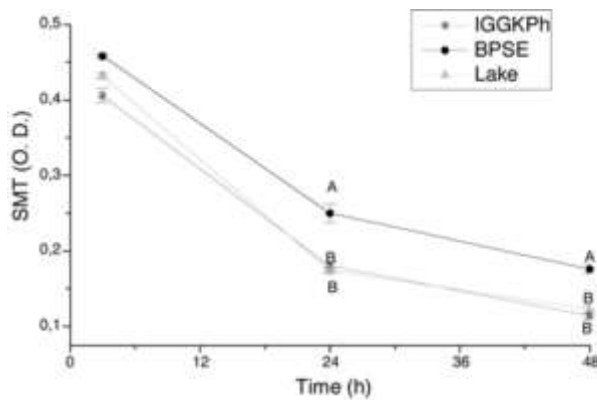


Figure 2. Viability of sperm diluted with BPSE (●) IGGKPh (■) and Lake (▲) extender at 3, 24, 48 h of *in vitro* storage at 5°C. Different letters indicate significant differences ( $P < 0.01$ ).

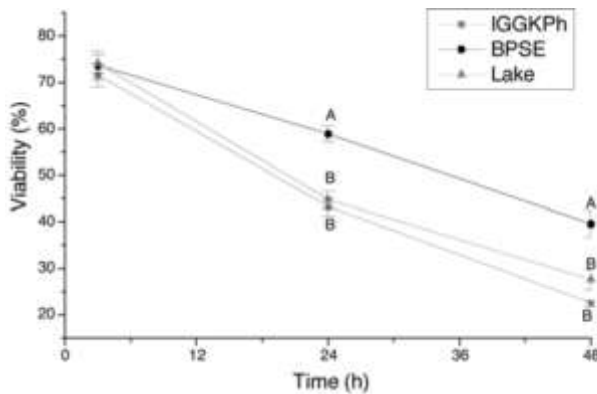
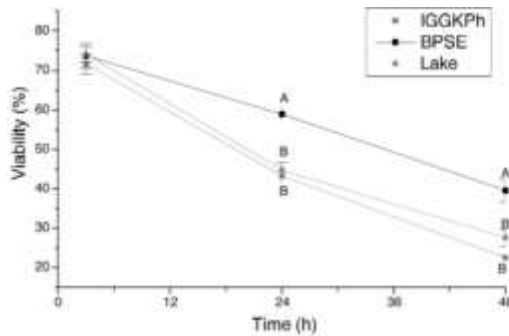


Figure 3. Hypo-osmotic membrane integrity of sperm diluted with BPSE (●) IGGKPh (■) and Lake (▲) extender at 3, 24, 48 h of in vitro storage at 5°C. Different letters indicate significant differences (A,B: P<0.01; a,b:c P<0.05).



After 24 h SMT (P<0.01), viability (P<0.01) and membrane integrity (P<0.05) were significantly higher with BPSE compared to IGGKPh and Lake extenders. The same occurred after 48 h: the SMT was higher (P<0.01) as well as the viability (P<0.01) and the membrane integrity (P<0.01) in BPSE with respect to IGGKPh. Our results indicated that the semen with BPSE extender showed better quality characteristics during storage compared to the other extenders used.

The extenders used for stored turkey semen are buffers appropriate for the immediate survival of spermatozoa because they provide osmotic pressure (330-400 mOsm) and pH (7.0-7.5) similar to those of seminal plasma but they also provide energy substrates as carbohydrates (glucose or fructose) or other components as citrate, glutamate and acetate.

The better quality of turkey semen that occurred when BPSE was used compared to others can be due to presence of all components ensuring energy and also for the higher concentration of acetate. Previous research indicated that acetate is an essential substrate, supporting the motility of spermatozoa more effectively than either fructose or citrate (Douard *et al.*, 2004). The exogenous substrates added to IGGKPh and Lake extender might not be sufficient or appropriate for the needs of gametes during in vitro storage. Moreover, further studies need to ascertain the effect of different extender on the fertilization ability in vivo of stored turkey semen.

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