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Short communication

Reproductive efficiency of a new modified boar semen extender for liquid storage

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ABSTRACT

In the present study the Authors developed a new modified boar semen extender for short-term liquid storage, based on the use of amikacin sulphate and fructose rather than gentamicin and glucose. The new extender (ME-S) was evaluated and compared in vitro to commercial ones (CRONOS™, TRIXcell™) and to a modified extender designated for long term storage (ME-L) for progressive motility. Progressive motility was not different ($P > 0.05$) among extenders until 120 h of storage, as differences among extenders became significant ($P < 0.05$) at 144 and 166 h. Motility data across time were better for ME-S than TRIXcell™ ($P < 0.05$). No differences were observed about the morphology and membrane integrity (ORT) among the new extender (ME-S) and the commercial ones. Following the results of the in vitro comparison, an artificial insemination field trial was performed for reproductive efficacy. In this trial ME-L was not used because it was not completely reliable yet. A total of 1011 sows were bred: 506 with ME-S and 505 with a commercial one (CRONOS™). The pregnancy rate for ME-S was 93.68% (474 pregnant sows), as the commercial extender resulted in 452 pregnancies (89.5%). The statistical comparison was significant ($P < 0.05$) and the number of live piglets born showed an increase of 52.

1. Introduction

Over the last 25 years artificial insemination in pigs has developed enormously, both as a means for increasing animal production as well for improving genetic quality in herds. During this time the number of sperm per artificial insemination (AI) dosage has steadily decreased from about 6×10^9 to less than 3×10^9, and this reduction of sperm numbers in the inseminate dose has greatly increased the efficiency of A.I. At the same time the shelf life of extended semen has become increasingly important for more flexible use of AI (Waberski et al., 2008). Swine sperm are very sensitive to the cooling, likely because of the low cholesterol/phospholipids ratio of their membrane (Dubè et al., 2004); so frozen semen accounts for less than 1% of all inseminations in swine industry (Johnson et al., 2000). Moreover slowed genetics gains, lowered fertility and additional costs may limit use of frozen-thawed semen (Knox, 2011). This condition leads to use mostly fresh semen diluted in short-term extenders (Vyt et al., 2004); thus in swine artificial insemination a major challenge is the maintenance of the viability of the sperm for several days in the extender (Dubè et al., 2004). Fertility of the diluted semen doses usually decline as their preservation period increases and the viability of preserved boar semen is strongly influenced by the composition of the extender used. To meet the different needs of the swine industry, several extenders are commercially available for short and long-term liquid storage. The function of the extender is to supply the nutrients needed

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for the metabolic maintenance of the sperm cells, to control pH, osmotic pressure of the medium and inhibit microbial growth. The source of energy most commonly used in semen diluents is glucose, although other sugars (galactose, fructose, ribose or trehalose) have been tested generally yielding worse results (Gadea, 2003). Sperm morphology gives an indication of sperm viability (Britt et al., 1999), while sperm motility in a medium is an indicator of an active metabolism and the integrity of membranes (Johnson et al., 2000) and it is considered essential for fertilizing ability (Ivanova and Mollova, 1993; Vyt et al., 2004). Sperm motility and viability are fundamental factors to the success of reproduction (Dubè et al., 2004). Motility can be assessed subjectively by visual scoring (Dimitrov et al., 2007; Tardif et al., 1999), as in most AI centres, and objectively by Computer Assisted Semen Analysis (CASA) systems, that provide accurate information (Lòpez et al., 2009) and some motion parameters related to capacitation changes and fertility (Garcià-Herreros et al., 2005; Vyt et al., 2008). The temperature during semen processing and storing, promotes the growth of most Gram negative bacteria (including *Escherichia coli* and some *Salmonella* and *Pseudomonas* species). Therefore, insemination with high contaminated sperm doses can interfere with fertilization resulting in high numbers of sows returning to oestrus (Kuster and Althose 1997). Thus adding an antibiotic at the appropriate concentration improves sperm survival and, in turn, improves fertility results (Gadea, 2003). The present study was aimed at investigating: the in vitro immediate and progressive motility of boar semen diluted for liquid storage with different formulations of a modified extender; the reproductive efficiency of the chosen extender for best motility results on increasing pregnancy rate in sows; the effects on number of piglets born alive.

2. Materials and methods

The study was carried out in accordance with the Italian Legislation on animal care (DL 116/92).

Two different extender formulations for swine, were evaluated, named ME-S and ME-L (Table 1), during 12 trials. The semen was collected and processed from 15 boars of different breeds: seven mature crossbred Landrace × Large White, three hybrids (C21 and Goland), one Pietrain, 4 Duroc, ageing from 10 months to 2 years (average age 17 months). Nine of the semen donors were from a specialized centre in boar semen production, and six were from a farm practicing integral pig production; in both farms the animals were housed in individual pens and managed under similar conditions. All the boars were of proved fertility, and they were normally employed in commercial production of semen for AI. Before semen collections the animals were properly cleaned. Their ejaculates were collected by farms personnel using gloved-hand technique with a dummy, wearing non-spermicidal gloves (nitrile). The semen was collected in a pre-warmed (38 °C) plastic container with 500 ml capacity, without insulated cover cup, with a disposable filter stretched across the opening to separate out the gel component. The presperm fraction and sperm poor fraction were not collected and only the sperm rich fraction

Table 1
Composition (% w/w) of the two modified extenders used in the study.

Component	ME-S[a]	ME-L[b]
Fructose	78.60	27.22
Potassium chloride	1.56	–
Sodium bicarbonate	2.67	4.47
Na EDTA	2.67	6.06
Sodium citrate	12.00	30.30
TRIS	–	16.72
Citric acid	–	11.94
Amikacin sulphate	2.50	3.29

Fructose (Acef, Italy), potassium chloride, sodium bicarbonate, disodium ethylendiaminetetracetate (NaEDTA), tri-sodium citrate dehydrate, citric acid, (Carlo Erba Reagens, Italy), tris-8hydroxymethyl aminometane (TRIS) (DBH Prolabo, Germany), amikacin sulphate (Sigma-Aldrich, USA).
[a] Modified extender Short-Term.
[b] Modified extender Long-Term.

was processed. For each trial both the extenders were used and compared with two commercial extenders (CRONOS™ by Medinova, Reggio Emilia, Italy and TRIXcell™ by IMV technologies, L'Aigle Cedex, France). Each trial was conducted with the following method: in the morning of each experimental session at the lab of Reproduction Unit in Parma the four extenders were prepared, by dissolving in 100 ml of purified water the powder formulations by means of a warmed magnetic stirrer, and maintained at 35 °C during the transport to the breeding centre until the semen collection. Immediately after collection each ejaculate was evaluated for colour (clear, cloudy, presence of blood, turbid or other uncommon colour), smell (typical, no smell, urine-smell, other), and weight/volume (g/ml). Sperm concentration was calculate by photometric means, using a spectrophotometer (Jenway LTD 60-51®, Bibby Scientific Equipment Division, Staffordshire, UK). A drop (10 μl) of undiluted semen was examined on a warmed (37 °C) microscope slide overlaid with a coverslip and observed for sperm motility by phase contrast microscopy at 200 × magnification in at least four fields on the slide taking the average of these readings to obtain the final motility estimate. The semen was then diluted in each extender at 30×10^6 spermatozoa/ml proportion to achieve a final concentration of 1.5×10^9 spermatozoa in a total volume of 50 ml. Within the next 40 min, the diluted samples were transported to laboratory in a thermostat at 23–24 °C, then allowed to cool down at room temperature for 1 h and finally stored at 16 °C. Each sample was investigated in the following days (at 24, 48, 72, 96, 120, 144 and 166 h of storage) for progressive motility and clumping phenomena using a light microscope at 100 × magnification, until motility decreased below 40%. The progressive motility was determined as described for raw semen. The controls were always done by the same trained technician. Morphology was assessed using eosin–nigrosin staining following standard procedures (Shipley, 1999) and Osmotic Resistance Test (ORT) was performed according to the Martin Rillo et al. (1996) technique at 24, 48, 72, 96, 120, 144 and 166 h of storage. An aliquot of crude and diluted semen was referred to the laboratory of the Unit of Infectious Diseases of Animals (Parma University) and investigated for routine microbiology testing and antimicrobial susceptibility tests. Bacteriological swabs were obtained on raw and