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EFFECT OF DIFFERENT SEMEN EXTENDERS AND ADDITIVES TO INSEMINATION DOSES ON EWE´S PREGNANCY RATE

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ABSTRACT

The aim of our study was to compare effects of two additives (glutathione or caffeine) to semen extenders (EquiPro or egg yolk-based diluent) on pregnancy rate of the cervically inseminated ewes. Semen was collected by an artificial vagina from 3 mature Lacaune and 1 Suffolck rams, pooled to eliminate individual differences and divided into five equal aliquots. The aliquots were diluted by either EquiPro or egg yolk-based extenders at 1:3 and supplemented with either glutathione or caffeine. The semen dose diluted in an egg yolk-based extender but without additives was served as a control. The pregnancy rate was significantly different ($P<0.05$) between the group of ewes inseminated with semen diluted in an EquiPro with glutathione (81.25%) and the group inseminated with semen diluted in a egg yolk-based extender added with caffeine (63.16%). No differences among the other groups have been observed, although glutathione, when added either to EquiPro (81.25%) or egg yolk-based (68.97%) diluent, showed a tendency to increase pregnancy rate when compared to caffeine.

These results demonstrate that egg yolk-based extender can be substituted by a synthetic EquiPro extender for the insemination of ewes without reducing fertility. The addition of glutathione (but not caffeine) has a positive effect on fertilizing ability of ram spermatozoa.

Key words: ram; semen; extender; glutathione; caffeine; pregnancy rate

INTRODUCTION

Artificial insemination with chilled-stored semen has become a technique in sheep breeding (Ax et al., 2000). In order to maintain fertilizing ability of semen for a long time semen extenders with various additives have been tested. Efforts to improve the preservation of ram semen are focused on the modification of extenders (Marti et al., 2003), as well as on the addition of various components to maintain motility, fertilizing capacity and preserve sperm membrane integrity (Riha et al., 2006; Sarlos et al., 2002). Egg yolk is generally accepted to be an effective agent in semen extenders for protection of spermatozoa against cold shock and the lipid-phase transition effect (Aboagla and Terada, 2004). However, the use of chilled-stored semen diluted in egg yolk-based semen extenders is limited by its relatively short-time fertilization capacity (Aurich et al., 1997) and individual differences in egg yolk due to different period of egg´s storage. Therefore, removal of chicken egg yolk from a semen extender would improve consistency in the components of semen extenders and eliminate hygienic risk. Therefore, it is reasonable to develop synthetic semen extenders free of egg yolk. A soybean lecitin-based extender (AndroMed, Minitube, Tiefenbach, Germany) has been developed and utilized for bovine (Muller-Schlosser et al., 1995; Aires et al., 2003), mountain gazelle (Saragusty et al., 2006) and sheep (Fukui et al.,

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2008) semen with satisfactory fertility results.

The potential cause of the decline in motility and fertility during hypothermic storage of liquid semen is an oxidative damage of spermatozoa (Ball et al., 2001). Therefore, to maintain sperm for longer period cool and cryopreserved, it is necessary to dilute semen in a protective solution (Ax et al., 2000). A wide variety of antioxidants, such as glutathione (GSH), oxidized glutathione (GSSG), cysteine, taurine, hypotaurine, bovine serum albumine, trehalose or hyaluronan have been tested to minimize the damage caused by cooling and freezing-thawing on bull (Stradaioli et al., 2007; Uysal et al., 2007), water bufallo (Ghosh and Data, 2003), stallion (Ball et al., 2001), goat (Salvador et al., 2006) and ram (Paulenzen et al., 2002; Uysual et al., 2005) semen.

Glutathione can influence cell metabolism through detoxication (De Matos and Furnus, 2000) and by preventing the formation of free radicals (Wijaya, 1996) in spermatozoa. The addition of glutathione to ram sperm diluents improved sperm motility, viability and plasma membrane characteristics, protected the spermatozoa from free radical damage (Triwulanningsih et al., 2003) and improved sperm survival following 6 h cooling storage at 5 °C (Bucak and Tekin, 2007).

Caffeine, when added to Tris-based diluent was beneficial for bull and goat sperm at doses of 10 or 20 mM (Sinha, 1995; El Gaafary et al., 1998), but this effect was dose-dependent. However higher doses caused a deleterious effect on spermatozoa.

The aim of this study was to test the effect of two additives - glutathione or caffeine given in combination with two extenders - egg yolk-based and synthetic EquiPro, on pregnancy rate of ewes following insemination.

MATERIAL AND METHODS

Semen was collected from 3 mature Lacaune and 1 Suffolck rams by an artificial vagina at the local farm (Trenčianska Tepla, Slovak republic). Following evaluation only ejaculates which fulfiled the following criteria were accepted: volume - 0.5 cm^3, sperm concentration > 2.8 x $10^6/mm^3$, motility >70%, morphological abnormalities < 20%.

The semen samples were pooled to eliminate individual differences and divided into 5 equal aliquots. The equal aliquots were diluted (1:3) by either EquiPro (Minitübe, Germany) or egg yolk-based extenders, containing either glutathione (1 $mg.ml^{-1}$) or caffeine (1 $mg.ml^{-1}$) (both from Sigma-Aldrich, St. Louis, MO, USA). The semen dose, diluted in a egg yolk-based extender but without additives, served as a control.

A total of 236 Lacaune x Cigaja cross-breed mature ewes (2-3 years old) were used for insemination. The ewes were treated with 40 mg fluoroprogesterone acetate (Cronolonum; Intervet International, Boxmeer, Holland) using an intravaginal sponge, inserted into vagina of ewes for 14 days. On the day of sponge removal, 500 IU of pregnant mare serum gonadotropin (Werfaser, Werff Chemie; Austria) were injected intramuscularly. Following 48-57 hours after sponge removal the ewes were cervically inseminated and after 24 h reinseminated with a sperm dose at the concentration of 100 x 10^6 spermatozoa.

Pregnancy was diagnosed 60 days after AI by a real-time ultrasonic scan device (Alloka SSD 500). The pregnancy rate (number of pregnant ewes to number of inseminated) in all groups was statistically analyzed using a chi-square test. A difference at $p<0.05$ was considered as significant.

RESULTS

Significant difference in pregnancy rate was observed between the groups EquiPro + glutathione (81.25%) and egg yolk-based extender + caffeine (63.16%; Table 1 and Fig. 1). Although differences between the other groups were not significant, pregnancy rate after the addition of glutathione either to the commercial EquiPro or to egg yolk-based diluents was higher than after the addition of caffeine to either extender (72.22 and 63.16% resp.; Table 1). Generally, higher pregnancy rates were recorded when glutathione or caffeine were used in combinantion with EquiPro extender.

Table 1: Ewe´s pregnancy rate after cervical insemination with ram sperm diluted in extenders in combination with glutathione or caffeine

Semen extender + additive	No of inseminated ewes (n)	No of pregnant ewes (n)	Pregnancy rate (%)
Egg yolk (control)	38	29	76,32
Egg yolk + glutathione	58	40	68,97
Egg yolk + caffeine	38	24	63,16b
Equipro + glutathione	48	39	81,25a
Equipro + caffeine	54	39	72,22

a,b Values with different superscripts are significantly different at $p<0.05$

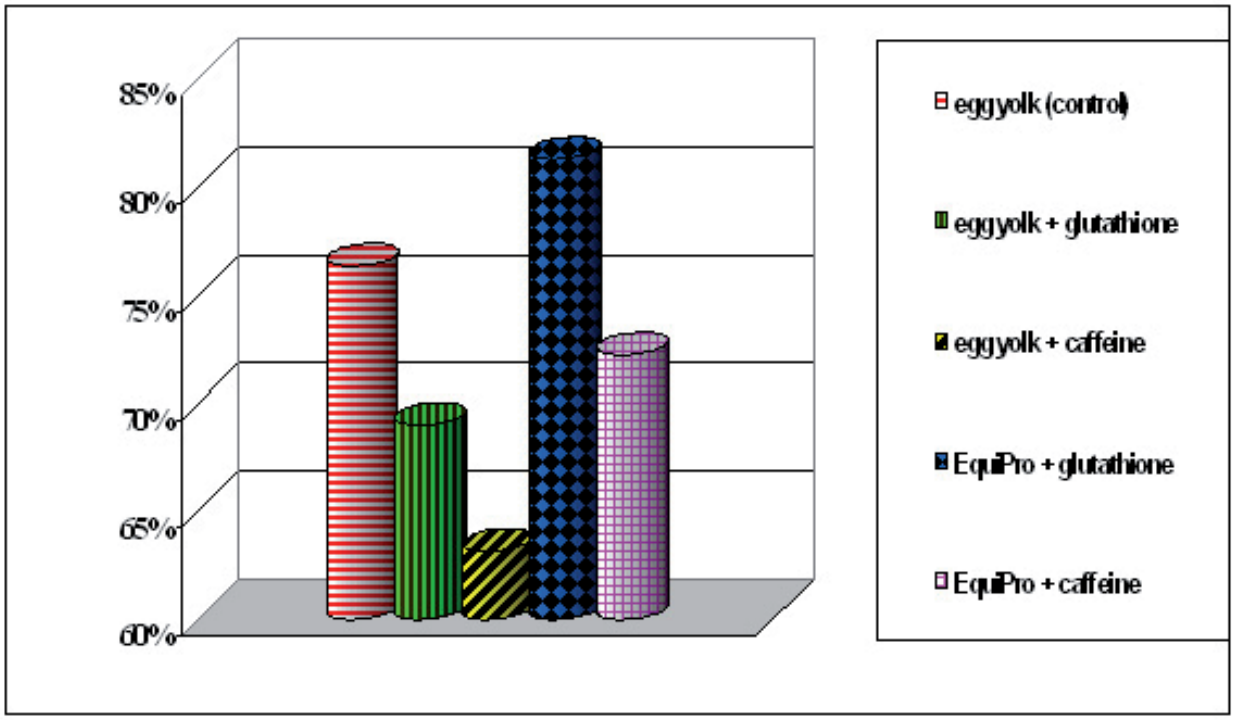

Fig. 1: Comparison of ewe´s pregnancy rates using Equi Pro or Egg yolk diluents in combination with additives

DISCUSSION

Although egg yolk-based extenders have been widely used for dilution and cryopreservation of semen from farm animals, it have serious restrictions because of the individual quality differences in an egg yolk due to the duration of the egg storage and the risk of microbiological contamination. Furthermore, it has been reported that the addition of egg yolk affects the acrosome integrity and the post-thawing viability of ejaculated spermatozoa in goats (Aboagla and Terada, 2004), rams (Watson and Martin, 1975) and water buffaloes (Kumar et al., 1993).

There are many efforts in order to substitute egg yolk-based extenders by other synthetic semen diluents, for example AndroMed, Biociphos Plus (Bielanski, 1997), Bioexcell® (Gill, 2003) - for bovine, ram and goat sperm or EquiPro-defined milk protein extender – for stallion sperm (Pagl et al., 2006). To minimize the damages to sperm during a long-term storage a wide variety of substances such as glutathione, caffeine, cysteine, taurine, hypotaurine, bovine serum albumine, trehalose or hyaluronan have been tested. In our experiments we used glutathione or caffeine as additives to semen extenders. Glutathione has many important functions in the cellular physiology and metabolism, including the protection of the cell from oxidative stress, synthesis of protein and DNA and gamete cell fertilization. Caffeine is a heterocyclic compound that has been reported to promote hyperactivation in bull and human spermatozoa (Ho and Suarez, 2001; Marques and Suarez, 2004) and to enhance sperm motility and improve fertilization (Ho and Suarez, 2001; Pavlok et al, 2001; Marquez and Suarez, 2004). However, Tatham et al. (2003) reported that the treatment of spermatozoa with caffeine and heparine inhibited embryo development to the blastocyst stage. They showed that caffeine, although can promote capacitation and fertilization, but at high concentration, it is detrimental to embryonic development. Caffeine, given into fertilization medium, caused decrease in fertilization rate, delay in pronuclei formation (Pavlok et al., 2001) and high incidence of polyspermic penetration (Mao et al., 2005). Glutathione, when added to spermatozoa diluents, decreased or prevented the emergence of free radicals which are harmful for the plasma membranes. As a result, the conceptional and pregnancy rates were improved (Triwulanningsih et al., 2003).

Caffeine addition in our study led to lower pregnancy rate in comparison to the addition of glutathione independent on the extender used. The addition of glutathione or caffeine to EquiPro commercial diluent provided higher ewe´s pregnancy rate in comparison to the egg yolk-based diluent. Similarly, Fukui et al. (2007) demonstrated that the use of the semen extender without egg yolk resulted in the fertility rate comparable to egg-yolk contained extender (63 and 67% lambing rates for

BSA- and egg yolk-contained extender, respectively). Their conclusion has been supported by the recent experiments using a commercial synthetic extender – AndroMed (Fukui et al., 2008).

In conclusion, our experiments demonstrated that an egg yolk-contained semen extender can be replaced with the synthetic extender EquiPro for the insemination of ewes without reducing fertility. Glutathione showed a positive effect on ram semen. It can be used as an additive to semen extender to enhance pregnancy rate of ewes after artificial insemination.

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