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Effect of different concentrations of egg yolk and virgin coconut oil in Tris-based extenders on chilled and frozen-thawed bull semen

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ABSTRACT

The aim of this study was to evaluate the effects of 8% virgin coconut oil (VCO) combined with different percentages of egg yolk in Tris extender on the quality of chilled and frozen-thawed bull semen. A total of 24 ejaculates from four bulls were collected using an electroejaculator. Semen samples were diluted with 8% VCO in Tris extender which contained different concentrations 0% (control), 4%, 8%, 12%, 16% and 20% egg yolk. The diluted semen samples were divided into two fractions: one was chilled and stored at 4 °C until evaluation after 24, 72, and 144 h; the second fraction was processed by chilling for 3 h at 4 °C to equilibrate, then packaged in 0.25 ml straws and frozen and stored in liquid nitrogen at −196 °C until evaluation after 7 and 14 days. Both chilled and frozen semen samples were then thawed at 37 °C and assessed for general motility using computer-assisted semen analysis (CASA), viability, acrosome integrity, and morphology (eosin–nigrosin), membrane integrity (hypo-osmotic swelling test) and lipid peroxidation (thiobarbituric acid-reactive substances (TBARS)). The results indicate treatments with 8%, 12%, 16% and 20% egg yolk with 8% VCO had greater sperm quality ($P < 0.05$) as compared with the control. The treatment with 20% egg yolk had the greatest sperm quality ($P < 0.05$) among the treated groups for both chilled and frozen-thawed semen. In conclusion, the use of 8% VCO combined with 20% egg yolk in a Tris-based extender enhanced the values for chilled and frozen-thawed quality variables of bull sperm.

1. Introduction

Cryopreservation of sperm has been used in highly effective ways for conserving genetic resources of animals. Since the 20th century, liquid and frozen storage methods have been applied for sperm cryopreservation. These methods, however, have significant implications: some factors have been found to affect the quality of sperm including the chemicals used in extenders, storage temperatures, and extent of bacterial contamination (Yoshida, 2000). The cryopreservation of sperm is a complicated procedure that results in several forms of cellular damage (Purdy, 2006) associated with cold shock, intracellular ice crystal formation, membrane changes, and osmotic alterations (Isachenko et al., 2003; Khalili et al., 2009) which may reduce the sprem motility and resulting fertilization rate when the sperm are used for AI (Matsuoka et al., 2006). The cryoprotectant agent is, therefore, included in the

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cryopreservation media to reduce the damaging effects of the freezing process (Purdy, 2006). Currently, egg yolk is used as a common component of non-penetrating cryoprotectant in semen extenders of domestic animals. Egg yolk has is valuable for semen cryopreservation as a protector of the plasma membrane integrity and the acrosome against temperature-correlated injury because of the presence of phospholipids, in association with other components (Purdy, 2006). Currently, different percentages of egg yolk are used in the cryopreservation procedure. The concentration of 20% egg yolk is, however, still used as a standard amount in most cases for bull sperm cryopreservation (Thun et al., 2002; Crespilho et al., 2012; Rahman et al., 2012). The use of egg yolk as a cryoprotectant, however, has recently been controlled in many countries due to fears of immunologic and hygiene hazards (Thun et al., 2002). Furthermore, some investigators (Amirat et al., 2005) have reported that extenders based on egg yolk can have harmful effects on sperm respiration and motility due to other specific ingredients of the extender. There is a heightened global concern about microbiological contamination of sperm. Due to these limitations, many researchers have focused on finding a more desirable cryoprotectant or at least a supplement that could address these limitations. Many saturated and unsaturated fatty acids have been tested and found useful for sustaining general sperm motility, viability and membrane integrity in chilled and cryopreserved semen of boars (Hossain et al., 2007) and bulls (Kiernan, 2012). Such fatty acids include oleic acids (OA), linoleic acid (LA), arachidonic acid (AA), palmitic acid (PA) and α-linolenic acid (ALA). When used all these fatty acids have, however, failed to solve the problem of microbial contamination. The VCO contains saturated and unsaturated fatty acids (Dosumu et al., 2010), and is also rich in lauric acid (50%) which is considered antibacterial, antiviral, antinociceptive, and anti-inflammatory (Zakaria et al., 2011). Moreover, VCO contains large amounts of antioxidant components such as tocotrienol, polyphenols, and tocopherols (Marina et al., 2009; Nevin and Rajamohan, 2006). With all the these beneficial properties, VCO appears to be a potentially desirable supplement that may have synergistic beneficial actions with egg yolk as an extender although it has not been previously assessed in combination with egg yolk. The present study was, therefore, conducted to evaluate the use of 8% VCO in Tris extender combined with different concentrations of egg yolk for bull semen cryopreservation.

2. Materials and methods

2.1. Animals

Semen samples were collected from four mature and fertile (as previously assessed when used for AI) Brangus-Simmental crossbred bulls from the Universiti Putra Malaysia (UPM) Farm. The bulls were 5–6 years of age and weighed about 625–650 kg. All bulls were managed in similar ways, fed *Brachiaria decumbens* grass and commercial palm kernel cake (PKC) containing approximately 16% crude protein and 2.6% crude fat at a rate of 3 kg/bull/day. Mineral blocks and water were provided *ad libitum*. Approval for the current study was given by the Institutional Animal Care and Use Committee (IACUC) at Universiti of Putra Malaysia (UPM) AUP No. R073/2015.

2.2. Semen collection and preparation of extenders

Ejaculates were collected twice a week, at 3-day intervals every week from each of the four bulls with an electroejaculator (Electrojac 6, USA). A total of 24 samples were collected and then transferred at 37 °C in a cooler box containing warm water to the Theriogenology and Cytogenetics Laboratory at UPM Serdang for evaluations. Ejaculates with more than 80% morphologically normal sperm, more than 60% general sperm motility (this was to confirm that there were no bull effects throughout all the treatment effects), and a concentration of more than 500 $\times$ 10^6 sperm/ml were used for the experiment (Khumran et al., 2015). Semen samples were then extended in one of the two fractions of Tris(hydroxymethylaminomethane) which was composed of Tris (2.42 g)–citric (1.48 g)–fructose (1 g)–glycerol (6.4 ml extenders per 100 ml) (Amirat-Briand et al., 2010) as the primary extender with different percentages of egg yolk (0%, 4%, 8%, 12%, 16% and 20%) and 8% VCO (a value chosen based on a previous preliminary study conducted in our laboratory to determine optimum concentration of VCO for superior post-thaw semen quality results, unpublished data). The VCO used is the product of Nano Xan Sdn. Bhd and Malaysia Agriculture Research and Development Institute, Malaysia (MARDI). Fraction one contains the main extender without glycerol for the extender of chilled treatments. The other fraction, besides the primary extender, contains glycerol (6.4 ml) for the treatments involving freezing. The treatment groups for chilled and frozen sperm were divided into seven groups (Table 1). Semen was extended to adjust the concentrations of sperm to 20 $\times$ 10^6 cells in a

Table 1
Dilution media (8% VCO + egg yolk%) used for each treatment groups.

Treatment groups	Composition of diluting medium
Control + (C+) (20% egg yolk)	Tris-based extender (Amirat-Briand et al., 2010)
Control − (C−) (8% VCO)	Tris + 8% VCO
Treatment 1	Tris + (4% egg yolk + 8% VCO)
Treatment 2	Tris + (8% egg yolk + 8% VCO)
Treatment 3	Tris + (12% egg yolk + 8% VCO)
Treatment 4	Tris + (16% egg yolk + 8% VCO)
Treatment 5	Tris + (20% egg yolk + 8% VCO)

Tris: Tris(hydroxymethylaminomethane).