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Pellet-freezing of Damascus goat semen in a chemically defined extender

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Abstract

During the breeding season of goats (12 bucks and 64 does) in Egypt, five experiments were conducted using a chemically defined cryoextender (CDE) to investigate: (1) the influence of rates of semen dilution (1:2, 1:4 and 1:19) and methods of thawing of frozen semen pellets (dry thawing versus wet thawing) on sperm progressive motility (SPM), sperm acrosome abnormalities (SAA) and rate of lipid peroxidation in semen as measured by malonaldehyde (MAL) production, and (2) the effect of insemination of does in natural ($n = 38$) and cloprostenol-synchronized ($n = 26$) estrus with frozen semen on their kidding rates and prolificacy. Semen (two successive ejaculates/buck) was collected twice a week via an AV and only ejaculates of $>2500 \times 10^6$ sperm/ml and 70% SPM were diluted in one step at 30 °C with the CDE, cooled to 5 °C over a 4 h-period, frozen in the form of 0.30 ml pellets and stored in liquid nitrogen for 72 h. The results revealed that post-thaw SPM of semen diluted at a rate of 1:4 was significantly ($P < 0.01$) higher than that of semen diluted at the other rates. Dilution of semen at a rate of 1:19 ($\leq 151 \times 10^6$ sperm/ml) not only minimized ($P < 0.01$) pre-freeze and post-thaw SPM, but also augmented ($P < 0.01$) pre-freeze and post-thaw rates of lipid peroxidation as evidenced by the high level of MAL production and the ability of antioxidants (1 mg/ml EDTA, 200 U/ml bovine liver catalase, 0.61 mg/ml reduced glutathione and 0.11 mg/ml sodium pyruvate) to restore ($P < 0.01$) pre-freeze and post-thaw SPM. Frozen semen pellets exposed to dry thawing had a greater percentage of SPM ($P < 0.01$) as well as lower values of SAA and MAL ($P < 0.01$) than those exposed to wet thawing. Although the kidding rates did not vary significantly among does in natural (55.26%) and

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synchronized (53.85%) estrus, a higher ($P < 0.05$) prolificacy was obtained after their insemination in natural (1.81 ± 0.16) rather than in synchronized (1.22 ± 0.11) estrus.

Keywords: Goat; Frozen semen; Chemically defined extender

1. Introduction

Constituents of animal origin, such as blood sera, egg yolk and milk, are still widely used in hypothermic storage of eutherian semen (Yoshida, 2000). Bovine serum albumin, low-density lipoprotein, phosphatidylserine and lecithin of egg yolk (Coulter and Foote, 1975; Watson, 1981; Graham and Foote, 1987) together with phosphocaseinate of milk (Batellier et al., 2001; Leboeuf et al., 2003) are the protective fractions during rapid cooling of spermatozoa. Nevertheless, over the past three decades, there was a growing interest in developing and employing chemically defined semen extenders (Coulter and Foote, 1975; Graham and Foote, 1987; Upreti et al., 1995; Leboeuf et al., 2003), particularly diluents free from additives of animal origin and containing soybean protein (Promine-D) (Coulter and Foote, 1975) or soybean lecithin (Aires et al., 2003; Gil et al., 2003a,b). In our preliminary work on conservation of Damascus goat semen (Khalifa and El-Saidy, 2003), we demonstrated that inclusion of 0.60 mM of butylated hydroxytoluene (BHT), as an alternative of egg yolk, in Tris-based extenders significantly sustained post-thaw motility (47.50%) and fertility (53.75%) of frozen sperm.

Membrane fluidity is a prerequisite for proper sperm functions (Graham and Hammerstedt, 1992). In addition to its antioxidative property (Killian et al., 1989), BHT is similar to other compounds, such as β-cyclodextrin (Zeng and Terada, 2000) and trehalose (Aboagla and Terada, 2003, 2004a), in its ability to maintain sperm membrane fluidity during freezing (Hammerstedt et al., 1976) and thawing (Bamba and Cran, 1988) of semen.

The objectives of the present study were to investigate (1) whether the rates of semen dilution and methods of thawing of frozen semen pellets could modulate some attributes of goat semen preserved in a cryomedium devoid of ingredients of animal origin, and (2) whether insemination of goat does in natural and synchronized estrus could influence the fertility of frozen semen.

2. Materials and methods

Unless otherwise stated, all chemicals used in this study were purchased from Sigma–Aldrich Co., Deisenhofen, Germany.

2.1. Semen extender

A chemically defined extender (CDE) was utilized in cryopreservation of goat semen (Khalifa and El-Saidy, 2003). It was composed of Tris (hydroxymethyl) amino methane (3.786 g), D-glucose anhydrous (0.625 g), citric acid monohydrate (2.172 g),