Animal Reproduction Science 118 (2010) 94–97

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Pregnancy and conception rate after two intravaginal inseminations with dog semen frozen either with 5% glycerol or 5% ethylene glycol

Ada Rota[a,*], C. Milani[b], S. Romagnoli[b], P. Zucchini[b], A. Mollo[b]

[a] Department of Animal Pathology, Faculty of Veterinary Medicine, University of Turin, via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy
[b] Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, University of Padua, Agripolis 35020 Legnaro (PD), Italy

ARTICLE INFO

Article history:
Received 17 March 2009
Received in revised form 22 May 2009
Accepted 8 June 2009
Available online 17 June 2009

Keywords:
Intravaginal AI
Dog
Frozen semen
Glycerol
Ethylene glycol

ABSTRACT

The primary goal of this study was to compare the effects of 5% ethylene glycol (EG) and 5% glycerol (G) on fertility of frozen–thawed dog semen following intravaginal insemination. The sperm-rich fraction of the ejaculate of three male dogs was collected, pooled and divided into two aliquots, and then frozen with a Tris–glucose–egg yolk–citric acid extender containing either 5% G or 5% EG. A total of 10 bitches were inseminated twice, five with G-frozen–thawed semen and five with EG-frozen–thawed semen; intravaginal inseminations were performed the 4th and the 5th day after the estimated LH peak; four straws, thawed in a 37 °C water bath for 1 min and diluted in a Tris buffer, were used for insemination (200×10^6 spermatozoa); the insemination dose was introduced in the cranial vagina of the bitch using a sterile plastic catheter. Ovariohysterectomy was performed in all bitches between days 29 and 31 after the calculated LH surge, and pregnancy status, and the number of conceptuses and *corpora lutea* were recorded. All bitches were pregnant. Neither the number of conceptuses, nor the ratio of conceptuses to *corpora lutea* (conception rate) was significantly different between groups. In this first screening, with a limited number of bitches, EG-frozen semen did not show a higher fertility than G-frozen semen when used for two intravaginal inseminations. Irrespective of the semen used, conception rate was 0.50.

1. Introduction

Fertility of frozen–thawed dog semen following intravaginal insemination is generally rather unsatisfactory (Niżański, 2006) and lower than following intrauterine insemination, regardless of whether this is achieved through cervical catheterisation (Fontbonne and Badinand, 1993; Linde-Forsberg et al., 1999; Thomassen et al., 2001) or using a surgical approach (Silva et al., 1996; Tsutsui et al., 2000). However, intravaginal insemination is easy to perform and does not require expensive equipment. Also, semen extenders induce uterine inflammation even if a small amount of extender passes through the cervix when semen is placed in the cranial vagina (Ribeiro et al., 2006); intrauterine insemination is likely to produce a higher degree of inflammation, while deposition of semen in the physiological place may keep it to a minimum. Although the place of frozen–thawed semen deposition strongly affects whelping rate and litter size (Linde-Forsberg et al., 1999), other factors contribute to fertility, such as semen quality (Thomassen et al., 2006), timing of insemination (Shimatsu et al., 2003; Thomassen et al., 2006), number of inseminations (Linde-Forsberg et al., 1999; Thomassen et al., 2006), post-thaw addition of prostatic fluid (Nöthling and Volkmann, 1993; Nöthling et al., 2005).

Freezing strongly reduces semen quality, firstly by decreasing the number of live spermatozoa and secondly by producing cellular modifications which alter spermato-

* Corresponding author. Tel.: +39 0 11 6709051; fax: +39 0 11 6709097.
E-mail address: ada.rota@unito.it (A. Rota).

0378-4320/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2009.06.007

zoa motility, longevity, membrane integrity and fertilizing ability. The large majority of the procedures suggested for freezing dog semen use glycerol as a cryoprotectant (Andersen, 1975; Peña et al., 1998; Rota et al., 1998); more recently, the effect of ethylene glycol on frozen–thawed dog semen quality has been investigated (Soares et al., 2002), and a significant improvement in semen quality at thawing was reported when 5% ethylene glycol was used instead of 5% glycerol in an egg yolk TRIS extender containing Equex STM Paste® (Rota et al., 2006).

The aim of the present investigation was to assess whether the higher initial quality of dog semen frozen with 5% ethylene glycol could give better fertility results compared to semen frozen with 5% glycerol when used in intravaginal inseminations.

2. Materials and methods

2.1. Semen freezing

Three healthy, privately owned, 3–6-year-old, mixed-breed, medium-size, male dogs, of proven fertility in natural matings were used as semen donors. The sperm-rich fraction of the ejaculate was collected by digital manipulation in the presence of an estrous female; a drop of semen was immediately placed on a warmed slide, covered with a warmed cover-slip and evaluated for total and progressive motility under light microscopy (400×). The ejaculates of the three dogs, which showed total and progressive motility higher than 85% and 70% respectively, were pooled, assessed for motility and spermatozoa concentration (Bürker counting chamber, 1:40 dilution with water), and then divided into two aliquots. Each aliquot was centrifuged at $700 \times g$ for 6 min, and then diluted (200×10^6 sp/ml) with a Tris–glucose–egg yolk–citric acid extender containing either 3% glycerol (G) or 3% ethylene glycol (EG) (Rota et al., 2006). After one hour of equilibration at 4 °C, the samples were diluted a second time with an equal volume of an extender containing 1% Equex STM Paste® (Nova Chemicals Sales, Scituate, MA, USA) and 7% G or 7% EG, respectively. After 10 min at 4 °C, 0.5 ml straws were filled with the extended semen and sealed with Seal-Ease® (Becton Dickinson and Company, Franklin Lanes, NJ, USA), placed horizontally 4 cm above the surface of liquid nitrogen for 10 min and then plunged into it. Semen was collected twice from each dog, at one week interval, to obtain enough material for the insemination program.

Frozen straws were thawed in a 37 °C water bath for 1 min and the content was emptied in a test tube where a Tris buffer (Rota et al., 1999) was added at 37 °C in a 1:2 (semen:buffer) ratio. A small drop of semen was used to subjectively assess total motility under light microscopy (400×).

2.2. Artificial insemination

Ten healthy, 1.5–5-year-old bitches (7 beagles, 1 boxer and 2 mixed-breed bitches of medium-size), with regular estrous cycles, were randomly assigned to either group G or group EG (insemination with semen cryopreserved with G or EG respectively) (Table 1).

After proestrus onset, vaginal smears were examined every other day and, when cellular cornification was greater than 80%, blood was collected daily by jugular venipuncture for progesterone determination by chemiluminescence (Immulite®, Diagnostic Products Corporation, Los Angeles, CA) (Kutzler et al., 2003), until insemination. Artificial insemination (AI) was performed twice, on the 4th and 5th day after the estimated LH peak (corresponding to a serum progesterone concentration between 1.0 and 2.0 ng/ml (Concannon et al., 1989). Four straws were used as insemination dose (200×10^6 spermatozoa, in a total volume of 6 ml), two from each semen batch. The insemination dose was aspirated into a 10 ml sterile syringe and deposited into the cranial vagina using a sterile 30 cm long insemination plastic catheter (Kruuse®, Langeskov Denmark). Bitches were maintained with their hindquarters raised for 10 min to prevent the reflux of semen.

Ovariohysterctomy was performed in all bitches between days 29 and 31 after the calculated LH peak. Bitches were given medetomidine (Domitor®, Orion Corporation, Espoo, Finland, 5 μg/kg iv) and methadone (Eptadone®, Molteni & C F.lli Allitti, Firenze, Italy, 0.3 mg/kg im); anesthesia was induced with an intravenous dose of propofol (Rapinovet®, Schering Plough Animal Health, Milan, Italy, 2 mg/kg) and maintained with isoflurane in

Table 1
Pregnancy and conception rates in bitches after two intravaginal inseminations with pooled canine semen frozen with either 5% glycerol (G) or ethylene glycol (EG) as cryoprotective agent.

Bitch	Age (years)	Cryoprotectant	Conceptus (N°)	*Corpus luteum* (N°)	Conception rate conceptus N°/CL N°
Beagle 1	3.0	G	2	5	0.40
Mixed-breed 1	5.0	G	1	7	0.14
Beagle 2	1.5	G	5	8	0.63
Beagle 3	2.0	G	7	7	1.00
Boxer 1	4.0	G	10	13	0.77
Mean ± SD	3.1 ± 1.4		5.0 ± 3.7	8.0 ± 3.0	0.59 ± 0.30
Beagle 4	3.0	EG	2	5	0.40
Mixed-breed 2	3.0	EG	9	14	0.64
Beagle 5	5.0	EG	4	6	0.67
Beagle 6	4.0	EG	1	5	0.20
Beagle 7	5.0	EG	3	8	0.38
Mean ± SD	4.0 ± 1.0		3.8 ± 3.1	7.6 ± 3.8	0.46 ± 0.20