Theriogenology 82 (2014) 844–850

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Comparison of endoscopic-assisted transcervical and laparotomy insemination with frozen-thawed dog semen: A retrospective clinical study

S.J. Mason*, N.R. Rous

Monash Veterinary Clinic, Victoria, Australia

ARTICLE INFO

Article history:
Received 6 July 2013
Received in revised form 12 June 2014
Accepted 18 June 2014

Keywords:
Intrauterine
Frozen-thawed
Semen
Dog
Laparotomy
Transcervical

ABSTRACT

The objective of this retrospective clinical study was to compare pregnancy rates obtained after the use of endoscopic-assisted transcervical catheterization (EIU) or laparotomy (SIU) for insemination of frozen-thawed dog semen. Healthy bitches from various breeds were inseminated with semen from multiple donors processed by different freezing centers. Data from 118 inseminations (78 EIU and 40 SIU) performed between 2009 and 2011 were analyzed. Insemination timing was based on vaginal cytology, serum progesterone concentrations, and vaginoscopy. A ureterorenoscope and a CH-5 Transcervical insemination catheter were used for EIU; 28 of the bitches in this group were inseminated twice with the second insemination less than 12 hours after the first. The numbers of live morphologically normal sperm (LMNS) were determined to characterize insemination doses. Overall, pregnancy rate was greater ($P < 0.05$) in the EIU group (65%) than in the SIU group (45%). Pregnancy rates were greater ($P \leq 0.06$) when more than 100×10^6 LMNS were inseminated regardless of insemination method; the greatest pregnancy rate was observed in the EIU group when this insemination dose was used (38/49; 78%). There was no significant difference in pregnancy rate whether one (69%) or two inseminations (64%) were performed in the EIU group. Complications in the SIU group included anesthetic-induced bradycardia during surgery, significant postsurgery pain, seroma formation over the abdominal incision, and delayed wound healing. No complications were noted during or after insemination in the EIU group. In conclusion, these results support the use of EIU as a noninvasive alternative to laparotomy for insemination of frozen-thawed dog semen. In addition, use of more than 100×10^6 LMNS is also recommended for insemination.

1. Introduction

Since the first reported successful pregnancy from frozen-thawed dog semen in 1969 [1], pregnancy rates and insemination methods have improved dramatically. Although initial reports adopted vaginal insemination [1,2], it is now well accepted that intrauterine deposition yields superior results [3–5]. Andersen [2] reported early success of laparotomy (SIU) and later developed a technique for nonsurgical intrauterine insemination using the Norwegian Elk Catheter, a technique that is still commonly used in many European Countries [6,7]. The catheter was developed "to adapt the technique for practical conditions" [6], thus providing an alternative to surgical intrauterine insemination in the bitch. The registration bodies of some countries have restricted the use of SIU, notably the United Kingdom and some European countries, for ethical concerns over the need for anesthetics and surgery to inseminate the bitch. Despite the lack of data supporting SIU over other nonsurgical methods of intrauterine insemination, SIU is still a

* Corresponding author. Tel.: +613 9544 7455; fax: +613 9543 4191.
E-mail address: mvc@monashvet.com.au (S.J. Mason).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.06.014

common practice in many countries. Although there are data to confirm that SIU can result in pregnancy in the bitch, there are few studies documenting its success [8,9], with only one containing clinical retrospective data [9].

The reported insemination techniques using frozen-thawed dog semen comprise intravaginal insemination and four intrauterine techniques (Norwegian Elk Catheter, laparoscopic assisted, endoscopic-assisted transcervical catheterization [EIU], and SIU). A higher pregnancy rate has been well reported with inseminations using Norwegian Elk Catheters compared with vaginal insemination with both fresh semen [10] and frozen-thawed dog semen [3,10], whereas laparoscopic techniques have been reported to have a similar pregnancy rate to vaginal inseminations with both fresh [5,11] and frozen semen [11]. Although there are a number of published retrospective studies using Norwegian Elk Catheter [3,12–16], EIU [3], SIU [9], or vaginal insemination [17] for artificial insemination in the bitch [3,9,12–17], no reports have compared the success of EIU and SIU. Most published studies on intrauterine inseminations performed using frozen-thawed semen have been undertaken using the Norwegian Elk Catheter [3,12–16]. Reports on EIU have either used small sample sizes (19/327; [3]) or were skewed toward one particular breed [18] or highly fertile dogs [19,20]. Of the few reports on SIU, most are experimental [2,21] or descriptive in nature [22]. There is one clinical retrospective study on SIU reporting success using fresh, chilled, or frozen semen [9], which reported similar overall pregnancy rates to other retrospective studies using noninvasive techniques [3,13–16].

A major influence on the pregnancy rate is the total number of live morphologically normal sperm (LMNS) inseminated [7]. In clinical practice, the insemination dose of frozen-thawed dog semen is usually determined considering only the progressive motility alone and not taking into account the morphologic characteristics of the sample [3]. Although motility is known to be correlated to fertility, the percentage of morphologically normal spermatozoa in the sample is also correlated to fertility [23]. The reported number of progressively motile spermatozoa required to attain pregnancy varies from 100 to 200 $\times$ 10^6 [3,12,24]. This number dates back to the earliest reports on use of frozen-thawed dog semen [6] and has changed little. There is some debate in the literature as to whether the insemination dose should be calculated on progressive motility alone, or whether morphologic characteristics of the spermatozoa should also be considered as in more recent reports [7,16].

The objective of this study is to compare the success rates of SIU and EIU, correlate them to LMNS inseminated, and evaluate the need for anesthetics and invasive procedures to attain pregnancy using frozen-thawed dog spermatozoa.

2. Materials and methods

2.1. Bitches

A total of 118 inseminations were performed on 115 bitches presenting to Monash Veterinary Clinic for routine insemination of frozen-thawed dog semen from November 2009 to March 2011. All bitches were clinically healthy, between the ages of 1.25 and 8.25 years (3.9 $\pm$ 1.63 years; mean $\pm$ SD) and of varying parity ranging from 0 to 4 litters, with most bitches having had either zero or one litter before presenting. Bitches that had a history of uterine disease or known infertility were not included in the study. German Shepherds (10.2%), Border Collies (7.6%), Newfoundlands (6.8%), Irish Setters (6.8%), and Bernese Mountain Dogs (5.1%) were the most represented breeds. Breeds considered to have higher fertility such as Greyhounds [25] and dogs from commercial working facilities (observed unpublished data) were not included in the study.

2.2. Insemination timing

Insemination timing was based on vaginal cytology, serum progesterone concentrations, and vaginoscopy [26–28]. All bitches were presented to the clinic for their first assessment 5 to 7 days after the onset of vaginal swelling or discharge being noted by the owner. Vaginal smears were collected by introducing a moistened cotton swab into the caudal vagina. Swabs were then gently rolled onto glass microscope slides and stained using Diff Quick (Australian Biostain, Pty Ltd., Victoria, Australia). Vaginal smears were evaluated during the first visit to help stage the cycle [29,30]. Further evaluation of vaginal smears was not performed unless there was concern about the cycle not progressing as expected based on progesterone changes.

Blood was collected *via* jugular or cephalic venipuncture into tubes with no additives and submitted to a commercial laboratory for analysis of serum progesterone concentrations using chemiluminescence. Serum progesterone concentration was determined at the first visit and every 3 to 4 days until the LH surge was detected (progesterone concentration >2 ng/mL). Subsequent to the LH surge, serum progesterone concentration was determined every 1 to 2 days until ovulation was deemed complete [31]. Once ovulation was determined (progesterone concentration of 5–8 ng/mL), vaginoscopic examinations were commenced and insemination was not performed unless ovulation was deemed complete (progesterone concentration >10 ng/mL). Serum progesterone assays were not continued subsequent to a concentration of greater than 10 ng/mL.

Vaginoscopy was performed using a Sigmoidoscope (32020 Fibreoptic Sigmoidoscope; WelchAllyn, Skaneateles, NY, USA) daily from the time of ovulation (progesterone concentration of 5–8 ng/mL) until the time of insemination [30]. Insemination was performed on the first day in which maximal crenulation (vaginal folds at their most shrunken and angular state) of the anterior vagina was detected [30] in conjunction with a progesterone concentration of greater than 10 ng/mL.

2.3. Semen handling

Frozen semen used in this study was obtained from a variety of sources both within Australian and international freezing centers and had been stored for varying periods of time (3 months to 22 years). Semen had been frozen in pellets using Camelot farms or International Canine Semen