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SCIENCE DIRECT
Theriogenology
Theriogenology 63 (2005) 150–159
www.journals.elsevierhealth.com/periodicals/the

In vitro fertilization as a predictor of fertility from cervical insemination of sheep

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Received 7 April 2003; received in revised form 7 April 2004; accepted 7 April 2004

Abstract

The objective of this study was to determine if the quality of frozen–thawed ram semen could be effectively evaluated through in vitro fertilization (IVF) procedures prior to insemination as a means of improving pregnancy rate. In experiment 1, frozen semen from four Belclare rams was assessed using IVF and was used for cervical insemination of ewes ($n = 181$) in 13 pedigree Belclare flocks. There was a significant association between IVF score (proportion of oocytes cleaved at 48 h post insemination) and non-return rate ($P < 0.001$). For experiment 2, semen from nine Belclare rams was evaluated by IVF and semen from rams with the highest ($n = 3$) and lowest ($n = 2$) IVF scores was used for cervical insemination of ewes ($n = 111$) under experimental conditions. Differences in pregnancy rates between individual rams did not reach significance. Experiment 3 was designed to determine if differences detected between rams at field level could be accurately identified via IVF evaluation and involved frozen semen from eight Norwegian rams of known field fertility (non-return rates ranged from 45.7 to 73.8%). IVF score did not reflect the differences in field fertility. In the final experiment six of the eight Norwegian rams involved in experiment 3 were selected based on IVF score (three highest and three lowest) and their semen was used for cervical insemination ($n = 90$ ewes). While significant differences in pregnancy rate were found between individual rams ($P < 0.02$, range: 12.9–65.8%) they were not associated with IVF score. Ewe breed had a significant effect ($P < 0.003$) on pregnancy rate in both experiments 2 and 4. In conclusion, there was no evidence from this study that the evaluation of semen quality through IVF provided a useful predictor of pregnancy rate under field conditions. It may be that the IVF procedures as used routinely, which are essentially designed to maximize blastocyst yields rather than for detecting differences in fertilizing ability between batches of sperm, need to be modified.

Keywords: Sheep IVF; Cervical insemination; Sire; Ram; Ewe

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doi:10.1016/j.theriogenology.2004.04.015

1. Introduction

Artificial insemination (AI) is probably the most important single technique devised to facilitate the genetic improvement of farm animals. The widespread use of AI in cattle has allowed accurate genetic evaluation and rapid dissemination of genetic merit and has enabled the use of sophisticated data analysis procedures to estimate genetic merit. The availability of an efficient sheep AI service would yield similar benefits and would greatly enhance the scope for pedigree and commercial breeders to respond to changes in market requirements [1].

The widespread use of sheep AI and the realisation of its full potential depend on the use of frozen semen and thus on the availability of techniques that results in acceptable fertility. While intra uterine insemination is effective in terms of achieving good pregnancy rates, the ideal would be to use cervical insemination rather than the invasive procedures associated with laparoscopy. However, the very low level of fertility obtained when frozen–thawed semen is used for cervical AI in sheep has limited its uptake. Although, a relatively high proportion (40–60%) of ram spermatozoa are motile after the freeze–thaw process, only about 20–30% remain biologically undamaged [2]. There are reports on differences in freezability of spermatozoa between breeds [3,4], individual rams [5–8] and ejaculates [9–12].

The availability of an in vitro assay to predict the field fertility of frozen–thawed semen would be of great benefit to the development of an effective protocol for cervical AI with frozen–thawed semen. Many laboratory procedures have been used to test different aspects of semen quality but the usefulness or otherwise of these techniques as predictive measures of fertility remains inconclusive (see [13] for review).

Based on previous work from our group [14] it was concluded that in vitro fertilization had the potential to assess the fertilizing capacity of frozen–thawed semen. This would enable the identification of individual rams whose semen is better able to survive the freeze–thaw process and yield high pregnancy rates. The objective of this study was to extend our previous results to determine if the quality of frozen–thawed ram semen could be effectively evaluated through in vitro fertilization procedures prior to insemination as a means of improving pregnancy rate.

2. Materials and methods

2.1. Semen collection and freezing

Semen was collected from individual rams by artificial vagina. Following collection, wave motion was assessed and graded on a scale from 0 to 5 (0, non motile; 5, dense rapidly moving waves). The concentration of spermatozoa was measured using a photometer (IMV, L'Aigle, France). Any sample with a wave motion below 3 was discarded. Semen was diluted up to 7 mL with an extender based on a skim milk/egg yolk extender (11% skim milk with 5% (v/v) egg yolk added) which was maintained at 32 °C. Samples were then placed in a cold room at 5 °C and allowed to cool to 5 °C for 45–60 min. A second