EFFECT OF TYPE OF DILUENT, STORAGE AND DEPTH OF INSEMINATION ON THE FERTILITY OF CHILLED RAM SPERMATOZOA

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ABSTRACT

A total of 240 ewes were inseminated at the cervix or deep into the cervix with fresh or chilled semen diluted in either Tris or Tris-Milk diluents. There was no significant difference ($P > 0.05$) in the non-return rates between Tris and Tris-Milk diluents (32.5 v 40.0% respectively). Chilled storage of the semen for 24h resulted in significant ($P < 0.001$) decline in non-return rates from 47.5 to 29.5%. Non-return rates were however, significantly higher ($P < 0.001$) following deep cervical inseminations than when inseminations were carried out at the cervix (45.8 v. 28.3% respectively). Lambing rates were significantly ($P < 0.001$) higher following deep cervical insemination than when cervical insemination were done (40.0 v 24.2% respectively). They were also significantly higher ($P < 0.001$) for fresh than chilled stored semen (41.7 v 22.5% respectively). There was no significant difference ($P > 0.05$) in lambing rates between diluents (27.5 v 36.5% for Tris and Tris-Milk respectively).

Keywords: Diluent, Storage, Insemination, fertility, Chilled spermatozoa.

INTRODUCTION

In vitro studies revealed that chilled ram spermatozoa can survive for up to 4 days in Tris diluent especially when 10mM of EDTA is added. A mixed diluent containing 50% Milk and 50% Tris diluents also gave good spermatozoa survival rates after 2 days of storage.

Under chilled storage at 5°C ram spermatozoa cannot last longer than 1-2 days without showing loss of fertility. Salaman and Robinson (1962) obtained a linear regression of

percentage fertility of ram spermatozoa on duration of storage. These losses vary with the type of diluent (Zlatarev, 1976; Ozkoca, 1980; Espana Garcia, 1987; Odaibo, 1990). The cervix constitutes an initial barrier to the ascent of the spermatozoa after insemination. Inseminations involving cervical by-pass have resulted in significant increase in fertility of stored spermatozoa (Anderson *et al.*, 1973; Inskeep, 1974; Fukui and Roberts, 1976; Killen *et al.*, 1982). Even within the cervix, deposition of semen at depths of 0.5 cm resulted in marked increase in conception rates (Boyarskii, 1986; Deniran, 1990).

The present study was designed to investigate the effects of type of diluent, storage and depth of insemination on the fertility of chilled ram spermatozoa.

MATERIALS AND METHODS

Semen

Semen was collected from 4 yearling Yankasa rams by artificial vagina method.

Diluents

Two types of diluents were used in this study; Tris (Hydroxymethylaminoethane) (Salamon and Robinson, 1972) and a mixed diluent containing 50% Tris and 50% milk (Salamon and Robinson, 1962a) diluents. All diluents were prepared in 100 ml volumetric flasks without warming and kept in polythene bags at - 18°C.

Semen processing and storage

Semen samples were analysed for initial spermatozoa motility and concentration. The motility was estimated subjectively by observing a drop of the semen on a glass slide with cover slip under a microscope at 400 X magnification (average motility in semen samples used were

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between 85 and 95%). Spermatozoa concentration was determined by photoelectric method using a spectrophotometer (Beckman, Model C-400) calibrated against haemocytometer counts at 600nm wavelength (sperm concentration in samples used ranged between 2.5 and $3.0 \times 10^9/\text{ml}$).

Semen dilution and storage were done in glass test tubes placed in a water bath at 30°C . A dilution rate of 1:4 (v/v semen: diluent) was used. The diluted semen was divided into two, one part used for immediate insemination (Fresh semen insemination) and the other kept in a refrigerator at 5°C for 24 hours before inseminating.

Oestrus detection

Ewes between the ages of 2 and 5 years were checked twice daily for Oestrus between 0800-0900 and 1500 - 1600 hours using vasectomized rams.

Insemination

Inseminations were carried out 24 hours after oestrus detection. Two types of insemination pipettes were used: Normal straw pipette and a modified version of the straw pipette - tapered at one end and fitted with a micro pipette tip for deep penetration of the cervix. Each pipette was fitted with a 1ml tuberculin syringe. The cervix was located by means of a self-illuminating vaginoscope. The volume of the inseminate was 0.2 ml containing approximately 250×10^9 spermatozoa. For the deep cervical insemination, the average depth of cervical penetration was 2.5cm.

Non-return to oestrus checks

Ewes inseminated were checked for non-return (NR) to oestrus 12- 21 days after insemination using vasectomized rams. Ewes not returning to oestrus were assumed to be pregnant.

Lambing rates

Lambing rates were determined by observing the percentage of the ewes inseminated that actually lambed.

Experiment:

The effects of the following factors on the fertility of chilled ram spermatozoa were investigated.

1. Type of diluent: Tris v Tris-Milk
2. Storage length: 0 v 24h
3. Depth of insemination: cervical v deep cervical insemination.

A total of 240 ewes were divided into 8 groups of 30 each. Each ewe was inseminated with 0.2ml of either diluted fresh or stored semen at the cervix or deep into the cervix (approximately 2.5 cm deep).

Statistical analysis

Chi-square analysis was used to test the relevant main effects.

RESULTS

Non-return rates: The result is summarized in figure 1.

Type of diluent did not have any significant effect ($X^2=0.142$, $df=1$, $P>0.05$) on the percentage of ewes not returning to oestrus (32.5 v 41.7% for Tris and Tris-Milk diluents respectively). Chilled storage of the semen for 24 hours resulted in a significant ($X^2=7.876$, $df=1$, $P<0.001$) decline in the non-return rate (47.5 v 29.5% for 0 and 24 hours storage respectively). Deep cervical insemination gave significantly higher ($\chi^2=6447$, $df=1$, $P<0.001$) non-return rate than when cervical insemination was carried out (48.4v 28.3% respectively).

Lambing rates

The lambing result is summarized in figure 2. Lambing rates followed the same pattern as the non-return rates. Type of diluent did not have a significant effect ($X^2=1.53$, $df=1$, $P>0.05$) on the lambing rate 27.5 v 33.5% for Tris or Tris-Milk diluents, respectively). Lambing rates were significantly higher ($X^2=6.35$, $df=1$, $P>0.001$), following deep cervical insemination than when cervical insemination was carried out (40 and 24.2% respectively). Lambing rate was also significantly higher ($\chi^2=8.32$, $df=1$, $P<0.001$) when diluted fresh semen was used than when stored semen was used (41.7 and

22.5% respectively).

DISCUSSION

This study shows that both non-return and lambings rates of ewes inseminated with chilled ram semen are not significantly affected by type of diluent. However, Tris-Milk diluent gave slightly higher (non-return and lambing rates (41.7 and 33.5%) than Tris-diluent (32.5 and 27.5%). Previous *in vitro* studies revealed that semen stored in Tris diluent had a higher % motile spermatozoa than that stored in Tris-Milk diluent. The present fertility trials show the Tris-Milk diluent preserved spermatozoa fertility better than the Tris diluent. This means that semen fertility is lost while good spermatozoa motility is retained. The better protection accorded the spermatozoa fertility by Tris-Milk diluent can be attributed to the presence of milk. Milk on its own has been reported to have better fertility results than other diluents. (Salamon and Robinson, 1962b;) asserted that lambing rates with semen diluted in milk were equal to those obtained with fresh undiluted semen.

Fertility of the semen generally declined with storage. Although the decline was common to both diluents, it was more rapid in Tris than in Tris-Milk diluent. The non-return rate declined from 45.0 to 20.0% in Tris of 45.0 to 33.3% in Tris-Milk, while the lambing rates fell from 41.7 to 13.5% in Tris v 41.7 to 31.7% in Tris- Milk diluents. The observed effect of storage on the semen fertility is in line with the reports of Salamon and Robinson (1962b), Wiggan and Clark (1967) Zlatarev (1970) and Firth (1976). This loss of fertility during storage may be as a result of loss of intra-cellular materials especially enzymes observed in stored semen (Gokhale (1980). Better conception results could be obtained with semen extended in diluents that caused minimum leakage of sperm intra-cellular materials.

Significant increases in both non-return and lambing rates of ewes followed deep cervical inseminations than that carried out at the cervix. The observation is similar to that reported by

Tervit *et al.* (1984), Kerten *et al* (1984), Boyarkii (1986) and Deniran (1990) Boyarskii (1986) stated that even within the cervix, insemination at a depth of 2.5cm resulted in significantly higher conception rate than when carried out at the normal 2.0 cm depth. The cervix constituted an initial barrier to spermatozoa ascent thus deep penetration increases the chances of more viable spermatozoa reaching and fertilizing the ovum. Similar increases in conception and subsequent lambing rates of ewes inseminated with techniques involving cervical by-pass have been reported (Futui and Robert, 1976; and Killdeen *et al* 1982).

IMPLICATIONS

The result of the study shows that fertility of chilled ram spermatozoa is better preserved in Tris- Milk than in Tris diluent. Storage results in significant decline in spermatozoa fertility though good results can be obtained if inseminations are carried out within 24 hours of semen collection and processing. Further increases in both conception and lambing rates can be achieved if ewes are inseminated deep into the cervix as against the normal cervical insemination.

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Fig 1. Effect of type of diluent, depth of insemination and storage of chilled ram semen on non-return rates

Fig.2. Effects of diluent type, depth of insemination and storage length of chilled ram semen on lambing rates