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Conception Following Transcervical Insemination with Predetermined Ejaculate Concentration in Synchronized West African Dwarf Does

O.O. Leigh and Z.O. Ajibade

Department of Veterinary Surgery and Reproduction,
Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Abstract: The possibility of achieving conception through artificial insemination with diluted semen sample of pre-determined ejaculate concentration was investigated in six (6) adult West African dwarf (WAD) goat does. Two adult WAD bucks certified as satisfactory potential breeders were involved in the study. The animals were maintained on 12% crude protein concentrate, greens and fresh water *ad libitum*. Semen was collected from the bucks via electroejaculation method and pooled. The semen collected was diluted with freshly prepared standard sodium citrate- egg yolk extender to contain 10^7 sperm cells/0.54ml of final ejaculate. The six does were synchronized using two intramuscular injections, seven days apart of Lutalyse$^{(R)}$ and inseminated transcervically with 0.54ml of the final ejaculate at the 84th and 96th hours following the second injection of Lutalyse$^{(R)}$. The results showed that all six does (i.e. 100%) failed to return to estrus within 22 days post insemination as evaluated via exfoliated vaginal cells. Only 66.7% (i.e. 4) of the does however were confirmed pregnant via ultrasonographic scan which was conducted with 3.5MHz transabdominal transducer at day 60 post insemination. The present study showed that transcervical insemination with freshly collected, citrate- egg yolk diluted buck semen containing 10^7 sperm cells achieved conception in synchronized West African dwarf does. This finding is a step that will lead to further research aimed at investigating the minimum concentration of ejaculate that can achieve conception in the WAD breed of goats.

Key words: Conception · Transcervical insemination · Predetermined ejaculate concentration · Synchronized WAD does

INTRODUCTION

The quest to combat the monstrous, ravaging and ageless challenge of malnutrition in the third world appears to be in the horizon. This is because many developing nations are beginning to embrace some of the technological advancements developed in the industrialized countries to boost agricultural productivity and also prevent depletion of their natural resource base. Long before now, agricultural practitioners, most especially, livestock farmers have tended indigenous species, relying on traditional breeding methods with its attendant enormous reproductive wastages. Again, more than 70% of food native to developing countries was produced by resource-poor farmers who did not have access to these new technologies [1]. Some of these technologies are no longer new to agricultural scientists and related professionals per se. Perhaps one of the most globally accepted technology in agriculture is artificial insemination [2] and another is oestrous synchronization. These techniques are often employed hand in hand at research stations or in the field. There are abundant literature on the procedure and success of oestrous synchronization and artificial insemination in domestic animals [3-6]. However, it appears that these technologies are used far less, particularly in Africa, than in developed countries. Their uses in developing countries have been limited largely to research institutions instead of taking the technologies to the field to upgrade indigenous stock. Despite the preponderance of available literature on these techniques, especially artificial insemination, there are only a few/none on the native West African dwarf (WAD) goat. Apart from the present study being centred on providing information on artificial insemination in the WAD goat doe, it is determined to also investigate the success of achieving conception in does through the use

Corresponding Author: O.O Leigh, Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, Tel: +8033266825, E-mail: damilareolufisayo@yahoo.com.

of diluted ejaculate sample of predetermined/known concentration. The result of the study is expected to provide information leading to further investigations directed at determining the minimum insemination dose for achieving conception through artificial insemination in the WAD goat doe using diluted semen samples.

MATERIALS AND METHODS

Experimental Animals and Management: Eight adult WAD goats consisting of six does and two bucks which had been kept for more than 36 months in the small ruminant unit of the Department of Veterinary Surgery and Reproduction, University of Ibadan were used for the study. The six does were housed together in a separate pen from the two bucks. The goats were fed with Elephant grass in the mornings and concentrate (12%CP) in the evenings. Feeds and fresh water were provided *ad libitum* throughout the study which was carried out between February and August, 2010.

Oestrous Synchronization: Oestrus was induced in the six adult WAD does as have been described earlier [3]. Briefly, the method consist of intramuscular administration of two injections of 5mg PGF2α (Lutalyse$^{(R)}$; Pharmacia & Upjohn) seven days apart to each doe. For the two injections per animal/doe, a total of 10mg Lutalyse$^{(R)}$ was used. According to the earlier report [3] the does will be in estrus between 72-96 hours following the second injection of Lutalyse$^{(R)}$.

Semen Collection and Evaluation: Semen was collected separately through electroejaculation method [7] from the two bucks which have been certified as Satisfactory Potential Breeders and pooled. Semen analysis was carried out using standard methods as have been described [8]. Morphological abnormalities of spermatozoa were equally evaluated according to methods earlier described [9].

Preparation of Extender and Semen Dilution: A standard sodium citrate buffer was prepared by dissolving 2.9g of sodium citrate ($Na_3C_6H_5O_7.2H_2O$) in 100mls of double distilled water. 4 parts of the prepared buffer was thoroughly mixed and shaken with 1 part of egg yolk from freshly laid chicken egg to make up the extender. These preparations were done under strict hygiene.

Determination of Insemination Dose (ml):
Calculation of Volume of Extender:

$$\text{Volume of extender} = \frac{\text{Concentration (x10}^9\text{ sperm cells) x motility (\%) x semen volume (mls.)}}{\text{Insemination dose (i.e. 10}^7\text{ sperm cells).}}$$

$$\text{Total volume of semen after extension} = \text{Volume of extender} + \text{total volume of semen ejaculated}$$

$$\text{Volume of diluted semen containing 10}^7\text{ sperm cells} = \frac{\text{Total volume of semen after extension (mls.) x Insemination dose}}{\text{Total concentration of sperm cells (x10}^9\text{)}}$$

Artificial Insemination: The does were kept tightly by two legs of one assistant while the other assistant held the tail and cleaned the vulva with clean cotton wool soaked in saline. The hind legs of the does were lifted so that the rear part was kept higher than the fore part of the body. 0.54ml of the diluted ejaculate sample containing 10^7 sperm cells was slowly introduced into the cervix of each doe using insemination catheter guided by a battery operated small ruminant speculum lubricated with K-Y jelly. Each doe was inseminated twice i.e. 84th and 96th hours during 72-96 hours following second intramuscular injection of Lutalyse$^{(R)}$.

Evaluation of Pregnancy: Failure to return to oestrus was monitored through the pattern of vaginal exfoliated cells during 22 days following insemination as was described earlier [3]. Also the does were subjected to ultrasonographic scan for confirmation of pregnancy using a 3.5MHz transabdominal transducer at day 60 post insemination as earlier described [10].

Data Analysis: Descriptive statistics such as percentages were used in summarizing the data.

RESULT AND DISCUSSION

Spermiogram:
Average progressive motility: 95%
Spermatozoa concentration: 2.53 x 10^9
Average percentage morphological abnormality: 19.26%
Average volume of collected ejaculate: 0.57ml

Conception/Pregnancy: The six does (i.e. 100%) which were inseminated at the 84th and 96th hours after the second Lutalyse$^{(R)}$ injection failed to return to oestrus as evaluated through vaginal exfoliated cells (Fig. 1) during 22 days following insemination.

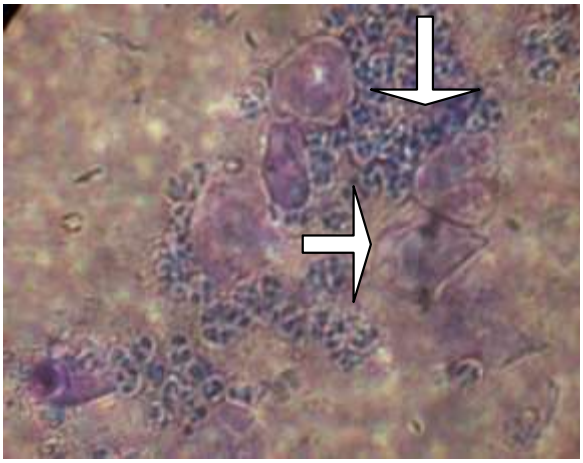

Fig. 1: Typical representation of vaginal exfoliates (depictive of diestrus) post insemination during the study. Vertical arrow shows numerous neutrophils while horizontal arrow shows few giant cells

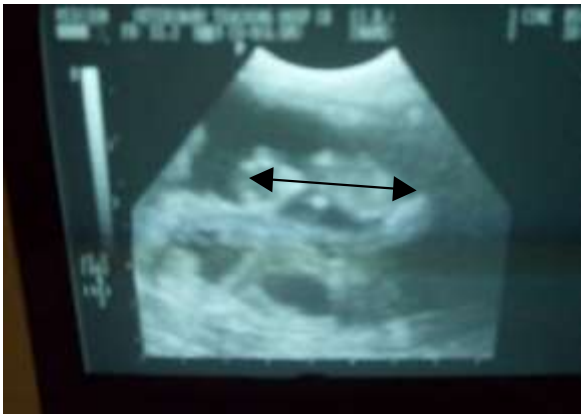

Fig. 2: Sonogram showing fully extended fetus (↔) with CRL= 3.3cm on day 60 of gestation

Only 4 (i.e. 66.7%) of the inseminated does were positive for pregnancy as evaluated by ultrascan pictures taken at day 60 post insemination (Fig. 2).

From the results of the present study, it may be said that the six does inseminated with freshly diluted semen in egg yolk containing 10^7 sperm cells were pregnant on the basis that they were not observed to return to oestrous as monitored by exfoliated vaginal cells during 22 days post insemination. This observation consolidates the validity of relying on the pattern of vaginal exfoliated cells in West African dwarf does as was earlier described [3] for detecting estrus and its application in breeding stations where estrus synchronization is carried out. Although the success of artificial insemination obtained in the study based on inseminating the does twice between 72 and 96 hours after 2nd Lutalyse® injection was good (66.7%) or comparable and even better than observation from a similar study in dairy goats [11], the achievement of pregnancy within the first 22 days following insemination and eventually in 66.7% in the study showed that conception could be achieved in WAD does with ejaculate sample containing 10^7 sperm cells. The cause of disappearance of pregnancy in only one doe (32.3%) in the study was not investigated. However, it has been reported that better success rate in artificial insemination is achieved by depositing semen at the bifurcation of uterine body than at other locations [12] such as the cervix as was done in the present study. Since none of the does returned to estrous during 22 days following insemination (Fig. 1), it is likely that the single doe in this study has a characteristic long diestrus or that the pregnancy was lost at the embryo stage. It has been reported that a higher rate of embryonic deaths usually occur in multiparous than nulliparous dams. This is usually due to the fact that the risk of genital infection is higher in multiparous animals [13-15]. The present study shows that conception can be achieved through transcervical deposition of caprine ejaculate samples containing 10^7 sperm cells and also confirmed that does synchronized with two injections of 5mg Lutalyse® (7 days apart) can be successfully inseminated at 84th and 96th hours following the last administration of Lutalyse®.

In conclusion, the present findings can be used as a springboard in determining the minimum concentration of ejaculate to be used for insemination in WAD does.

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