

CONCEPTION FOLLOWING ARTIFICIAL INSEMINATION OF WEST AFRICAN DWARF DOES USING FRESH BUCK SEMEN EXTENDED WITH PAWPAP JUICE - EGG YOLK CITRATE.

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ABSTRACT

A conception study was conducted via artificial insemination of fifteen West African dwarf (WAD) goats (does) using Pawpaw juice as 10% part replacement of citrate buffer in Citrate-egg yolk extender. 0.5ml of semen with estimated spermatozoa motility and concentration of 90% and 2.15×10^7 cells/ml respectively was collected through electroejaculation from a proven WAD buck for the study. The semen was diluted in the 10% Pawpaw juice-egg yolk citrate extender. The extended semen (containing 10^7 sperm cells/ml) was introduced into the cervix of 'in-heat' does through sterilized and lubricated catheter. For 22 days following insemination, none of the does was observed to return to 'heat'. Subsequently, signs of developing pregnancy were observed in the does and these climaxed with kidding by the 15 does at about 145 $\pm$ 25 days. Our findings suggest that incorporation of Pawpaw juice as 10% part replacement of citrate buffer in citrate-egg yolk extender is not harmful to goat spermatozoa fertilizing capacity and will not prevent conception in does following artificial insemination.

INTRODUCTION

The adoption of Artificial insemination technique has continued to receive favorable recognition among breeders all over the world. The widespread acceptance is connected with its numerous advantages including reduced risk of infection, reduced cost and its potential at improving livestock production (Tawseck and El-Gaafary, 1991), thereby increasing the percentage of protein consumed daily among individuals. Several studies are also being generated to achieve user friendliness of the technique of artificial insemination. In tropical Africa, a lot is being done to making artificial insemination programmes more affordable. One of such is investigation into the efficiency of using pawpaw juice as semen extender prior to insemination (Ajala and Oyeyemi, 1997). Two of the most widely used semen extenders include those made from egg yolk or pasteurized and homogenized milk (Gadea, 2003). Examples of these include Illinois Variable Temperature Extender, Coconut Milk Extender, Cornell Universal Extender, Pasteurized Homogenized milk Extender and Ovogenized Milk Extender. The limiting factor to the use of these however has to do with their availability and cost. The ready accessibility of pawpaw (*Carica papaya*) and its juice in the tropics increases its relevance and use as a semen extender. Ajala and Oyeyemi, (1997) have reported that the replacement of egg yolk in part by Pawpaw juice in extenders will reduce cost. Successful extension of buck semen has been reported

using goat milk (Akusu *et al*; 1995). Also, vegetable materials such as orange and grape juice have been found suitable for semen extension in the bull (Bonadona *et al*; 1962). The aim of this present study is to further consolidate on the usefulness of pawpaw juice as semen extender by artificially introducing semen extended with pawpaw juice-incorporated citrate-egg yolk diluent/extender into the cervix of 'in-heat' does and observing them for conception by return or non-return to estrus and kidding.

MATERIALS AND METHODS

Sixteen adult West African dwarf goats with average age and weight of 21 months and 13.0kg consisting of one buck and fifteen does were purchased from a local breeder for the study. The does were housed in groups of five in each pen while the buck was kept in a separate compartment within the large animal unit of the Department of Veterinary Surgery and Reproduction, University of Ibadan. Throughout the study, they were fed on concentrate supplemented with dried cassava and yam peelings, roughage and hand cut grasses. Clean fresh drinking water was provided *ad libitum* throughout the study. Following a stabilization period of four weeks, each doe was administered 5mg Lutalyse^R (Dinoprost tromethamine, Pharmacia & Upjohn Co. Kalamazoo, MI) intramuscularly. Fresh ripe pawpaw after peeling and removing the seeds was homogenized using mortar and pestle. The homogenate was filtered using a sterilized cloth. The juice collected was heated

at 80° for 10 minutes in a water bath to denature enzymes capable of destroying spermatozoa. The juice was cooled to room temperature (28°C). A standard Sodium citrate buffer for semen extension was prepared by dissolving 2.9g of Sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) in 100ml of double distilled water (Arthur et al; 1992). The 10% Pawpaw juice replacement of Sodium citrate for the Pawpaw juice-egg yolk-citrate extender used in the study was prepared containing Pawpaw juice (0.5 Parts), 2.9% Sodium citrate (4.5 Parts) and Egg yolk (1 Part). This mixture was centrifuged at 10,000 G for 10 minutes and 500 units each of Penicillin and Streptomycin was added per 100ml of the supernatant. Semen was collected from a proven buck via an Electroejaculator. Semen evaluation for Colour, Volume, Mass activity, Motility, Livability and Concentration and dilution in the test extender (i.e. 10% Pawpaw juice replacement of citrate buffer in egg yolk-citrate extender) was done as described by Zemjanis, (1970). Briefly, the dilution involves determination of the number of females that can be inseminated from a semen sample based on sample volume, motility and the minimum insemination dose. Twenty hours into eliciting estrual signs (e.g. mucous vulvar discharges, mountings among pen mates and standing heat), the does were restrained by raising their hind limbs such that their long axis made 60° contacts with the floor. The perineum and vulva were cleaned with sterile water and dried with sterile towel. With the aid of a lubricated speculum and catheter, 1ml of the extended semen containing 10^7 spermatozoa was carefully introduced into the cervix of each doe. The does were then kept each in separate pen to prevent fighting and they were closely (6 times daily) observed for any sign of estrus following insemination, pregnancy and then parturition.

RESULTS AND DISCUSSION

The semen collected from the proven buck in the study had the following characteristics: Color (cream), Volume (0.5ml), Motility (90%), Mass activity (+++), Livability (95%), Concentration (2.15×10^9 sperm cells/ml). Signs of estrus were also observed about 48 hours in the 15 does following treatment with a single dose of 5mg Lutalyse^R. Following insemination and close observation for 22 days, none of the 15 does was observed to return to estrus.

The pre-dilution semen characteristics were comparatively satisfactory and similar to values reported earlier in the tropics for the West African dwarf (WAD) breed of goat (Akusu et al; 1984), though a bit lower than values for temperate breeds (Sartas and Stembach, 1982). This difference may be due to many factors including climate, nutrition management and genetics. The response observed in the does to Lutalyse^R treatment was similar to that reported by Akusu et al. (1984) in WAD does. Although in their own case, Lutalyse^R was administered twice intramuscularly (i.e. 11 days apart) with signs of estrus following within 4-6 days after the second injection. However in this study, estrus was observed in the does at about 48 hours following a single injection. This observation is interesting as it suggests that the 15 does were around the same point in their sexual cycle when the Corpus luteum (CL) is already regressing and hence is not affected by exogenous luteolysin (Arthur et al; 1992). This is because responses to luteolysins have always been associated with estrus occurring 4-8 days post treatment regardless of single or double injection (Whitley and Jackson, 2004). Such occurrences are seen when treatment is done at the time of CL sensitivity to luteolysins (Arthur et al; 1992).

The length of the estrous cycle in does vary between 19-22 days (Akusu, 1994; Al-Mestari et al; 2003; Jackson et al; 2003), therefore, the observation of non-return to estrus following natural coitus or insemination is usually suggestive of conception. Beyond this period in this study, signs of developing pregnancy were observed in all the does until kidding occurred at about 145 ± 1.25 days after insemination. The observed non return to estrus in the does as well as the pregnancy and kidding at about 145 ± 1.25 days are good indicators to the adaptability of Pawpaw juice as a constituent of semen extenders. It shows that its inclusion at the level of 10% replacement of citrate buffer is not hazardous on semen or spermatozoa fertilizing capacity and on conception. This reflects its usefulness as a medium for the transfer of spermatozoa to the female tract during artificial insemination in does.

CONCLUSION

The semen diluted in Pawpaw juice- egg yolk-citrate extender in the study achieved conception in the does post insemination, we therefore conclude that the inclusion (as 10%

part replacement of citrate buffer) of Pawpaw juice in egg yolk citrate extenders for the purpose of artificial insemination is not harmful to spermatozoa fertilizing capacity and will not prevent conception in goats. It is however worthy to note that studies are on-going to know if there are limits to the level of inclusion of pawpaw juice in semen extenders beyond which the sperm cells may be adversely affected and then prevent or reduce conception.

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