Research Article/Artículo de investigación

CONCEPTION IN WEST AFRICAN DWARF DOES INSEMINATED WITH NORMAL SALINE DILUTED BUCK SEMEN

CONCEPCIÓN EN CABRAS WEST AFRICAN DWARF INSEMINADAS CON SEMEN DILUIDO EN SOLUCIÓN SALINA NORMAL

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ABSTRACT

This study investigated fertility response of West African Dwarf (WAD) does artificially inseminated with semen diluted with normal saline and the dilution ratio for achieving an optimum conception. Semen was collected from seven proven adult WAD bucks via electro ejaculation method and pooled. The semen collected was divided into 5 portions and each diluted with normal saline at 1:0 (T1, control), 1:1(T2), 1:2 (T3), 1:3 (T4) and 1:4 (T5) corresponding to five treatments. The treatments were evaluated for motility, livability and concentration. Twenty eight does aged 12-24 months with an average weight of 12.15±1.51kg were allotted to the five treatment groups with unequal replicates in a completely randomised design. Oestrus in the does was synchronised using prostaglandin (PGF2α) (Lutalyse(R) Pharmacia and Upjohn CO. NY). Oestrus was detected at 72 to 96 hours and insemination was carried out at 78th and 90th hours with respective treatments at 0.4 mL of diluted semen through the cervix using insemination gun. Conception was monitored by inspection of those that returned to oestrus and with the use of ultrasound scanner at day 65. Collected data were subjected to descriptive statistics and ANOVA. Results indicated that spermatozoa motility ranged from 81.67±2.87% (T1) to 90.00±5.00% (T4). T1 and T5 had lower motility than other treatments. Spermatozoa concentration ranged from 6.38±0.51x10^9/mL (T5) to 31.87±2.58x10^9/mL (T1). The number of motile sperm cells inseminated ranged from 5.33±0.59x10^9/mL (T5) to 26.05±2.75x10^9/mL (T1). Total live spermatozoa ranged from 5.94±0.58x10^9/mL (T5) to 29.48±1.51x10^9/mL (T1). Does inseminated with semen diluted at ratio 1:1 (T2) had the highest percentage conception, while T1, T4 and T5 had the least percentage conception. The present study showed that conception was enhanced when normal saline was used to dilute goat semen at ratio of 1:1, while dilution up to 1:4 also had a comparable percentage conception with the undiluted semen.

Keywords: Goat semen, saline solution, fertility, conception, artificial insemination.
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RESUMEN

En este estudio se investigó la respuesta a la fertilidad en cabras West African Dwarf (WAD) que se inseminaron artificialmente con semen diluido en solución salina normal y la relación de dilución para lograr un nivel de concepción óptimo. El semen se recolectó de siete machos WAD adultos probados, mediante electroeyaculación y fue mezclado. El semen recolectado se dividió en 5 alícuotas y cada una se diluyó con solución salina normal a 1:0 (T1, control), 1:1 (T2), 1:2 (T3), 1:3 (T4) y 1:4 (T5).) correspondiente a cinco tratamientos. Los tratamientos fueron evaluados por motilidad, vitalidad y concentración. Se usaron veintiocho cabras, con una edad entre 12 a 24 meses y un peso promedio de 12.15±1.51 kg, para los cinco grupos de tratamientos con réplicas desiguales en un diseño completamente al azar. El celo se sincronizó usando prostaglandina (PGF2α) (Lutalyse(R) Pharmacia y Upjohn CO. NY) y se detectó a las 72 a 96 horas y la inseminación se realizó entre 78 y 90 horas con 0,4 ml de semen diluido y a través del cuello uterino utilizando una pistola de inseminación. La concepción se monitorizó mediante la detección del celo y con el uso de ultrasonografía el día 65. Los datos recopilados se sometieron a estadísticas descriptivas y ANOVA. Los resultados indicaron que la motilidad de los espermatozoides osciló entre 81.67±2.87% (T1) y 90.00±5.00% (T4). T1 y T5 tuvieron menor motilidad que otros tratamientos. La concentración de espermatozoides osciló entre 6.38±0.51x10^9/mL (T5) y 31.87±2.58x10^9/mL (T1). El número de espermatozoides mótiles inseminados varió desde 5,33±0,59x10^9/ml (T5) hasta 26,05 2,75x10^9/mL (T1). El total de espermatozoides vivos osciló entre 5.94±0.58x10^9/mL (T5) y 29.48±1.51x10^9/mL (T1). Las hembras inseminados con semen diluido en una proporción de 1:1 (T2) tuvieron el mayor porcentaje de concepción, mientras que T1, T4 y T5 tuvieron el menor porcentaje de concepción. El presente estudio muestra que la concepción se mejora cuando se utiliza solución salina normal para diluir el semen en una proporción de 1:1, mientras que la dilución 1:4 tuvo una concepción porcentual comparable con el semen sin diluir.

Palabras clave: Semen caprino, solución salina, fertilidad, concepción, inseminación artificial.
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INTRODUCTION

Management of goats for reproductive soundness is one of the most important roles in goat breeding especially in intensive system of production. This can be used as a tool to control reproduction and to improve the production of milk, hair and meat (revised by Udeh & Oghenesode, 2011). The producer can use a number of tools and steps to enhance reproductive performance, such as using exogenous hormone, nutritional approaches and mechanical manipulation, estrus synchronization, artificial insemination, embryo transfer, sex sorting among others.

Synchronization of estrus cycle is a technique used to bring large number of animals in a flock into overt heat at the predetermined time. The technique offers an opportunity to increase the efficiency of animal production in different ways such as reducing the time needed for detection of estrus, allowing insemination on a predetermined schedule with the help of a procedure for controlling the time of ovulation, which in turn enables feeding of goats in uniform groups (Gogol, 2004).

Artificial insemination (AI) is perceived as another reproductive tool to accelerate the provision of rapid animal protein (Morrel, 2011). It is the deliberate introduction of semen into a female's vagina or oviduct for the purpose of achieving pregnancy through fertilization by means other than copulation (Morrel, 2011). It is an alternative to natural mating. The technique offers significant benefits including genetic selection, prolonged fertility even during unfavorable times of the year, cycle based production, more efficient breeding programs and improved health monitoring (Bergonzoni et al., 1994). Adoption of AI technique in goat production in Nigeria has been generally low. This has accounted for the scanty documented information on storage and practice of AI technique for goat reproduction in Nigeria. The success of any artificial insemination program depends on the appropriate management of semen collection, storage and use (Etches, 1996; Lunak, 2005).

Extension of semen using suitable extenders is an important ingredient in the success of AI in goat reproduction. Extended buck semen can be used frozen, thawed, chilled or fresh (non-refrigerated), but when fresh semen is used, it offers better fertility and conception rate (Hackett & Wolynetz, 1981). In addition, Blash et al. (2000) stated that the process of freezing and thawing of goat semen reduces the percentage of live sperm cells and acrosome integrity. Conventional extenders are rather expensive and sometimes not readily available for farmers; therefore, it is vital to identify reliable on- farm AI diluents in Nigeria. This research aimed at evaluating the suitability of normal saline diluent and minimum insemination dose to achieve optimal conception through AI in the WAD goat does.

MATERIALS AND METHODS

Study location

The experiment was conducted at the Small Ruminant Unit of Teaching and Research Farm, University of Ibadan, while the analysis of semen was done at the Physiology Laboratory of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental animals and management

Twenty-eight does aged 12-24 months with an average weight of 12.15±1.51 kg were allotted to five treatment groups of unequal replicates of 5 does (T1), 7 does (T2), 6 does (T3), 5 does (T4) and 5 does (T5) in a completely randomised design. Seventeen adult bucks aged 1 -3 years were used for the study. The goats were fed 60% concentrate containing crude protein of 12.60 %, crude fiber of 10.00% and digestible energy of 3202.28 kcal/kg and 40% guinea grass and *Gliricidia sepium*. Fresh water was provided regularly. The goats were given preventive treatment against ecto and endo parasites during the acclimatization period.

Semen collection and evaluation

In the weeks previous to the experiment, semen samples were taken from all bucks to assess their characteristics and suitability for reproductive purposes. Semen was collected using electro ejaculator (Tingari et al., 1986) and pooled. The semen was collected into a collecting tube in a warm flask and temperature was maintained at 37°C. The semen collected was divided into 5 portions and each diluted with normal saline at 1:0 (T1, control), 1:1(T2), 1:2 (T3), 1:3 (T4) and 1:4 (T5) to constitute five treatments. The experimental layout is shown in Table 1.

Sperm motility, spermatozoa concentration, percentage livability and acrosome integrity were assessed as described by Ewuola & Egbunike (2010).

Estrus synchronization and insemination

Estrus was synchronized in the WAD does as has been described by Leigh and Ajibade (2010). All does were injected intramuscularly with 5mg of prostaglandin (PGF2α) per doe seven days apart to synchronize estrus. After the second injection of 5mg of prostaglandin (PGF2α), according to Leigh and Ajibade (2010) the does were in estrus between 72-96 hours following the second injection of Lutalyse(R) (Dinoprost, trometamina: 5 mg). Estrus was detected at 72-96 hours and insemination was carried out at 78th and 90th hour. Each doe was inseminated twice (12 hours interval) with respective treatments through the cervix using inseminating gun. Pregnancy diagnosis was based on the number of non-returns to estrus at 17 to 22 days after AI followed by ultrasound scanning of the does at 65 day post insemination (Figure 1). Ultrasound procedure was carried out as described by Hesselink & Taverne (1994). Crown-rump length was also measured. Percentage conception was determined as follows:

$$\text{Percentage conception} = \frac{\text{Number of pregnant does}}{\text{Number of inseminated does}} \times \frac{100}{1}$$

Table 1: Experimental layout

Treatments	Dilution ratio (semen:normal saline)	Volume of semen (mL)	Number of motile sperm cells x10^9/mL
T1	1:0	0.4	26.05
T2	1:1	0.4	14.09
T3	1:2	0.4	9.07
T4	1:3	0.4	7.18
T5	1:4	0.4	5.33

Statistical analysis

Data collected on conception were subjected to descriptive statistics, while data on characteristics of diluted semen were analyzed using one-way analysis of variance procedure of SAS (Inst. Inc.; Carry, NC. EUA, 2011) and means were compared using Duncan's multiple range test of the same software, while $P < 0.05$ was considered significant.

RESULTS

Presented in Table 2 is the baseline assessment of semen quality of bucks used for the experiment. Results showed that the quality of the semen was good and the bucks were fit for the experiment. Sperm motility values ranged from 75%-90% , mass activity ranged from 75-100% , livability was in the range of 70-90%, percentage of normal sperm cells ranged from 75-90%, acrosome integrity ranged from 70-90% and sperm concentration ranged from 25.6 x10^9/ml-34.4 x 10^9/ml.

Table 2: Baseline assessment study of semen quality of bucks used for the experiment

Buck number	Mass activity	Sperm motility (%)	Livability (%)	Sperm concentration (10^9)	Acrosome integrity (%)	%Normal sperm cells
1	++++	90	80	33.1	85	90
2	++++	80	75	28.7	75	80
3	++++	80	90	33.0	80	95
4	++++	85	75	34.2	85	85
5	++++	95	70	25.6	75	90
6	+++	75	85	32.0	80	85
7	++++	90	90	30.0	80	90
8	++++	90	80	26.7	75	85
9	++++	90	80	31.5	85	90
10	++++	85	85	34.4	80	80
11	++++	75	79	31.0	75	85
12	++++	80	75	30.0	90	90
13	++++	85	80	30.2	75	75
14	+++	75	70	31.0	70	85
15	++++	80	75	33.0	85	95
16	++++	80	70	32.0	90	90
17	++++	75	80	33.0	85	90

The characteristics of semen diluted with normal saline at different ratio are shown in Table 3. Results showed that sperm motility ranged from 81.67±2.87% (T1) to 90.00±5.00% (T4) and was not significantly ($P>0.05$) different among the treatments. Spermatozoa concentration ranged from 6.38±0.51x10^9/mL (T5) to 31.87±2.58x10^9/mL (T1), these values were significantly reduced ($P<0.05$) as the dilution ratio increases. The number of motile and live spermatozoa in the treatments significantly ($P<0.05$) declined as the dilution rate increases with T1 having the highest ($P<0.05$) value and T5 recorded the least.

Table 3: Characteristics of semen diluted with normal saline and inseminated

Parameters	Treatments				
	T1 (1:0)	T2 (1:1)	T3(1:2)	T4 (1:3)	T5(1:4)
Sperm Motility (%)	81.67±2.87^a	88.33±2.89^a	85.00±5.00^a	90.00±5.00^a	83.33±2.87^a
Sperm Livability (%)	92.67±3.06^a	92.67±3.06^a	92.33±3.21^a	92.67±3.06^a	93.00±2.65^a
Sperm concentration (x10^9/mL)	31.87±2.58^a	15.92±1.32^b	10.6±0.87^c	8.0±0.61^d	6.38±0.51^e
Number of motile sperm cells (x10^9/mL)	26.05±2.75^a	14.09±1.60^b	9.07±0.87^c	7.18±0.27^d	5.33±0.59^e
Total live spermatozoa (x10^9/mL)	29.48±1.51^a	14.77±1.58^b	9.78±0.48^c	7.72±0.25^d	5.94±0.58^e

T1: 1:0 (control), T2: 1:1 (S:NS), T3: 1:2 (S:NS), T4: 1:3 (S:NS), T5: 1:4 (S:NS) where S: semen, NS: Normal saline
Means in the same row with different superscripts are significantly different ($P<0.05$).

Percentage conception in WAD does inseminated with diluted semen is presented in Fig.1. Results showed that percentage conception was 60%, 71.43%, 66.65, 60% and 60% in T1, T2, T3, T4 and T5, respectively. The T2 had apparently highest percentage conception.

Crown-rump length of developing fetus at 65 days post insemination is presented in Fig. 2. The plate showed an image of a developing fetus with crown rump length of 6.8cm at day 65 of gestation. A fluid filled sac was seen with an embryo flowing inside the fluid. Eyelids were visible, the limbs were becoming slenderer, and the neck elongated. The fetus was still very tiny and a little hair appearing on the eyelids and lips.

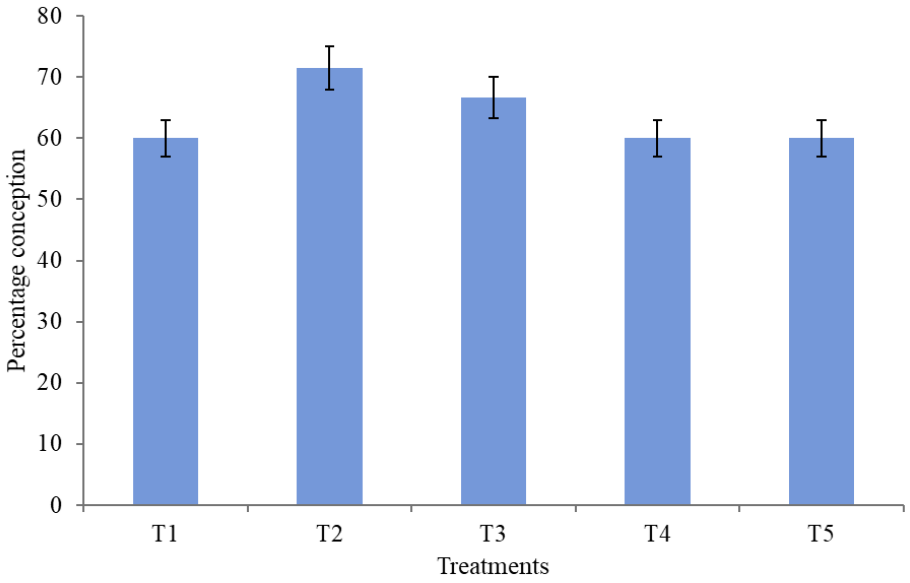

Figure 1. Percentage conception of does artificially inseminated with semen diluted with normal saline
T1: Undiluted (control), T2: 1:1 (S:NS), T3: 1:2(S:NS), T4: 1:3 (S:NS), T5: 1:4 (S:NS) where S= Semen, NS=Normal saline

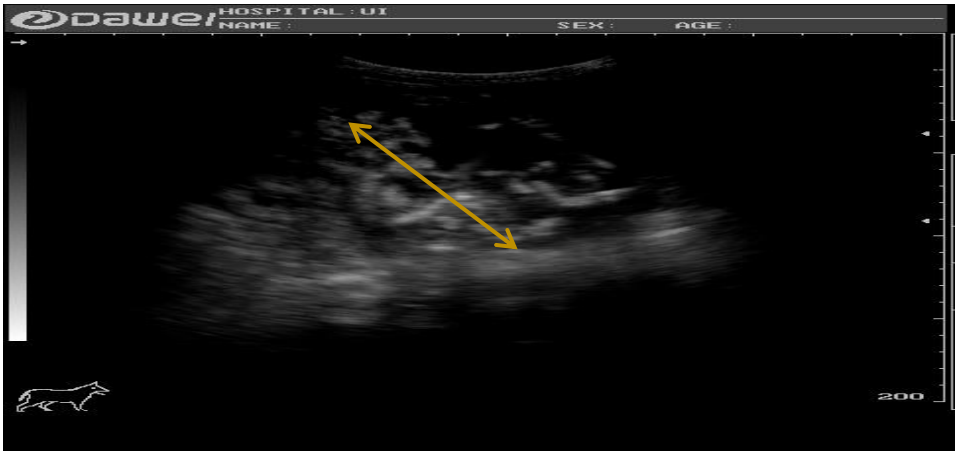

Figure 2. Image showing fully extended fetus with crown rump length of 6.8 on day 65 of gestation.

DISCUSSION

The similar sperm motility and livability observed between treatments in this study affirmed that the use of normal saline as semen diluent did not adversely affect the viability of the spermatozoa. This could also be attributed to the ability of the diluent to serve as buffer needed for the metabolic maintenance of the sperm cells and modulate the medium for spermatozoa survival (Peterson et al., 2007; Udeh & Oghenesode, 2011). However, the proportional decrease in the sperm concentration and number of motile sperm cells is expected due to dilution ratio involve in this study, since the number of spermatozoa is express per mL of the semen. Results revealed that percentage conception was 60%, 71.43%, 66.65%, 60% and 60% in WAD does artificially inseminated with undiluted semen, semen diluted with normal saline at 1:1, 1:2, 1:3 and 1:4, respectively. The 1:1 diluted semen improved percentage conception than others probably because the sperm cells are free to swim independently in the diluted medium than the clog experience in undiluted semen and also the number of motile sperm cell was significantly higher than others diluted with same diluent. The results obtained in this study was higher than what was recorded by Bigirwa et al. (2015) who observed conception rate of 56.7% following cervical insemination using fresh diluted semen of boer buck semen and 53.3% conception with indigenous buck semen. Conception rates resulting from artificial insemination was 50% in Palestinian goats, 70.8 and 80% in Barbari and Jamnapari breeds of India, respectively (Devendra and McLeory, 1982). Conception rates of 66.7%, 75% and 100% were reported following AI in Angora goats in Turkey (Ozsar et al., 1988) and were 45% in artificially inseminated local Brazilian dairy goats (Simplicio & Machado, 1991). Aryal et al. (1992) reported conception rate of 31 % in Kiko goats. Conception rates following AI of goats in Switzerland was 74.7% (Summermatter, 1993), it was 58.4% in estrus-induced goat versus 49.8% in natural estrus. Optimum conception rate in artificially inseminated goats in France using fresh semen were reported to be 70% (Leboeuf, 1993). The result obtained in treatment 2 was higher than the percentage conception reported by Leigh & Ajibade (2010) who recorded 66.7% conception in transcervical inseminated WAD does with freshly collected citrate-egg yolk diluted buck semen containing $1x10^7$ sperm cells. Ajala et al. (2012) recorded 100% conception when 10% coconut milk–egg yolk citrate was used as extender in WAD does.

Moreover, percentage conception recorded in this study was higher than what was observed by a similar study in dairy goats by Boué & Sigwald (1995). Ambrose (1996) recorded 37.50% conception using semen from Saanen buck on Sudanese Nubian goat does. It was reported that better success rates in artificial insemination are achieved by depositing semen at the bifurcation of uterine body than at other locations (Seguin, 1986) such as cervix as was done in the present study. However, the extenders used by these researchers mentioned above were different from the normal saline diluent used in the present study.

CONCLUSIONS

Normal saline is efficient as an on-farm diluent in maintaining the viability of goat semen at dilution ratios of 1:1 to 1:4 at ambient temperature. Conception was enhanced when normal saline was used to extend goat semen at ratio up to 1:2 and dilution at 1:3 and 1:4 compared favorably with the non-extended semen which implies that dilution of semen with normal saline up to 1:4 increases the number of does to be inseminated without adverse effects on the conception rate.

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