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ORIGINAL RESEARCH ARTICLE

An improvised artificial vagina for rabbit semen collection and the characteristics of the extended rabbit semen as panacea for artificial insemination

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ABSTRACT
Rabbit production has been greatly improved upon by the use of artificial insemination. There are several reports on characteristics of semen collected with the use of artificial vagina. This study developed an unsophisticated and improvised artificial vagina (IAV) using locally fabricated materials and its efficiency in semen collection was evaluated. The IAV was used to collect semen from 35 bucks with total attempt of 700 over a period of 10 weeks. 692 attempts were successful with adequate ejaculate collections giving an efficiency of 98.86%. Result reveals that the use of this IAV is possible. This study also show that dilution ratio reduced sperm concentration and extension above 1:2 ratio (semen : extender) declined sperm progressive motility and reduced mass activity in in vitro assessment of extended rabbit semen.

Keywords: Artificial vagina, semen collection, Rabbits,

INTRODUCTION
Artificial Insemination has been introduced in industrial rabbitries mainly to improve breeding management (Castellini, 1996). Artificial insemination (AI) has also become a routine practice in rabbit production (Alvariño, 2000). The technique offers significant benefits, including genetic selection, prolonged fertility even during unfavourable times of the year, cycle based production, more efficient breeding programmes and improved health monitoring (Bergonzoni *et al.,* 1994). An essential factor to the practice of AI is semen collection and the most commonly used means of collection is through the use of an artificial vagina. The AI in rabbits is generally performed with 0.5 mL of extended semen (Carluccio, 2004). Theoretically, it is possible to obtain 30-40 doses per ejaculate, but in everyday practice it is preferable to have a dilution rate from 1:5 to 1:10, meaning approximately 10-15 doses/ejaculate, to ensure that there are at least 10 million viable, non-damaged spermatozoa (Castellini and Lattaioli, 1999).

Independent of semen dilution, the type of extender used will have an impact on the reproduction rate (Kiprianidis and Facchin, 1994). Many extenders have been developed for rabbit semen storage in liquid state. Of these, extenders containing Tris, citric acid and glucose, as well as commercial extenders such as Galap (IMV, France) have found wide application in practice (Alvarino, 2000). Nevertheless the diluents are not able to completely prevent changes to a range of essential features for sperm functions which rapidly occur under *in vitro* conditions. Therefore, studies are under way to improve extenders for storage of rabbit semen at above-zero temperatures (Piotr, and Agnieszka, 2009). Extenders differ in composition depending on the species, use, temperature at which the diluted semen is to be stored; and the duration of storage desired. All extenders are based on a particular buffer, which has provided the best results for a given species (Hopkins *et al.*, 1989). Semen extenders containing sodium citrate, egg yolk, glycerol, sugar etc in different proportions have been reported (Samad, 1985).

Semen evaluation must be able to provide necessary information required to forecast the fertilizing ability of spermatozoa. Relevant parameters correlated with the fertility rate are the number of spermatozoa inseminated, intact acrosome integrity, sperm morphology and progressive motility, although the use of a single attribute is not sufficiently accurate to predict the fertilizing ability of the semen (Lavara *et al*., 2005). Additional semen traits or composite indexes better predict the fertilizing capacity of spermatozoa (Castellini, 2008). The evaluation of the ejaculate, however, relies on the ability to reliably and effectively collect semen from the animal (Naughton *et al.,* 2003). Although the collection of rabbit ejaculates with the aid of an artificial vagina has been previously reported by Naughton *et al.* (2003), a commercially available, inexpensive device was not available at the time of the study. The purpose of this study was to develop an

unsophisticated and less expensive artificial vagina for semen collection from rabbits. This could be constructed from locally available materials and used for semen evaluation of rabbit spermatozoa which would be used to predict the fertilizing potential of the semen collected with the improvised artificial vagina.

MATERIALS AND METHODS

Description of an improvised Artificial Vagina (IAV) for rabbit

The locally fabricated IAV was constructed using varying dimensions of polyvinyl chloride (PVC) pipe inserted into one another and joined with a top bond gum as shown in Figure 1. Four different sizes and length of the PVC pipe were used for the fabrication. The length of Tubes 1, 2, 3 and 4 were 2.6cm, 3.5cm, 4.0cm and 4.6cm respectively (Fig. 1) while the thickness of each of the tube was 0.25cm (Fig. 2). The depth of tubes 2, 3 and 4 relative to tube 1 was 0.5cm, 0.9cm and 1.3cm (Fig 2). The depth between tubes 2 and 3, and tubes 3 and 4 was 0.4cm (Fig. 2). The inner diameter of tubes 1, 2, 3 and 4 were 2.3cm, 1.9cm, 1.5cm and 1.1cm respectively (Fig. 1). When fixed and fastened together using top bond gum or super glue, the total length of the IAV was 5.9cm (Fig. 3). For the hand usage of the IAV, glove latex or condom latex was inserted through the pipe to serve as inner lining with a little cushion material (foam/tissue/cotton wool) at the tip end of the IAV to reduce friction effect when the buck inserts its pennies into the locally fabricated IAV during intromission. The inner lining was made to fit and to see through the open end. The inner lining was tightly fastened around the pipe and held bound with a rubber band (Plates 1, 2 and 3). A small collection tube such as eppendorf was attached to the narrow end of the IAV (Plate 4) to store the harvested semen before dispensed into a sample bottle for subsequent analysis.

Stepwise construction of mainframe of an IAV for Rabbit

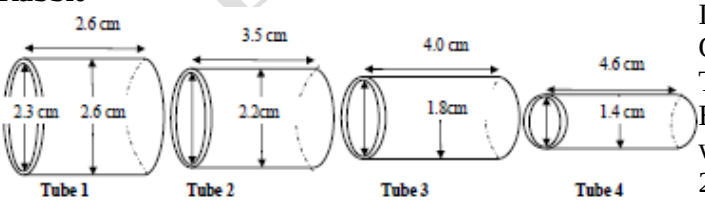

Figure 1: Schematic structure showing the length and diameter of different sizes of PVC tubes for Rabbit AV

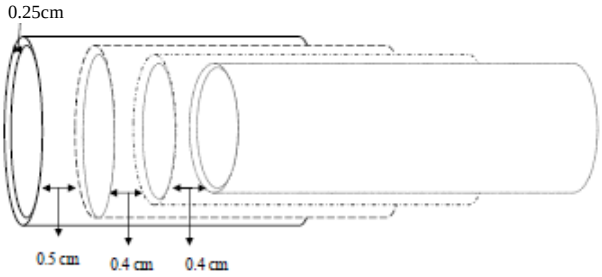

Figure 2: Schematic structure showing the thickness and depth of each tube when coupled

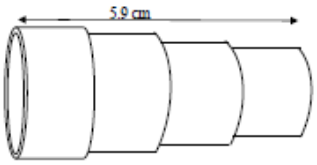

Figure 3: Schematic structure showing total length of the IAV after construction

The application of the IAV for ejaculate collection

The IAV was warmed in warm (slightly hot) water to 34-38 ^{o}C (approximately body temperature of the animal) and hand-held beneath a restrained doe with the open end pointed in a caudal direction (Plate 5). As the buck began to mount, the IAV was placed to allow penetration of the rabbit penis into the IAV. Since ejaculation occurs rapidly within few seconds (less than one minute if the male is sexually aggressive and mount well), the IAV was removed immediately after ejaculation.

Assessment of the efficiency of the IAV

The experiment was carried out at the Rabbitry unit of the Teaching and Research Farm of the University of Ibadan, Ibadan, Nigeria. Mature New Zealand white × Chinchilla rabbit bucks aged 12–13 months were used. The IAV was used to collect semen from 35 bucks. Each buck was ejaculated ones per day and twice in a week for 10 weeks consecutively. Total collections of 20 attempts were made per buck with total attempt of 700 for all the 35 bucks. Out of these attempts, 692 attempts were successful with adequate ejaculate collections. Percentage efficiency was estimated using the formula:

$$\frac{\text{Total attempt-unsuccessful attempt}}{\text{Total attempt}} \times 100$$

Semen collection and extension

Semen collections were done twice a week between 8.00 am and 9.00 am to ensure that optimum quality semen was obtained. A mature doe was used to tease the buck. Semen collection was done using the IAV. Prior to semen collection, the IAV was warmed in warm water to about 36 ^{o}C ± 2.0 ^{o}C mean temperature and thereafter drained while retaining its heat (36 ^{o}C on the average). Precaution was taken so as to prevent water from seeping into the IAV by using latex material (such as the hand glove latex) before putting same anterior end inside the hot water. This was done to further prevent contamination and reduction in semen quality when mixed with water. The latent heat was transferred into the IAV to acquire the required temperature. Care was taken not to allow the IAV get too hot (36 ^{o}C) as this may result to decreased efficiency of semen collection and a possible contamination of the semen with urine or injury of the pennis. The IAV was adequately positioned on the dorsum of the stimulus female to allow penis intromission and subsequent ejaculation into the IAV.

Semen extension and evaluation

The ejaculates were pooled and divided into 4 parts. Glucose-egg yolk-sodium citrate extender (Table 1) was used to extend a portion of the semen at ratio 1:1 (Treatment 2), ratio 1:2 (Treatment 3) and ratio 1:3 (Treatment 4), while treatment 1 (control) was unextended semen. The ratio was semen: extender. The extended and unextended semen were assessed for mass activity, motility, sperm concentration and percentage live spermatozoa. Sperm motility was determined by putting a drop of freshly collected semen with a drop of sodium citrate and was placed on glass slide on a warm stage at 37 ^{o}C. Observations were made at × 400 magnifications on the sample under microscope to determine sperm motility. Mass activity was also determined and scored on a scale of 0-3 (no wave and movement to high turbulent wave). Sperm concentration was assessed by Neubauer haemocytometer after dilution with formal saline (1:20 v/v). Percentage live sperm cells after staining and total sperm concentration were derived by calculation as described in Ewuola and Egbunike (2010).

Table 1: Composition of Glucose Yolk Citrate Extender

Composition	Proportion
Distilled Water	100 mL
Glucose	58 mg
Sodium Citrate	5 gm
Egg yolk	20 mL
Penicillin G (Procaine Propen-G ®)	1 mg/1000 mL
Streptomycin (Matostrep ®)	1.00 mg/mL

Modified from Chaudhari and Mshelia , (2002).

Data analysis

Data were subjected to statistical analysis using descriptive statistics and one-way analysis of variance of statistical analysis software (SAS, 2003) program. Treatment means were compared using Duncan's option of the same software.

RESULTS AND DISCUSSION

The fabricated IAV is shown in plates 1, 2 and 3. The use of latex as inner lining and cushion at the anterior tip end of the IVA was to prevent injury and friction effect during mating when buck inserts its penile organ during intromission. The efficiency of the IAV was 98.86 %. The unsuccessful attempt could be attributed to inadequate maintenance of the IAV temperature required for efficient recognition, intromission and ejaculation since it was meant to mimic the natural female genital tract environment, most especially, the temperature. When the temperature of IAV is high, it could lead to decrease in the efficiency of semen collection which may cause injury to the male copulatory organ and there is possibility of semen contamination with urine.

Also, when the IAV temperature is cold, that is lower than expected female genital temperature. In most cases, the male refuse to ejaculate when the IAV temperature is below 30 ^{o}C. The present observation corroborates the findings of Naughton *et al*. (2003) who reported 98% efficiency for an inexpensive rabbit artificial vagina elsewhere.

Images of the Improvised Artificial Vagina for Rabbit

Plate 1: Front view of the improvised artificial vagina

Plate 2: Arial view of the improvised artificial vagina

Table 2: Characteristics of unextended and extended rabbit semen *in vitro*

Parameters	T1	T2	T3	T4	SEM
Mass Activity	3.00	3.00	3.00	2.00	
Sperm Motility (%)	91.25^{a}	94.25^{a}	82.50^{b}	76.25^{c}	1.82
Sperm Livability (%)	94.25	91.00	100.00	93.00	3.55
Sperm Concentration (x 10^{6})	52.96^{a}	29.80^{b}	21.52^{bc}	15.96^{c}	0.10

abc: Means along the same row with different superscripts are significantly (P<0.05) different

Plate 3: Back view of the improvised artificial vagina

The *in vitro* assessment for quality attributes of extended and unextended rabbit semen is shown in Table 2. The result showed that progressive motility decreased significantly (P < 0.05) from T2 to T4 as the dilution ratio increased.

Plate 4: Complete improvised artificial vagina with collection tube

Plate 5: Positioning of the improvised artificial vagina for semen collection

Percentage livability was not significantly different across the treatments, while sperm concentration in T1 was significantly ($P < 0.05$) higher than those in T2, T3 and T4. The sperm concentration in T4 was not significantly different from T3 but significantly ($P < 0.05$) lower than that of T2. Mass activity was reduced in T4 compared to other treatments.

The result revealed that the higher the dilution ratio, the lesser the progressive motility of the sperm cells in the medium. This result corroborates the findings of Lahnsteiner *et al.* (2004) and Sadeghi *et al.* (2013) who reported that increasing dilution ratio causes sperm plasma to lose its protective effect thereby resulting in reduced sperm viability. Highest motility percentage of sperm cells was also obtained for treatments with the dilution ratio of 1:1 which also agrees with Liu *et al.* (2006) who obtained maximum motility percentage at the same dilution ratio. The observed decreased in the sperm concentration may be attributed to the dilution factor. The trend was expected since the concentration was expressed per mL of the sample. The higher the rate of dilution, the lesser the sperm cells in the sample (Kondracki, 2003). This is due to increase in the volume of seminal plasma when the sperm cells in the medium remain constant. It was logical because the dilution ratio is inversely related to the concentration (Kondracki, 2003). This result agreed with the observation of Kommisrud *et al.* (2002) who reported that as the amount of seminal plasma decreases, the concentration of the spermatozoa increases.

CONCLUSION

The study showed that the use of an unsophisticated, less expensive and available materials for construction of artificial vagina for semen collection from rabbits at a reduced cost and high semen collection efficiency is possible. The use of an improvised artificial vagina increased the ease of semen collection in rabbit and subsequently reduced the time of on-farm artificial insemination in rabbit. Based on the result from the study, dilution ratio reduced sperm concentration and extension above 1:2 ratio (semen : extender) apparently declined sperm progressive motility and reduced mass activity in *in vitro* assessment of extended rabbit semen.

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