Asian Journal of Research in Animal and Veterinary Sciences

9(1): 8-15, 2022; Article no.AJRAVS.75628

Comparative Study of Semen Collection from Rabbits Using Locally Assembled and Conventional Artificial Vaginas

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Editor(s):
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(3) Iqra Bano, Pakistan.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/75628

Original Research Article

Received 09 November 2021
Accepted 11 January 2022
Published 14 January 2022

ABSTRACT

Artificial insemination has been used in time past to improve the reproductive performance of animals and has a great potential in rabbit production. The cost of purchasing sophisticated artificial vagina is a challenge for rabbit producers in developing countries like Nigeria, therefore, this study was carried out to compare the quality of semen collected using the Locally-assembled Artificial Vagina (LAV) with that collected using the Conventional Artificial Vagina (CAV). 3 matured bucks were housed separately in a Completely Randomized Design and each type of Artificial Vagina (AV) was used to collect 4 semen samples from each buck over a period of 4 weeks. A total of 24 samples were collected and analysed. The results showed that the viscosity and odour of all samples are the same, while the colour was Slightly yellowish white and milkfish white for LAV and CAV respectively. It was observed that the Liquefaction time, pH and Massal Motility of semen collected with both AVs were not significantly different ($p \geq 0.05$), but the Volume, Individual Motility, Sperm Vitality and Concentration of the semen collected, were significantly higher ($p < 0.05$) for CAV at 0.47ml, 66.33%, 71.33% and 455.18 x 10^6 respectively. Although, there was a little difference in the quality of both semen, the semen collected using LAV are within acceptable range.

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It was concluded that unsophisticated LAVs made from cheap and available materials, is a good option for local rabbit breeders to collect semen from rabbits in an AI process, in order to save cost for rabbit farmers in developing countries.

Keywords: Artificial insemination; rabbit; reproduction; artificial-vagina; semen.

1. INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are raised for a variety of purposes across the world in small scale and commercial levels. Rabbit production (Cuniculture) and its market in Nigeria have unlimited potentials, most of which are still untapped. This tremendous opportunity is because the rabbit has so many usefulness; ranging from meat production, to use as pets, for hide and skin, production of fur, for exhibitions and research purposes [1]. Low animal protein intake is a major nutritional problem in Nigeria, especially for the low-income and non-wage earners [2], and although rabbit meat is not as readily available in the open market as beef, chevon, mutton, pork, poultry, fish and "bush-meat", researches over time show the growing interest in adopting rabbit production as a veritable means of economic initiatives for the poor and alternative to high cost meat products of other farm animals and bush-meat [3,4]. It has since been identified as an economic livestock for small-scale rural farmers/dwellers, capable of producing about 47kg of meat, enough to solely meet the animal protein requirements of a medium size family [5,6]. Rabbits can also be kept in the backyard in small unit of 2-4 does (females) and a buck (male) to supply the family with an additional source of animal protein [7]. Therefore, there is an urgent need to develop rabbit production as an alternate source of animal protein, to bridge the gap between animal protein supply and consumption. Rabbit production is relatively cheap when compared to other livestock and the meat has been found to be palatable, nutritious, low in fat and cholesterol; fine-grained, suitable alternative to poultry meat, purely white, bristle, highly nutritious and a convenient source of high-quality protein [8]. The animal has a relatively short gestation period ranging from 28-32 days and can have a litter size as large as 8. But several challenges have limited the production in Nigeria, of major concerns are: availability of feed ingredients, feed wastage, poor management techniques and also, insufficient and high cost of giant and fast-growing breeds [9]; heat-stress-which can lead to sterility in males and fetal ingestion in pregnant does [10]. The early history of Artificial Insemination (AI) dates back to 1677 when Leeuwenhoek discovered spermatozoa but progress rested in anticipation of the first successful bitch insemination by Spallanzani in 1780 [11], AI was the first great biotechnology applied to improve reproduction and genetics of farm animals and it has also had an enormous impact worldwide in many species, particularly in dairy cattle [12]. AI has been used in other species of farm animals to improve their reproductive potentials, and it was reported by [13] that AI of rabbit does appeared on European farms in the late 1980's.

The technique offers significant benefits, including genetic selection, prolonged fertility even in the unfavorable times of the year, a more efficient breeding program, and improved health monitoring [14]. However, artificial insemination has not been widely explored to improve rabbit production in Nigeria, probably due to the cost. The artificial vagina (AV) is one of the most commonly used methods for the collection of semen. However, artificial vaginas are not readily available to local rabbit farmers in Nigeria. Therefore, there is need to develop a cheap and cost-effective way of collecting semen from rabbits in order to improve the use of artificial insemination in rabbits. The construction of artificial vaginas from cheap and locally available materials, will be a cheap means of achieving the aim of improving the reproduction and breeding activities of rabbit production in Nigeria, thus increasing the availability of the rabbit meat to local farmers and low-income eraners in the country. This would also help develop a new channel of study, that would further improve the performance and efficiency of LAVs.

1.1 Objectives of the Study

- To examine the characteristics of rabbit semen collected using a locally-assembled artificial vagina (LAV)
- To compare the quality of semen collected using the locally assembled artificial vagina with that collected using the conventionally artificial vagina (CAV)

- To further verify the effects of LAV and CAV on fertility of sperm-cells during Artificial Insemination

- To determine if the LAV can be an effective substitute for CAV due to availability and cost imolication, without reducing the quality of the semen.

2. MATERIALS AND METHODS

2.1 Experimental Site

This research was conducted at the University of Benin Teaching and Research Farm, University of Benin, Benin City, Nigeria. The University of Benin is located in the ancient city of Benin, which is approximately 320km from Lagos, Nigeria's former capital state. Benin is in the Tropical Savannah Climate, experiencing 2 major seasons – wet and dry seasons.

2.2 Experimental Animals

Three matured bucks of a composite breed of rabbits were used for this experiment. Both types of artificial vaginas were used to collect semen from each of the rabbits. Each rabbit used was housed in a separate cage for ease of semen collection.

2.3 Experimental Design

Completely Randomized Design

2.4 Treatments

Treatments were the two different kinds of artificial vaginas used.

- Conventional Artificial Vagina:

  The conventional AV used in this study was made with materials that consist of two pipes of varying lengths, a glass collection tube, and a silicon liner. The AV was imported by the Department of Animal Science, University of Benin.

- Locally-assembled Artificial Vagina:

  The locally assembled AV used in this study was constructed with materials that include 3" T-connector polyvinyl chloride (PVC) pipe with ¾" x ¾" x 3½" openings, a rubber band (1/2" x 1/8"), cut bathroom slipper, glue, a pipe made from a pen container and silicon condom as a collection device. A flip-flop slipper was cut to the size of the opening of the ½" T opening of the PVC connector. A narrow opening was created in the middle of the cut slipper to allow the entering of the pipe made from a pen container. The cut flip-flop with the pipe was then fitted into the 3 ½" of the T connector pipe. An unrolled condom was washed thoroughly with warm water to remove the spermicide and passed through the PVC T-connector's two ¾" openings. The condom was rolled over the ends of the pipe and was firmly held with a rubber band to have a water-tight jacket between the condom layer and the pipe. The closed end of the condom was folded to have a collecting cone to collect the semen. Warm water and air (45 – 50®C) are introduced through the pipe in the cut flip flop into the jacket formed by the condom and the pipe with a syringe.

2.5 Collection of Sample

The semen collection process was similar to the process stated by [15,16]. AV was warmed in a water bath of about 40 to 60^{o}C for 10 to 15 minutes in a container. A mature non-pregnant doe was used as a teaser. The doe was first kept in a hutch next to the buck for the buck to see and sniff, the doe was then brought into the cage of the buck. The device was hand-held beneath a doe with the open end pointed in a caudal direction. False mounting was allowed and encouraged for 2 or 3 times. As the buck attempts to mount, the device was positioned more posteriorly and inferiorly for ease of penetration of the male rabbit into the artificial vagina. Ejaculation occurred very quickly, and the device was removed. After ejaculation, the AV is removed and disassembled with the warm water carefully removed and the silicon condom drawn out from the PVC pipe with caution taken to avoid water contamination of the sample. The semen is collected into a weighed disposable plastic container after it is allowed to settle down into the narrow tip of the condom. The semen is immediately analyzed afterward.

The conventional and the locally made AVs were used to collect 4 (four) semen samples each from the three animals at 2 ejaculates weekly (one ejaculate on each of these days) over a period of 4 weeks, making a total of 12

ejaculates collected per AV. The collection was evenly distributed using both AVs weekly and alternating in the following week in order to eradicate bias in the collection.

2.6 Data Collection

2.6.1 Semen colour

After collecting the semen, a visual test was carried out to determine the colour of the ejaculate.

2.6.2 Viscosity

This was determined immediately after liquefaction by gently aspirating the sample into a wide bore (approximately 1.5 mm diameter) plastic disposable pipette. The semen sample was allowed to drop by gravity, and the length of the thread formed was measured.

2.6.3 Volume

The volume of each sample was measured using the following parameters;

Weight of collection tube (W_1)
Weight of collection tube with semen (W_2)
Estimated volume of semen collected (W_3) = $W_2 - W_1$

$$\text{Since density of semen} = \frac{\text{Mass}}{\text{Volume}}$$

Where semen density is approximately 1g/mL (Omoyakhi JM (2018) Personal Communication)

2.6.4 Liquefaction time

After collection, the sample was kept erect at room temperature in the collection tube without agitation, and the time it took the semen to liquefy was observed using a stopwatch.

2.6.5 pH

A digital pH metre was used to determine the pH of the semen sample

2.6.6 Motility

To determine the Massal Motility, the semen sample, without dilution, was placed on a microscopic slide and covered with a coverslip. The progression (waving motion) of spermatozoon mass was analyzed with an X10 optical lens in a bright field microscope.

To determine the Individual Motility, the sample was diluted with physiological saline prepared by adding 1gram of salt in 99.9ml of distilled water. A drop of the diluted sample was then placed in a microscopic slide and covered with a coverslip. Ten spermatozoa were counted on ten different areas of the slide. The number of spermatozoa progressively moving was taken as the estimate of the set motility.

2.6.7 Vitality

Sperm vitality test was carried out to estimate the percentage of live and dead spermatozoa.

Eosin solution was the only dye used to stain the spermatozoa. A drop of semen was taken and placed on the microscopic slide. An equal volume of the eosin solution is also taken and dropped on the semen on the microscopic slide. This was smeared on the glass slide and allowed to dry. The dried slide was examined with the aid of a microscope at a magnification of X200

2.6.8 Spermatozoa concentration

The haemocytometer was used to determine the spermatozoa concentration, which is the estimate of the spermatozoa cells in the total volume of semen. The haemocytometer was cleaned with distilled water. A petri dish with wet serviette paper or tissue paper was used to support the haemocytometer. The semen sample was diluted with distilled water to kill the sperm cells and stop mobility to enable easy counting.

The coverslip was pressed firmly onto the chambers to avoid iridescence (Newton's ring) between the two glass surfaces. The coverslip was then firmly held on the counting chambers of the haemocytometer by lightly wetting either side of the wells using a capillary tube. 10µL of the mixed, diluted semen was transferred to each of the counting chambers of the haemocytometer. Adequate care was taken that the chambers were not over or under filled and the cover glass not moved. The haemocytometer was allowed to stand for about 5 minutes in the humid petri dish containing the wet paper to prevent it from drying out.

The cells were then counted at a magnification of 200 to 400X. Sperm cells in 5 of the 25 squares were counted as representative of the 25 squares. This process is repeated for the two chambers. Both values were summed up and averaged then multiplied by 5 to get the

spermatozoa in the total volume of the haemocytometer, i.e., 0.1mm^3. This value was then multiplied by the dilution factor (100) to estimate the total spermatozoa concentration of the undiluted semen.

2.7 Data Analysis

The data collected were subjected to a one-way analysis of variance using GenStat 2009 (12th Edition) statistical package. The means with significant differences were separated by Duncan Multiple mean test at a 5% probability level.

3. RESULTS

Table 1 shows the qualitative data of the various parameters of the semen samples collected with the locally fabricated artificial vagina (LAV) and the conventional artificial vagina (CAV). The result shows that the viscosity (Normal) and odour (Fresh Milk) of the semen collected using LAV and CAV are the same, while the colour of the semen are slightly different (slightly yellowish-white and creamy white for LAV and CAV respectively.

The data of the quality of the semen is presented in Table 2. From the analysed result, it shows that there is no significant difference ($p \geq 0.05$) in the Liquefaction Time, Massal Motility and pH. There is a significant difference ($p \leq 0.05$) in the Volume of semen collected, Individual Motility, Sperm Vitality and the Concentration- where Conventional Artificial Vagina is higher in all cases recorded – 0.47, 66.33, 71.33 and 455.18 respectively.

4. DISCUSSION

The macroscopic examination showed that semen samples collected with the conventional AV had a clearer milky white/ creamy white colour than those collected with the local AV, which was less milkish-white. This colour could result from some residue in the silicon condom used to collect semen in the local AV. A transparent glass tube was used to collect semen in the conventional AV, thus making the colour of the semen more apparent. However, both samples conformed to the creamy white colour reported by [17,18]. The odour and viscosity of all samples collected (both with CAV and LAV) remained fresh milk and normal respectively, this shows that both semen samples are of good quality. The pH of both semen samples was also measured and found to be 8.36 for the local AV and 8.00 for the conventional AV. A higher volume (0.473 ml) was recorded for the conventional AV as opposed to that of the local AV (0.4067 ml). This increase in volume is probably because, the semen was collected directly in a glass tube for the conventional AV, while the semen was first collected in a silicon condom before being transferred to a sample bottle for the local AV, thus retrieving all the semen ejaculate was quite difficult.

Sperm concentration was higher for semen collected with the conventional AV. This may be attributable to the use of the condom reservoir. The vitality of the sperm cells was also higher in semen collected with conventional AV than that collected with the local AV. This could be attributed to how the semen samples were retrieved into the sample bottles from the silicon condom in which the ejaculate was first collected. In trying to hurry up the collection of the semen sample into the bottles for analysis, they were often pressed out from an aperture made in the condom. This process may have damaged some of the sperm cells.

Although the parameters were of higher values for the conventional AV, those for the local AV were still within the acceptable range for artificial insemination.

Table 1. Qualitative Parameters of Semen Collected with Locally Assembled Artificial Vagina and Conventional Artificial Vagina

	LAV			CAV		
Parameters	**Animal A**	**Animal B**	**Animal C**	**Animal A**	**Animal B**	**Animal C**
Viscosity	Normal	Normal	Normal	Normal	Normal	Normal
Odour	Fresh milk	Fresh milk	Fresh milk	Fresh milk	Fresh milk	Fresh milk
Colour	Slightly yellowish white	Slightly yellowish white	Slightly yellowish white	Creamy white	Creamy white	Creamy white

Table 2. Quality of Semen Collected with Locally Assembled Artificial Vagina and the Conventional Artificial Vagina

Parameters	LAV	CAV	SEM
Volume (ml)	0.41[a]	0.47[b]	0.01
Liquefaction Time (min)	13.33	13.00	0.33
Massal Motility (%)	2.00	2.00	0.00
Individual Motility (%)	59.67[a]	66.33[b]	1.33
Sperm Vitality (%)	66.67[a]	71.33[b]	0.88
Concentration (x 10^6)	434.22[a]	455.18[b]	3.74
pH	8.36	8.00	0.28

[ab] *Means along the same row with different superscript are significantly different (p <0.05)*
LAV: Locally Assembled Artificial Vagina
CAV: Conventional Artificial Vagina

5. COMPARISON IN PRICE AND AVAILABILITY

Most of these materials used in making the Local AV are inexpensive, and some of them (rubber band, cut bathroom slipper, and pen container) can be obtained without cost as by-products of other household products. Unlike the conventional artificial vagina, the materials used to construct the local artificial vagina make it very accessible and cost-effective to rabbit farmers and breeders in Nigeria. The reusable portion of the local AV costs about 270 (~$0.5). The non-reusable portion of the AV is the silicon condom, and it costs 100 (~$0.2) for a pack of 3, which can be used for three different collections. This study conforms with [19] that available and less expensive materials can be used in the construction of artificial vagina for semen collection from rabbits at a reduced cost.

Some other artificial vaginas have been constructed with diethylene glycol used to fill the space between the casing and liner in order to provide pressure [20] and a small amount of glycerol smeared on the open end of the AV to provide sufficient lubrication to cause ejaculation [20] and as antifreeze [15] for the ejaculate. This is not the case for the locally assembled AV used in this study, as warm water was introduced between the PVC pipe and the liner to provide sufficient pressure. In contrast to the report of [21] that 5-6mls of glycerol should be poured into the space between the held liner and the tube to keep the AV warm, only warm water was used in this study. Using only warm water in place of diethylene glycol and glycerol helped to save cost and thus make the AV very cheap and affordable to construct. This agrees with another report that warm water was used in preparing the AV [22,19]. There was also no need to keep the AV in warm water of 45-60ºC for 10-15 minutes or in an incubator of 50 ºC for 30 minutes [15,20] as the warm water was injected directly into the AV with the help of a syringe and the device was immediately used to collect semen samples thus conserving money and time. One AV was used throughout this study, with only the Silicon condom used as liner replacement for each collection, opposed to earlier suggestions [23]. This further reduced the cost and conserved time.

6. CONCLUSION

The study shows that although the quality of rabbit semen collected using the conventional artificial vagina is higher, the locally assembled artificial vagina is a good option for local rabbit breeders as the quality of semen collected is still within the acceptable range. The locally assembled artificial vagina is made from materials that are readily available and inexpensive to the local farmer. As such, it can go a long way in improving artificial insemination by reducing the cost through the use of cheap and available materials.

It is recommended that this study be further experimented on the field. Studying the reproductive impact of the semen collected by both AVs in a reproductive field study on matured does.

7. LIMITATIONS OF THE STUDY

Due to the available facilities at our disposal during the study, the method used in determining the “Semen Colour” is not as relible as new methods, using sophisticated laboratory equipments.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ISSN: 1115-2540

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/75628