



# PROCEEDINGS OF THE 11<sup>th</sup> WORLD RABBIT CONGRESS

Qingdao (China) - June 15-18, 2016

ISSN 2308-1910

## Session Reproduction

***Ola S.I.***

**OLIRAV: A SIMPLE, DISPOSABLE RABBIT ARTIFICIAL VAGINA  
DEVICE/PROCEDURE.**

**Full text of the communication**

*How to cite this paper :*

*Ola S.I., 2016 - OLIRAV: A simple, disposable rabbit artificial vagina device/procedure. Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China,209-212.*



## **OLIRAV: A SIMPLE, DISPOSABLE RABBIT ARTIFICIAL VAGINA DEVICE/PROCEDURE**

**Ola S.I.**

Department of Animal Sciences, Obafemi Awolowo University, 22005, Ile-Ife, Nigeria  
idowuola@oauife.edu.ng

### **ABSTRACT**

In an attempt to make rabbit semen collection simpler and easier, an artificial vagina device/procedure was developed termed OLIRAV (OLa Improvised Rabbit Artificial Vagina). The materials required for OLIRAV includes a 20 ml disposable sterile syringe, 5 ml disposable sterile sample collection tube, a latex condom and a rubber band of 1.5 - 2 cm inner diameter. These materials after coupling try to simulate the conditions of the natural vagina of rabbit doe. The device was tested with twelve bucks studying three effects on seminal parameters: 1) the live body weight of bucks; 2) the time of day the test was performed; and 3) the type of the device (OLIRAV or commercial artificial vagina). Results indicated that semen collected with OLIRAV were largely within the normal reference values for rabbit semen volume (0.3 – 0.9 ml), sperm concentration (250 – 600 million spermatozoa/ml), progressive motility (30 – 90 %) and abnormal sperm cells (<10 %). Additionally, the time interval between mounting to ejaculation was similar between OLIRAV (0.25 seconds) and the commercial artificial vagina (0.18 seconds). The study concluded that OLIRAV is a cheap and very effective device or procedure to collect rabbit semen, both for AI purpose and andrologic studies.

**Key words:** Rabbit, Artificial Vagina, Semen Collection, OLIRAV

### **INTRODUCTION**

The Artificial Insemination (AI) technique is hinged on successful collection of quality semen from the male and, in all livestock specie, the use of artificial vagina (AV) is the preferred method of semen collection. The first type of AV for rabbit semen collection was described by Macirone and Walton (1938), which was later improved upon by others (Walton, 1958; Bredderman *et al.*, 1964). There have also been attempts to simplify the rabbit AV design, especially to collect semen intended for laboratory andrologic studies (Herbert and Adejumo, 1995; Naughton *et al.*, 2003).

A common disadvantage associated with all the available rabbit AV types (including commercial brands) is the need to wash and/or sterilize between semen collections. This could pose a serious hindrance where large numbers of samples are needed, either for AI or research purposes, especially in the developing countries where basic and essential facilities are inadequate.

Thus a simple, inexpensive and disposable rabbit AV device that also offers a sterile environment for semen collection was conceived. The device results from the coupling of readily available and cheap laboratory consumables in a simple procedure that is hereby termed OLIRAV (Ola Improvised Rabbit Artificial Vagina). Our objective is to determine if OLIRAV make rabbit semen collection easier, especially under laboratory condition and in developing countries where commercial AV are not readily available and basic research facilities are still short of requirement.

## MATERIALS AND METHODS

### Materials and Assemblage of OLIRAV

The materials required for OLIRAV include a 20ml disposable sterile syringe, 5 ml disposable sterile sample collection tube, one latex condom (appropriate for man) and one rubber band of 1.5 - 2cm inner diameter. To assemble, the 20 ml syringe is cut into two with a sharp razor at the 12 ml mark and the lower portion of the syringe is discarded. The top of the cover of 5 ml collection tube is also cut out completely to make a free passage to the tube. Next, the latex condom is removed from its sterile casing, unrolled completely and then cut into two equal halves and, the lower portion discarded. The cut condom with its lubricated surface forming inner lining is passed through the cut syringe to extend out at both ends of the syringe. The condom is folded backward over the cut end of the syringe and the collection tube (with its cut-open cover) is screwed into the syringe at this end to hold the folded condom in place and thus sealing the syringe. For immediate use, the space formed between the condom lining and the syringe body is filled with 8 - 10 ml of warm water (55 – 60 °C). The extended end of the condom is then folded backward over the top of syringe body and a rubber band is used to hold the condom fold in place. The device is ready and can be used in same way as any other AV for semen collection from properly stimulated buck.

A properly assembled OLIRAV is shown Figure 1 on the right side of a commercial rabbit AV. For multiple semen collection in a day, the latex condom (if damaged), collection tubes and warm water filling could be replaced between collections, while the entire AV is disposed off at the end of the day collections

### Semen Collection Trials

A total of twelve heterogenous rabbit bucks from crosses between New Zealand White, Chincilla and California breeds, maintained at the rabbitry of Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Nigeria were used to test the efficacy of the designed OLIRAV in three independent studies. All animals were fed *ad lib* forage mixture of *Moringa oleifera*, *Centrosema pubescens* and *Panicum maximum*, with a supplement of concentrates containing 16 % crude protein and 2478 Kcal DE/kg. Water was supplied *ad libitum* and routine health management practices were observed.

#### *Trial 1: Semen collection from bucks of different sizes*

The bucks ranged in live body weight from 1640 g to 2520 g, mainly as a result of age difference. The smaller animals were younger, about 7 month old and the bigger ones ranged from 16-20 month old. The bucks were categorized into three body weight groups namely: small (1600 – 1800 g); medium (1801 – 2000 g) and big (2001 – 2520 g). All the bucks were adapted to mounting females and confirmed to be capable of mating before actual trial commenced. Semen collection with OLIRAV was performed on all the bucks in successive turns, twice a week for three consecutive weeks, with 2 – 3 days intervals between successive collections. OLIRAV was carefully held between the hind legs of an oestrus doe for the buck to mount and ejaculate.

#### *Trial 2: Semen collection at different time of the day*

During this trial all the bucks were ejaculated with OLIRAV twice a week for four consecutive weeks. The two collections in a week were done at either 7.00 – 9.00 hrs (morning) or 17.00 – 19.00hrs (evening), during which animals appear more comfortable and responsive, as a result of lower atmospheric temperature.

#### *Trial 3: Comparison of OLIRAV to commercial AV*

In this third trial the 12 bucks ejaculated twice a week for another four consecutive weeks. The two collections in a week were done with either OLIRAV or a commercial brand of rabbit artificial vagina



**Figure 1:** OLIRAV (on the right) and commercial rabbit artificial vagina used for rabbit semen collection

(CAV) procured for the purpose. The ejaculation lag i.e time interval between mounting of doe by the buck and ejaculation into the AV was determined with a stop watch.

### Semen Evaluation, Data Collection and Statistical Analyses

After each semen collection, the collection tube was carefully unscrewed from the AV body, the semen plug was removed with a clean forceps where applicable and, the semen volume was immediately read off in the graduated collection tube. Semen samples were visually checked for colour and urine contamination. Further analyses done in the laboratory includes sperm motility under the light microscope at  $\times 400$ , percent live and normal spermatozoa after eosin-nigrosin staining and sperm concentration using the Neubauer improved haemocytometer. Data collected for trial 1 were analyzed using Repeated ANOVA while the T-Test was used for trials 2 and 3, with SPSS version 17 software package. Statistical differences between treatments with Duncan test were considered significant at 5 % level of probability.

## RESULTS AND DISCUSSION

According to the reference value provided by the International Rabbit Reproduction Group (2005), freshly collected rabbit semen of good quality should vary from 0.3 – 0.9 ml, containing 250 – 600 million spermatozoa/ml with 30 – 90 % progressive motility. The results obtained with OLIRAV in this study (Table 1) fell largely within these normal ranges and even better in the case of semen volume. Compared to the results obtained with improvised AV by Herbert and Adejumo (1995) with rabbits raised under similar climatic condition as Ile-Ife, OLIRAV showed better performance in terms of semen volume, sperm motility and concentration. Herbert and Adejumo (1995) reported 0.71 ml, 67.50 % and  $110.5 \times 10^6$  spz/ml, respectively, although nutrition, method of semen evaluation as well as animal factor, other than collection device could also play significant roles in the differences. Compared to the results obtained with the improvised AV by Naughton *et al* (2003) which was tried on heavier bucks (4.0  $\pm$  0.3 kg), OLIRAV again proved to be better in terms of semen volume although the sperm concentration was similar. Naughton *et al* (2003) attributed the low semen volume (0.3 ml average of 50 collections) to the poor retrieval from the condom lining of the AV, a situation that was not recorded with OLIRAV. However, a cursory look at the improvised AV of Naughton *et al* (2003) made with 3” T connector PVC pipe suggest that it could have been too long and wide to allow efficient ejaculation and collection of the semen. These factors were clearly expatiated and addressed by Bradderman *et al* (1964) in their design of rabbit AV. Also the noticeable urine contamination reported by Naughton *et al* (2003) and attributed to the condom lining was not observed with OLIRAV.

**Table 1:** Ejaculate characteristics of rabbit semen collected with OLIRAV

Trial	Buck size				Collection period			Collection device		
	Small	Medium	Big	$\pm$ SEM	Morning	Evening	$\pm$ SEM	OLIRAV	CAV	$\pm$ SEM
Rabbits, no.	4	4	4	-	12		-	12		-
No of collections	24	24	24	-	48	48	-	48	48	-
Live weight (g)	1738.22	1954.41	2283.27	45.33	1958.64		64.58	1907.05		60.28
Semen volume (ml)	0.78 <sup>a</sup>	1.19 <sup>b</sup>	1.16 <sup>b</sup>	0.22	0.99	1.17	0.34	0.83	0.94	0.32
Progressive sperm motility (%)	81.25	80.96	81.32	1.20	80.25	78.55	2.30	85.70	87.00	1.50
Spermconcentration ( $\times 10^6$ /ml)	235.07	222.30	200.70	21.40	287.0	296.5	18.55	408.70	421.93	25.20
Live spermatozoa (%)	98.44	98.68	98.48	0.33	98.39	98.68	0.27	98.22	98.50	0.22
Abnormal spermatozoa (%)	4.57	3.94	5.03	0.20	5.28	3.48	0.35	4.25	4.09	0.24
Ejaculation lag (sec)	NA	NA	NA	-	NA	NA	-	0.25	0.18	0.02

Means with different letters on the same row within the same trial group differ significantly ( $P < 0.05$ , Duncan test).

OLIRAV- Ola Improvised Rabbit Artificial Vagina; CAV - Commercial (Rabbit) Artificial Vagina; NA – Not applicable.

The efficacy of OLIRAV for rabbit semen collection from bucks of varying sizes ejaculated at different period of the day and in comparison to a commercial AV has been well proven by this study. The only significant variation obtained was in semen volumes between bucks of various sizes, with smaller and

younger bucks having lower ejaculates, in agreement with known trend (Castellini, 2008). The higher sperm concentrations of the third experiment could be due to the advanced age and adaptability of the bucks to the semen collection procedure. It is quite important to mention the unique advantages of OLIRAV over previous rabbit AV designs, including the commercial brands. OLIRAV is really cheap (costing less than \$USD 1 to build), can be readily assembled and used for multiple collections without contaminating each sample and is also disposable. In addition the materials to be used for OLIRAV all come in aseptically sterile condition, which eliminate the necessity to sterilize before use.

## CONCLUSIONS

The results from this study proved that OLIRAV is a cheap and very effective device or procedure to collect rabbit semen both for AI purpose and andrologic studies. Semen collected with OLIRAV met the benchmark for volume, sperm motility, sperm concentration and abnormality, irrespective of the buck size and period of semen collection.

## ACKNOWLEDGEMENTS

The contributions of Michael O. Faleti., Oluwafemi A. Fadoju, Solomon O. Awolope, Samson G. Adejumo, Emmanuel B. Mustapha, Olatunde O. Ilori and Ishola O. Adeyosoye to data collection and the supply of the commercial rabbit artificial vagina by the OAU iLINOVA project are greatly appreciated.

## REFERENCES

- Bradderman P.J., Foote R.H., Yassen A.M. 1964. An improved artificial vagina for collecting rabbit semen. *J. Reprod. Fert.*, 7, 401-403.
- Castellini C. 2008. Semen production and management of rabbit bucks. In: *Proc. 9<sup>th</sup> World Rabbit Congress, 2008 July, Verona, Italy*, 265-278.
- Herbert U., Adejumo D.O. 1995. Construction and evaluation of an artificial vagina for collecting rabbit semen. *Delta Agric* 2, 99-108.
- International Rabbit Reproduction Group. 2005. Guidelines for the handling of rabbit bucks and semen. *World Rabbit Sci.*, 13, 71-91.
- Macirone C., Walton A. 1938. Fecundity of rabbits as determined by dummy matings. *J. Agric. Sci.*, 21, 122.
- Naughton C.K, Nelson, D.R., Thomas (JR) A.J. 2003. Development of an inexpensive artificial vagina for semen collection from rabbits. *J. Androl.*, 24, 712-715.
- Walton, A. 1958. Improvement in the design of an artificial vagina for the rabbit. *J. Physiol.*, 143, 26.

=====