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Influence of Doe Exposure on the Spermiogram of Rabbit Bucks

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Target audience: *Livestock farmers and Reproductive Physiologists*

Abstract

The spermiogram of rabbit bucks exposed to rabbit does was evaluated. Seventy five mature rabbits comprising of 50 nulliparous does, 6-7 months old and 25 bucks, 8-10 months old, mixed breeds (New Zealand white, Carlifonia white, Chinchilla and Grey) were used for this study. The animals were allocated to five treatment groups in a completely randomised design (CRD). Each treatment consisted of 10 nulliparous does and five bucks. The does were placed in cages opposite the bucks separated by a distance of 2.5 cm at a ratio of 2 does: 1 buck. The treatment effect consisted of exposing the does to the bucks' pheromonal cues at different durations (days) to synchronize the does prior to mating. Treatment 1 served as the control group, the does in this group recieved instant mating without buck exposure. In Treatment 2, the rabbit does were exposed to the bucks for seven days and then mated. Treatment 3 had does and bucks which were mated after 14 days of exposure. Treatment 4 were exposed for 21 days and mated thereafter, while Treatment 5 had rabbit does and bucks which were mated after 28 days of exposure. Semen was collected from the bucks on weekly basis for evaluation. The result showed that there were significant ($P<0.05$) differences in reaction time, sperm motility, percentage live sperm, percentage dead sperm and percentage coiled tails in the bucks's semen exposed to the does from the control, indicating a positive influence of the doe exposure on the spermiogramic characteristics of rabbit bucks. Hence, this study suggests that exposing rabbit does to bucks prior to mating improves the reproductive performance of the bucks.

Keywords: Bucks, Does, Exposure, Mating, Rabbits,.

Description of Problem

The increasing demand for animal protein has aroused so much interest in the production of animals with short generation intervals like rabbits (1). Rabbits are commonly raised to provide tender meat for human consumption. Besides, the high prolificacy is the most important economic factor in rabbit production (2,3). To ensure a sustained rabbit production and development of the sector, rabbit breeds and breeding practices should be explored and challenges identified. The profitability of rabbit production depends on the reproductive intensity and the number of kits weaned from a litter (4). Optimal conditions of rearing rabbit bucks can improve the quality of semen resulting in the production of more doses with higher and more stable fertilizing ability (5). Many factors affect seminal traits (5) and thus it is crucial to define suitable protocols to improve spermatozoa characteristics (6,7). However, normal reproductive activity in the

male is comprised of the production of semen containing normal spermatozoa in adequate numbers, together with the desire and ability to mate (8). Sex drive is also largely dependent on endogenous male hormones. Daily peaks of mating activities are apparently associated with fluctuations in the male sex drive. The most widespread form of depressed fertility and sterility, with the exception of the pathological types, is that resulting from physiological causes. These directly or indirectly affect the general endocrine balance within an animal, causing changes in the levels of the controlling hormones. There are many instances where the reproductive potential of an animal is reduced by an endocrine imbalance. Disturbances in the endocrine system, which seem to influence reproduction, centre around the gonadotrophic hormones of the anterior pituitary (FSH, LH and prolactin), and the hormones of the testes. It is assumed that animals producing low numbers of spermatozoa may be deficient in the pituitary hormone, FSH, which stimulates spermatogenesis. Male effect is a natural and less expensive alternatives to improve the reproductive performance of animals. The male effect is described as the influence of a male on oestrus behaviour and ovarian activity in female co-species. Such methods have been found or tested in bulls (9), bucks (10), hamsters (11) and rams (12). However, the response of rabbit bucks to doe's visual, acoustic/auditory and olfactory stimuli has not been properly elucidated. This study aimed at evaluating the influence of doe exposure on the spermiogramic parameters of rabbit bucks.

Materials and Methods

Experimental Site

The study was conducted at the Rabbit Research Unit of Swine and Rabbit Research Programme, National Animal Production Research Institute (NAPRI), Shika, Zaria. Shika lies within the Northern guinea savannah Zone of Nigeria and located on latitude 11^0 12'N and longitude 7^0 33' E with an altitude of 691 m above sea level. Annual rainfall range is between1100-1200 mm, while mean temperature is about 24.4 ^{0}C (14.5-39.3 ^{0}C), with the lowest temperature occurring during the early dry season (November-January), while, the highest temperatures are experienced during late dry season between February-April. (13)

Experimental Animals and Design

A total of 75 mature rabbits, which comprised of 50 nulliparous does of about 6-7 months of age and 25 mature bucks of about 8-10 months of age (mixed breeds: New Zealand white, California white, Chinchilla and Grey) were used for this study. The animals were divided into five treatment groups comprising of ten nulliparous does and five bucks per group in a Completely Randomized Design (CRD). The study was planned such that there was an exposure ratio of one buck to two does, implying five replications per treatment and each replicate containing two does and one buck. However, the buck in the control group were not exposed to any doe.

Experimental Diets and Management

The animals were fed standard basal rabbit diet (2700 kcal ME and 18 % CP) with a mixture of *Panicum maximum* and *Brachiria decumbens ad libitum*. The does and bucks were individually housed in flat deck metal cages measuring 60 x 60 x 50 cm, placed in a ventilated house of 5ft walls. The bucks in treatment 1 were not exposed prior to mating, they served as the control group, while bucks in treatments 2, 3, 4 and 5 were exposed to the does by placing them in opposite cages at a distance of 2.5 cm from the does for 7, 14, 21 and 28 days respectively prior to mating. Semen was collected from the bucks on weekly basis for evaluation.

Data Collection

Reaction time(s): This was determined using a stop watch by recording the time interval (in seconds) between the introduction of a doe into the buck's cage to a successful copulatory thrust with ejaculation.

Semen collection and evaluation

Semen was collected from the exposed bucks once weekly between 08-10am using an artificial vagina and the ejaculates were examined for:

Semen colour: This was determined by visual appraisal of the semen and coded as 5 Excellent, 4 very good, 3 good, 2 fair, 1 poor and 0 very poor

Semen Volume: This was measured by collecting the ejaculate in a calibrated test-tube and the volume of each ejaculate recorded.

Semen P^H: This was measured with a P^H meter immediately after the collection of the semen. The P^H of each individual sample collected was determined using Chemo craft P^H paper. In each case, one inch of the paper was inserted into the semen while in the tube for five seconds and removed and properly air dried. The colour change observed after drying was placed to the colour chart on the P^H paper. The corresponding P^H number for the colour on the chart was recorded as the P^H of the sample.

Sperm Motility: This was determined by observing a drop of semen on a glass slide with cover slide under a microscope (type; electric, model; CX21FS1, manufacturer; Olympus Corporation,Tokyo, Japan) at x400 magnification, with a warm stage maintained at 37^0C. The wave patterns were recorded in percentages. Gross motility was estimated as percentage scores according to the procedure outlined by (14)

Sperm Concentration: This was determined using the red blood cell counting chamber of a haemocytometer that was crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares placed on a microscope (type; electric, model; CX21FS1, manufacturer; Olympus Corporation, Tokyo, Japan) and viewed at x 400 magnification. The total number of small squares on the haemocytometer is 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares. Sperm cells counted were multiplied by a dilution factor (19mls of diluents /1ml of semen) and a multiplication factor. The value obtained was recorded as sperm concentration (14).

Percent Live and Dead Spermatozoa: The percentage live and dead sperm was estimated by preparing a smear of individual semen sample using Eosin-nigrosin stain immediately after collection. A drop of semen was placed on a clean glass slide using a pipette. A drop of the Eosin-nigrosin solution was placed alongside the semen on the slide. The slide was gently turned to allow a uniform mixture of the two samples. One-quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide and allowed to dry. The principle is that the dead sperm cells accept the stain and appear pink or red while the live sperm cells reject the stain and remain unstained (15).

Percentage abnormalities: The percentage abnormalities (morphology) were determined by viewing the stained slide under a microscope.

Data Analysis

Data generated were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure (16). Significant means between treatment groups were separated using Dunnette's Procedure (17).

Results and Discussion

Table 1 shows the percentage composition of the feed ingredients while Table 2 shows the proximate composition of the experimental diet**.** Table 3 shows the reproductive performance of rabbit bucks exposed to rabbit does at different durations**.** At the point of mating, it was observed that the control group displayed a copulatory thrust with ejaculation in 37 seconds, while in 7, 14, 21 and 28 days exposed bucks, copulatory thrust with ejaculation occurred within 16, 23.4, 27 and 25 seconds respectively. Differences in the durations (days) of exposure might be responsible for these variations. The reaction time of the control group differed significantly ($P<0.05$) from the experimental groups with the shortest reaction time of 16 seconds recorded in bucks exposed to does 7 days prior to mating. The non exposed rabbit bucks had the longest reaction time of 37 seconds. The shortest reaction time of 16 seconds recorded in this study is lower than 27.9 s obtained by (18) but higher than14.2 s found by (19). Therefore, it seems that doe exposure improved the reaction time of the bucks.

Also, sperm motility, percentage live sperm, percentage dead sperm and percentage coiled tails of bucks exposed to does differed significantly ($P<0.05$) from the control and across treatments. The differences in sperm motility, percentage live sperm, percentage dead sperm and percentage coiled tails across the treatment groups may be due to a variation in the degree of perception of the does' odour by the bucks. In line with this observation, (20) reported that controlled male exposure to estrous females, viewing and periodical change of females enhances male sexual performance in several mammalian species. However, (21) reported that pheromones detected by the olfactory system stimulates male and female sexual behaviours in mammals. This positive effect of the does' stimuli on the bucks reproductive parameters (increased sperm motility, sperm concentration, and percentage live sperm) agrees with responses found in bulls (9), boars (22), hamsters (11) and rams (12). This finding is also in close agreement with the reports of (23) who reported a higher semen quantity and quality in rams exposed to odour and estrous manifestations from ewes. Therefore, it appears that pheromones (olfactory), auditory and visual cues can mediate increased reproductive potentials of rabbit bucks in physical close proximity to rabbit does with limited contact. However, the bucks' testosterone peak of 13.0ng/ml on the 14^{th} day suggests that this exposure might have exerted a higher stimulation on the hypothalomo-hypophyseal gonadal axes of the bucks for an increased secretion of gonadotropins (FSH and LH) which accelerated a higher testosterone secretion with higher sperm live-ability (higher motility, percentage live and percentage normal sperm cells) in the treatment groups than in the control. Differences in the magnitude of testosterone secretions across the treatments are a measure of the different degrees of stimulation received by the bucks from the does' stimuli at different time intervals. Therefore, longer duration of buck exposure seems to improve reproductive traits of rabbit bucks. This finding agrees with the work of Kishk (24) who reported higher testosterone secretion in rams exposed to odour from cycling ewes. This result showed that a close male-female proximity in rabbits prior to mating has a potent effect in increasing the androgenic activities in rabbit bucks.

The exposure also conferred in the exposed bucks' semen a higher degree of fertility which improved some reproductive traits of the does. Does mated to the exposed bucks had shorter gestation length, higher pregnancy rate, higher average litter size and higher average weaning weight than the control. It implies that exposing a rabbit doe to

a rabbit buck prior to mating improves their reproductive traits.

Conclusions and Application

1. Libido in rabbit bucks can be enhanced by keeping rabbit does and bucks closer to each other for about 7-14 days before mating without physical contact.
2. The hormonal profile of rabbit bucks can be enhanced by controlled doe exposure for higher testosterone concentrations.
3. Exposing rabbit bucks to does prior to mating has a potent androgenic activity in the bucks.
4. Doe-Buck exposure of 7-14 days prior to mating is a veritable tool for improving reproduction in rabbits while exposure longer than 7-14 days may adversely affect their reproductive performance.
5. To improve serum testosterone concentration of rabbit bucks, 14 days exposure to the does is recommended.
6. More research should be conducted to evaluate the effect of doe exposure on the attainment of puberty in rabbit bucks.

Table 1: *Percentage composition of the experimental diet*

Ingredients	(%)
Maize	55.00
Wheat Offal	25.00
Soya cake	18.00
Mineral & Vitamin Premix	0.25
Salt	0.25
Lysine	0.25
Methionine	0.25
Total	100.00
Calculated Analysis	
Metabolisable Energy (Kcal/Kg)	2707.00
Crude Protein (%)	18.15
Ether Extract (%)	3.61
Crude Fibre (%)	11.70
Calcium (%)	1.08
Available Phosphorus (%)	0.66
Methionine (%)	0.52
Lysine (%)	1.13

***Biomix premix supplied per kg of diet: Vit.A, 10,000 iu; vit D_3, 2000 iu; vit E, 23 mg; vit.k, 2mg, vit B_1, 1.8; vit B_2, 5.5 mg; Niacin, 27.5mg; pantothenic acid,7.5mg; vit B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; chloride, 300mg; cobalt, 0.2; Copper, 3mg; Iodine 1mg; Iron, 20 mg; Manganese, 40 mg; selenium, 0.2 mg; Zinc, 30 mg; Antioxidant, 1.25mg.(Manafactured by: Bio-organics Nutrient System Limited, Ibafo Ogun State, Nigeria.*

Table 2: *Proximate composition of the experimental diet*

Energy (Kcal)	2783.00
Crude Protein (%)	17.64
Crude Fibre (%)	8.35
Ether Extract (%)	5.81
Dry Matter (%)	91.74

Table 3: *Semen characteristics of rabbit bucks exposed to rabbit does at different durations of biostimulation*

	Durations						
	00	07	14	21	28	SEM	Pvalue
Parameters							
Reaction time (s)	37.00^{c}	16.00^{a}	23.40^{ab}	27.20^{b}	25.00^{b}	3.99	0.0211
Semen volume (mls)	1.50	2.50	1.00	3.00	2.00	0.84	0.2178
P^{H}	8.00	7.00	7.50	8.00	7.00	0.97	0.9191
Sperm motility	70.00^{c}	80.00^{ab}	85.00^{a}	80.00^{ab}	75.00^{c}	4.71	0.0500
Sperm concentration ($X10^{6}$)	152.00	159.00	166.00	156.00	151.00	15.5	0.3662
Percentage live sperm	75.00^{b}	80.00^{ab}	90.00^{a}	85.0^{ab}	80.0^{ab}	5.94	0.0364
Percentage dead sperm	25.00^{b}	20.00^{ab}	10.00^{a}	15.0^{ab}	20.0^{ab}	5.90	0.0264
Normal sperm cells (%)	69.00	73.00	89.00	73.00	79.00	8.03	0.1206
Detached heads (%)	17.00	13.00	3.00	12.00	8.00	3.81	0.3027
Coiled tails (%)	10.00^{b}	09.00^{b}	06.00^{a}	11.00^{b}	7.0^{ab}	2.52	0.0543
Bent tails (%)	04.00	05.00	2.00	4.00	6.00	1.87	0.4548

Means with different superscripts differed significantly ($P < 0.05$), 00, 07, 14, 21, 28 = treatments 1,2,3,4 and 5, representing weekly/durations of exposure respectively. SEM=standard error of the mean

Table 4: *Reproductive performance of rabbit does exposed to rabbit bucks at different durations of biostimulation*

	Durations (days)						
	00	07	14	21	28	SEM	Pvalue
Parameters							
Pregnancy rate (%)	30.00 ^{c}	80.00^{a}	70.00^{ab}	40.00^{b}	50.00^{b}	5.69	0.0112
Gestation length (days)	31.00^{c}	30.20^{ab}	29.10^{a}	31.25^{c}	31.20^{c}	0.688	0.0213
Kit mortality (%)	36.36	36.58	54.54	36.36	43.33	19.23	0.8558
Average litter size at birth(n)	3.67	5.12	4.70	8.25	6.00	2.703	0.6518
Average litter birth weight(g)	46.36	40.24	40.78	45.45	45.83	3.427	0.1616
Average litter size at weaning(n)	3.00	3.25	2.14	3.50	1.00	1.130	0.4409
Average litter weaning weight(kg)	0.99^{b}	0.98^{b}	1.09^{ab}	1.20^{a}	0.85^{c}	0.101	0.0215

Means with different superscripts differed significantly ($P < 0.05$), 00, 07, 14, 21, 28 = treatments 1,2,3,4 and 5, representing weekly/durations of exposure respectively. SEM=standard error of the mean

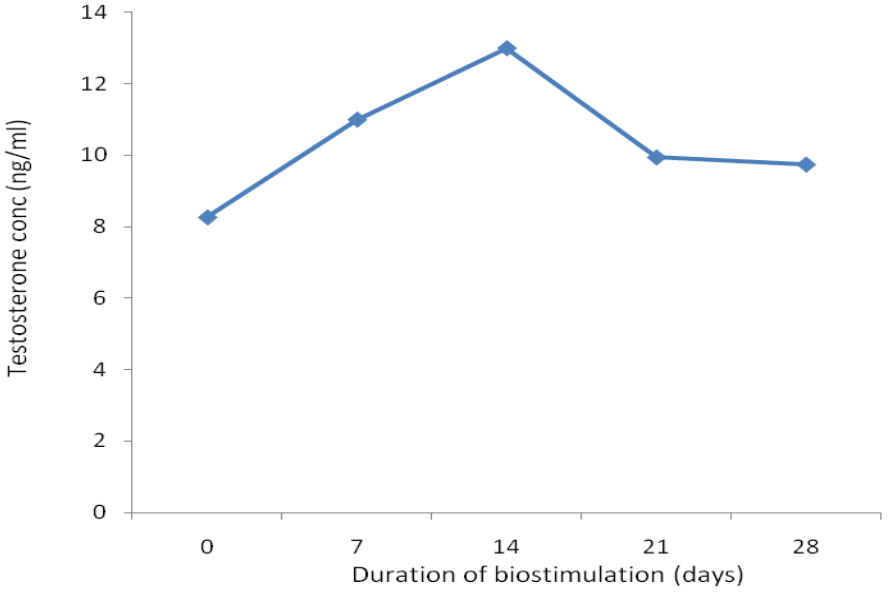

Figure 1: Testosterone concentration (ng/ml) of rabbit bucks under different durations of exposure

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