

Research paper

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RESPONSE OF NATURAL MATING FREQUENCY AND ARTIFICIAL INSEMINATION ON FERTILITY IN RABBITS AND THEIR CYTO-GENETIC PROFILE (X-CHROMATIN)

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ABSTRACT

A study was conducted to determine the Cyto-genetic profile of breeding rabbits and the impact of natural mating frequency and artificial insemination on its fertility status. Twenty four (post-pubertal) does aged 8-9 months and four matured (aged 10-12 months) fertile bucks of New Zealand white breed, were randomly assigned to four treatment groups designated A, B, C and D respectively in Completely Randomized Design (CRD). Treatment A-C indicated increasing levels of daily mating of once a day (treatment A- the control), twice daily (treatment B) and thrice daily (treatment C) mating pattern. Treatment D was subjected to artificial mating pattern. The experiment lasted for six months, during which two parities were obtained. The mean conception rate, kindling rate and litter size differed highly significantly ($P<0.001$) among the treatment groups according to increasing levels of frequency of daily mating. Significant differences ($P<0.05$) were also observed on litter weight, by decreasing order of daily mating frequencies. No statistical difference ($P>0.05$) was seen on gestation length. The X-chromatin incidence of does with zero conception and their males were within a normal range of 2-12% and 0-2% respectively. It was concluded therefore that daily mating frequency has a favorable influence on reproductive parameters examined in this study.

Keyword: Breeding Pattern, X-chromatin, Rabbit doe, Artificial mating, fertility, vagina, Pseudo-pregnancy, Post-Pubertal, Teaser Doe

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INTRODUCTION

Reproduction is a complex biological phenomenon which occurs in all living organisms.

Its significance and manipulations are the secret tools for the improvement of animal fertility [1, 2].(~~Berepubo et al., 1993; Dalton 1987~~).

Rabbit's sexual behavior and breeding potential are all influenced by a wide range of external and internal stimuli [3,4].(~~Berepubo and Umanah, 1995; Ajuogu and Ajayi 2010~~).

~~Female~~The female rabbits are reputed to have complex and peculiar reproductive pattern.

Apart from having high incidence of pseudo-pregnancy, the female rabbits are also considered ~~poly-estrous, poly-oestrous~~, or as having no cyclic or regular estrus [3,4].(~~Berepubo et al., 1993, Ajuogu and Ajayi 2010~~). These biological features seems to presuppose that the non-pregnant female rabbits would accept her male counterpart for mating at every presentation and indeed conceive at each mating since it is an induced ovulator. [3] ; [5]~~Berepubo et al. (1993) ; Fielding (1991)~~ reported that other estrous signs in female rabbits even though less visible, than other farm species undergoes a rhythmic weekly (5-7days) estrous cycle. Influence of mating frequency is important in the reproductive performance of farm animals including rabbits [6, 4]. [7] (~~Ajuogu 2002; Ajuogu and Ajayi (2010). Pitkajanen (1955)~~ reported a 0.285 increase in ovulation rates of the mated groups of sows. [8]~~Reed (1985)~~ compared fertile results of sows inseminated three times in each estrous period with those of sows inseminated twice. He reported both an increased conception rate and an increased litter size. [9] ~~Randel et al. (1973)~~ reported that clitoral stimulation following artificial insemination of beef cows hastened the onset

of ovulation with cervical stimulation reducing the time from the beginning of estrous to the luteinizing hormone (LH) surge suggesting that a neural path way may exist between the genital system and the hypothalamic pituitary axis.

The use of X-chromatin (drumstick) in diagnosing major X-related reproductive irregularities and stunted growth in the animal industry has contributed greatly in the detection of kind of reproductive anomalies and stunted growth in the animal industry [10,11]. The technique could thus be used in culling individuals exhibiting numerical or morphological abnormalities of X-chromatin appendages early in life so as to reduce the cost of production [12,3]. In modern genetic parlance, X-chromatin evaluation refers to the analysis of the X-chromosome only, without reference to the Y chromosome. The X-chromosome has successfully used been used in domestic animals to predict the cytogenetic or genetic merit of various economically important species. These include early detection of sex chromosomal and developmental anomalies which considerably impair fertility, prediction of the growth potential of neonates [13,14]. The principle is that there are as many drumsticks or Barr bodies (i.e. X-chromatin appendages) for the existence or presence of two X-chromosomes in a normal female animal cell. This implies that the normal fertile animal is expected to show at least one X-chromatin appendage (Drumstick or Barr body) in a preponderant proportion ie, in at least 90% of its cells [10]. The objectives of this research are:

- (1) To establish or ascertain normal fertility in female rabbits through X-chromatin evaluations
- (2) To evaluate the influence of natural mating frequency on the Reproductive performance of female rabbits
- (3) To compare the influence of natural mating and artificial mating on

[Conception rate, gestation length, litter size, litter weight, survivability and weaning weight/age in rabbits](#)

MATERIALS AND METHODS

Twenty four Post Pubertal female rabbits aged 8-9 months and four mature fertile bucks aged 10-12months of New Zealand white breeds were randomly assigned to four treatment groups A,B,C and D in a Completely Randomized Experimental design (CRD). The females were further replicated twice with three rabbit's per-replicate. The treatments were properly tagged for easy identifications indicating the breeding pattern and timing illustrated thus;

- A =Morning mating 8-9am, (once mating per day) (control)
- B =Morning mating 8-9am, afternoon mating 1-2pm (twice mating per day)
- C =Morning mating 8-9am, afternoon mating 1-2pm and evening mating 7- 8am (thrice mating per day).
- D =Artificial mating (i.e insemination by artificial or mechanical means)

A reinforced polyvinylchloride (PVC) tube (inner diameter 2.8cm, outer diameter 3.8cm) was cut to a length of 3.0cm and referred to as the lager tube (LT). A rigid plumbing hose (inner diameter 1.7cm outer diameter 2.7cm) was also cut to the same length and called the smaller tube (ST). These were use to construct artificial vagina according to[\[15\].](#)
[\(Herbert and Adejumo, 1996\)](#)

The artificial vagina (AV) unit was warmed by putting it in warm water at 60°C for 10-15munits in a rubber container according to [\[15\].](#)[Herbert and Adejumo \(1996\).](#) A mature

non gravid doe was used as the teaser animal. As the buck mounts and makes a thrust on the teaser doe, the pre-warmed AV was quickly applied from side of the erect penis of the buck prior to intromission of the penis on the AV, which elicited ejaculation within a few seconds. The semen volume (in mills) was read directly from the collection tube and used for the insemination.

The females were taken to the males for service and returned thereafter. The males belonging to each treatment group were used to serve the females in that group. Groups A-C females were naturally mated with males in their various groups while group D females were artificially inseminated by the semen collected from the male of that group.

Blood samples were collected from the females that could not conceive after mating. Sterile syringes and hypodermic needles were used to aspirate blood from the ear veins of the rabbits. After the collection, it was immediately decanted into a sterile heparinized sample bottle to avoid coagulation and immediately taken to the laboratory for evaluation. A drop of the whole blood was dropped on a glass slide and a smear was made and allowed to dry. After that, it was fixed in 90% ethanol for 3 minutes and stained after drying for 5 minutes with Leishman' s stain. It was then rinsed with tap water, and left to dry. It was examined under oil immersion (x 100) for drum stick The males belonging to the treatment groups that could not conceive were also screened for X-chromatin status

Two hundred polymorpho neutrophils (PMN) per slide were counted in order to determine the percentage incidence of drumstick, as follows:

$$\% \text{ incidence} = \frac{\text{Number of drumsticks observed}}{\text{Number of PMN observed}} \times 100$$

~~Each doe was weighed before mating with the aid of weighing balance to know the initial weight and weekly after mating until kindling.~~ Variables used to measure reproductive parameters were conception rate, gestation length, litter size, lifter weight, pseudo-pregnancy, weaning weight and mortality. Two parities were obtained. Data on reproductive parameters were collected and subjected to analysis of variance according to ~~[16]Steel and Torrie (1981)~~ and their means separated by Duncan multiple range test by ~~[17].Duncan (1955).~~

RESULTS AND DISCUSSION

The results of the effect of mating frequency and artificial insemination on reproductive parameters in parity one and two, are summarized in table 1. The result showed that conception rate was significantly influenced ($P<0.05$) by natural mating frequency in parity 1 and 2 than the artificial mating group. In parity one, treatments C and D recorded the highest and the least conception rate of 83.3% and 16.7% respectively (table 1) while in parity two, the highest and lowest conceptions rates were in treatments C and A (I.e. 100% and 33.3%). The average gestation length had no significant ($P<0.05$) effect between the treatment groups on both parties. Average kindling rate showed significant increase ($P<0.05$) except in artificially inseminated group i.e. 33.3%, 66.7%, 91.65% and 8.38% for treatments A, B, C, and D respectively. Also, the results of the average litter size and lifter weight showed significant difference amongst the treatment groups ($P<0.05$) for litter size. It progressively increased according to the mating frequency in both parities (3, 0, 4.67, 5.8, 4.0) (3.0, 4.25, 6.17, 0.0) for treatments A, B, C, and D for parities 1 and 2 respectively. But the effects on the litter weight were negatively affected by the mating pattern i.e. the higher the frequency of mating, the lower the litter weight.

Pseudo-pregnancies were also observed among the treatment groups in both parities. The highest was recorded in treatment D in both parities. Mortality of does and kids were also observed.

The results of X-chromatin (drumstick) incidence are presented in table 2, The average drumstick incidence of the females evaluated was [4.45%](#)~~4.447%~~, while the average % X-chromatin (drumstick) incidence of the males was [0.00%](#). The X-chromatin incidence in one of the females (F6) in treatment A was 0.00%.

Fairly high conception rates ranging from 33.3-100% were observed with the increasing frequency of mating in this study. This finding supports that of [\[18\]Paufler \(1985\)](#) who reported increased reproductive performance, conception rates and litter size of rabbits inseminated more than once.

However, this group recorded the least conception rate of 16.7% during the first parity and 0% during the second parity. This observation may be related to the insufficient physiological stimulation of the females [\[19\];\[20\]\(Murdoch and Dunn, 1982\), Fielding \(1991\)](#) resulting in low conception rate or non- conception. This supports the assertion of these workers;[\[1\];\[18\] Berepubo et al \(1993\); Paufler \(1983\)](#) that rabbits are induced ovulators. This implies that endocrinal gland could not receive enough stimuli (signal for concomitant release of ovulation hormone; Leutenizing hormone and Follicle stimulating hormone [\[19\]\(Murdoch and Dunn 1982\)](#) for induction of ovulation, it is possible therefore, that the ovum or ova were not released for fertilization by the semen introduced through artificial insemination, hence the poor or no conception observed. Also [\[4\]Ajuogu and Ajayi, \(2010\)](#) reported that frequency of mating either natural or

artificial to improves some reproductive parameters (conception rate, litter size and kindly rate). However, the reports of [\[18\];\[5\]Paufler \(1985\); Fielding \(1991\)](#) and [\[1\]Berepabo *et al.* \(1993\)](#) revealed that rabbits undergo a rhythmic weekly (5-7 days) oestrus cycle. This may be the reason for 16.7% conception rate recorded in treatment D among the artificially bred does. The insemination may have been co-incidental on their oestrus period since there was no tactile or physiological stimulation. The 0.0% conception rate of the same does in group D in the second parity seems confirmatory to this assertion.[\[20\] Rahajo *et al.* \(1986\)](#) similarly observed poor conception rates in artificially inseminated rabbits. He implicated lack of induction of ovulation as being responsible.

The result of no significant influence ($P < 0.05$) on gestation length, suggest that mating frequency and artificial insemination had no effect on gestation length. This corroborate [\[21\];\[22\]Omole and Sonaiye \(1991\); Aduko and Olukosi \(1990\)](#) who reported a mean gestation length of 31.6days in a well managed rabbit breeding outfit. The result of the kindling rate with significant influence ($P < 0.05$) from the treatment (natural mating frequency) agrees with the assertion of [\[23\]Drugociu \(1996\)](#) who implicated vaginal uterine Stimuli (tactile Stimulation through coitus) resulting in higher conception rate, kindling rate and greeter viability in-utero.

Also litter size which showed significant difference ($P < 0.05$) among the groups (mating frequency and artificial insemination) with the highest values recorded in treatment B) (5,4) and C (6,6) could probably be due to increase in neural and endocrinal release of Leutenizing hormone and Follicle stimulating hormone during copulation, which act on ovary causing more release of ova. This supports [\[24\]Cheek *et al.* \(1987\)](#) who observed

that copulation in rabbits stimulate the immediate release of Leutenizing hormone with peak serum levels reached in 1-2hours post copulation. This implies that the more the number of eggs released due to coital stimulation, the more the available sperm cells in the reproductive system will fertilize them. This will by and large affect the number of litters and other parameters (Conception rate, litter size and kindling rates).

The mean percent drumstick incidence obtained from animals in treatments A, B C and D, that had zero conception ranged from 2.5 to 15.0% with a mean value of 4.5%. This observation agrees with that of [\[25\] Basur, \(1984\)](#) who documented that some mammals such as humans and cattle showed an X-chromatin incidence of 2% - 12% in a normal female. Also [\[26\] Davidson and Smith \(1954\)](#) reported an X-chromatin incidence of 0.5% per 200pmn. These observations therefore suggests that the females in treatments D (F1 - 5), A (F6-9) and B (F10-11), that had zero conception (Table 2) may not have been affected by any form of health or management problems. According to this study, the treatment effect (artificial insemination and natural mating frequency) was implicated in the observed variations. Therefore, it could be implied that the influence of mating once, (treatment A) twice (treatment B) and artificial mating without artificial induction (treatment D) may have been the possible cause of zero conception observed within the groups in reference. Nevertheless, the zero percent (0.0%) X-chromatin (drumstick)' incidence of one of the females in treatment A (F6) may be suggestive of a fundamental genetic [\(sex-chromosomal\)](#)~~(sex-chromosomal)~~ disorder with the consequence of reproductive failure [\[27,28,29,11,14\].\(Long and Williams, 1980;Hare, et al., 1980; Bhatia and Shanka, 1982; Berepubo, et al., 1993; Wekhe, 1998\)..](#)

Furthermore, the ~~X-chromatin~~X-chromatin of the males (ml-3) had ~~(0%)(0.0%~~ drumstick) was in line with ~~[26]Basur (1984)~~ who reported an X-chromatin appendages of 0.0% -2.0% for normal mammalian males. The above observation therefore, suggest that genetic reproductive abnormalities may not have contributed in the observed differences in reproductive and productive performances

CONCLUSION

It was demonstrated that increased natural mating frequency has better influence on reproductive parameters (Conception rate, litter size and kindling rate) than artificial mating pattern in rabbits.

Pseudo-pregnancy; one of the major constraints in effective reproduction in rabbits, can be reduced greatly by increasing frequency of natural mating.

The influence of artificial insemination on reproductive performance of rabbits may be enhanced when the doe in question are properly induced physiologically (tactile induction) ~~[18].(Pauffer 1985).~~

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Table 1: Reproductive performance of does in the first and second parity

Reproductive	Parity 1				Parity 2			
Parameters	<u>Experimental groups (mean +SEM)</u>				<u>Experimental groups (mean +SEM)</u>			
	A	B	C	D	A	B	C	D
Av. Concept.rate(%)	33.3±0.0 ^c	66.7±0 ^a	83.3 ±0 ^a	16.71±0.0 ^d	33.3±0.0 ^c	66.7±0 ^b	100.0±0 ^a	-
Av. Gest. Length(days)	30.5±1.0	31.3±0.0	30.6±00	1.0±0.0	31.5±16	31±1.0	31.0± 0	-
Av. kindling rate (%)	33.3±0.0 ^a	66.7±0.0 ^d	91.65±1.0 ^c	8.38±0.0 ^d	-	-	-	-
Average litter size (Number)	3.00±0.0 ^a	4.67±1.0 ^c	5.8±1.0 ^c	4.00±0.0 ^b	3.0±1.0 ^a	4.25±0.0 ^{ab}	6.17±0.0 ^c	-
Av. litter weight(g)	85.20±1.9 ^a	72.80±2.4 ^b	68.00±2.4 ^b	5.70±0.0 ^{ab}	81.7±57 ^a	71.28±31 ^{ab}	67.35±2.3 ^{b-}	-
No of pseudo-pregnence	2	2	0	-	-	-	3	-
No of still births		--	-	-	-	-	-	-

Table 2 X-chromatin status of the animals used for Breeding

Animals	No of Neutrophils	% incidence of Drumstick
A1	200	3.6
A2	200	6.67
A3	200	15.4
A4	200	2.04
B5	200	2.04
B6	200	0.00
B7	200	4.17
F8	200	7.14
F9	200	4.55
F10	200	5.13

F11	200	2.5
F1	200	0.00
F2	200	0.00
F3	200	0.00
Mean	200	4.44