

EFFECT 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 experimental treatment groups (designated A, B, C and D) respectively in a Completely Randomized Design (CRD). Groups Treatment A to C had been subjected to different 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. Animals of groups Treatment D were subjected to artificial insemination mating pattern. The experiment lasted for six months, during which two parities were obtained. The mean conception rate, kindling rate and litter size showed significant differences highly significantly ($p < 0.001$) among the experimental treatment groups according to increasing levels of frequency of daily mating. Significant difference ($p < 0.05$) was also observed on litter weight when, by decreasing the order of daily mating frequency. The frequency of daily mating had no influence. 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 can be concluded therefore that daily mating frequency has a favorable influence on reproductive parameters examined in this study.

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Keyword: Breeding pattern, X-chromatin, Rabbit doe, Artificial mating, fertility.

1 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]. Rabbit's sexual behavior and breeding potential are all influenced by a wide range of external and internal stimuli [3,4].

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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, or as having no cyclic or regular estrus [3,4]. These biological features seems to presuppose that the non-pregnant female rabbit would accept her male counterpart for mating at every presentation and indeed conceive at each mating since it is an induced ovulator. [3] ; [5] reported that other estrous signs in female rabbits even though less visible, than other farm species undergoes rhythmic weekly (5-7days) estrous cycle. Influence of mating frequency is important in the reproductive performance of farm animals including rabbits [6, 4]. [7] reported a 0.28 increase in ovulation rates of the mated groups of sows. [8] compared fertile results of sows inseminated three times in each estrous period with those sows inseminated twice. He reported both an increased conception rate and an increased litter size. [9] reported that clitoral stimulation following artificial insemination of beef cow 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 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 ~~insemination~~ mating on ~~c~~Conception rate, gestation length, litter size, litter weight, survivability and weaning weight/age in rabbits.

2 MATERIALS AND METHODS

Twenty four ~~p~~Post ~~p~~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 ~~experimental~~treatment groups A,B,C and D in a Completely Randomized Experimental design (CRD). ~~Each experimental group~~The females were further replicated twice with three female rabbits per replicate. The treatments were properly tagged for easy identifications indicating the breeding pattern and timing, ~~as detailed below; illustrated thus;~~

A ——— = ~~m~~Morning mating 8:00-9:00 am, (once mating per day) (control),

B ——— = ~~m~~Morning mating 8:00-9:00 am, afternoon mating 1:00-2:00 pm (twice mating per day),

C = ~~m~~Morning mating 8:00-9:00am, afternoon mating 1:00-2:00 pm and evening mating 7:00– 8:00 am (thrice mating per day),

D ——— = ~~a~~Artificial ~~insemination~~mating (i.e insemination by artificial or mechanical means).

~~For collection of rabbit semen, 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 larger tube (LT). A rigid plumbing hose (inner diameter 1.7cm ~~and~~ outer diameter 2.7cm) was also cut to the same length and called the smaller tube (ST). These were used to construct artificial vagina (AV) according to [15].—

The artificial vagina (AV) unit was warmed by putting it in warm water at 60°Ce for 10-~~15~~ minutesunits in a rubber container according to [15]. A mature non gravid doe was used as the teaser. 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 few seconds. The semen volume (in m~~l~~ills) was read directly from the collection tube, and used for the insemination.

The female was taken to the male for service and returned thereafter. The males belonging to each ~~experimental~~treatment group were used to serve the females in that group. ~~Females of g~~Groups A-C ~~females~~—were

naturally mated with their respective males in their various groups while females of group D females were artificially inseminated by the semen collected from the male of that group.

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Blood samples were collected from the females that could not conceive after mating. Sterile syringes and hypodermic needles were used to collectaspirate blood from the ir ear veins of the rabbits. After collection, s, blood samples-it wereas immediately decanted into a sterile heparinized sample tubesbottles to avoid coagulation and immediately taken to the laboratory for X- chromatin evaluation. A drop of the whole blood was dropped on a glass slide and a smear made and allowed to dry. After that, samples wereit 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. Finally, blood slides#t wereas examined under oil immersion (x 100) for visualization of Barr bodiesdrum-stick. The males belonging to the treatment groups that could not conceive were also screened for X-chromatin status.

Two hundred pol⁴ymorphon^{uclear} neutrophils (PMN) per slide were counted in order to determine the percentage incidence of drumstick, as follows:

% incidence = Number of drumsticks observed x 100

Number of PMN observed I

Variables used to measure reproductive parameters were conception rate, gestation length, litter size, litter weight, pseudo-pregnancy, weaning weight and mortality. Two parities were obtained. Data ofn reproductive parameters were collected and subjected to analysis of variance according to [16] and their means separated by Duncan multiple range test by [17].

3 RESULTS AND DISCUSSION

The results of the effect of mating frequency and artificial insemination on reproductive parameters in both paritiesparity 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 group handled with artificial inseminationmating group. For firstth parity group-one, treatments C and D recorded the highest and the least-lowest conception rates with values of 83.3% and 16.7%, respectively (table 1) while in parity two, the highest and lowest conceptions rates were found in groups in treatments C and A (i.e. 100% and 33.3%, respectively). The average gestation length had no significant ($pP<00.05$) effect amongbetween experimental the treatment groups on both parties. Average kindling rate showed a significant increase ($pP<00.05$), except forin artificially inseminated group (-i.e. 33.3%, 66.7%, 91.765% and 8.438% for groupstreatments A, B, C, and D, respectively. Also, the results of the average litter size and litter weight showed significant difference amongbetween the treatment groups ($pP<0.05$). L for litter size, It progressively increased according to the mating frequency in both parity (parity 1: 3, 0, 4.67, 5.8, 4.0, and parity 2:) (3.0, 4.25, 6.17, 0.0) for treatments A, B, C, and D, for parities 1 and 2, respectively). But the litter weight wasere negatively affected by the mating pattern, i.e. the higher the frequency of mating, the lower the litter weight. Pseudo-pregnancy was also observed among the treatment groups in both parities. The highest value was recorded in treatment D in both parities. Mortality of does and kids was also observed.

The results of X-chromatin (Barr bodydrumstick) incidence are presented in table 2. The average drumstick incidence inof the female rabbits evaluated was 4.45%, while the average values in% X-chromatin (drumstick) incidence of the males was 0%. It was found no The X-chromatin incidence in one of the females (F6) ofin group treatment A was 0.00%.

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

However, inseminated this group recorded the lowestleast conception rates of 16.7% during the first parity (16.7%) and 0% during the second parity (0%). This observation may be related to the insufficient physiological stimulation of the females [19];[20] resulting in low conception rate or non-conception. This supports the assertions of these workers;[1];[18] that rabbits are induced ovulators [1];[18]. This fact implies that endocrinal gland could not receive enough stimuli (signal for concomitant release of ovulation hormone; Leutenizing hormone and Follicle stimulating hormone [19] 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] reported that frequency of mating either natural

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or artificial, improves some reproductive parameters (conception rate, litter size and kindly rate). However, the reports of [18];[5] and [1] revealed that rabbit undergo a rhythmic weekly (5-7 days) estrus cycle. This may be the reason for low ~~16.7%~~ conception rate (16.7%) recorded in treatment D among the artificially bred does. The insemination may have been co-incident on their estrus period since there was no tactile or physiological stimulation. The null ~~0.0%~~ conception ~~recorded for rate of~~ the same does (~~in group D~~) in the second parity ~~is support confirmatory to~~ this assertion. [20] similarly observed poor conception rate in artificially inseminated rabbits. He implicated lack of ovulation induction ~~of ovulation~~ as being responsible.

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The result ~~showed of no significant influence (P<0.05) on gestation length,~~ suggest that mating frequency and artificial insemination has no effect on gestation length (p<0.05). This ~~result agrees corroborate with~~ [21];[22] who reported a mean gestation length of 31.6 days in a well-managed rabbit breeding outfit. The natural mating frequency has significant influence (P<0.05) on result of the kindling rate, which with significant influence (P<0.05) from the treatment (natural mating frequency) agrees with the assertion of [23] who implicated vaginal uterine sStimuli (tactile sStimulation through coitus) resulting in higher conception rate, kindling rate and greeter viability in-utero.

Also, litter size ~~which showed significant differences (P<0.05) among experimental between the groups, (mating frequency and artificial insemination)~~ with the highest values recorded in group treatments B (5,4) and C (6,6). This ~~fact~~ could probably be due to increase in neural and endocrinal release of LLeuteinizing hormone (LH) and fFollicle stimulating hormone (FSH) during copulation, which act on the ovary causing more ova release ~~of ova~~. This ~~agrees with supports~~ [24] who observed that copulation in rabbits stimulates s the immediate release of LLeuteinizing hormone with peak serum levels reached in 1-2 hours 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 obviously ~~will~~ affect the number of litters and other reproductive parameters (cConception rate, litter size and kindling rate).

The mean percent drumstick incidence obtained from animals of experimental groups in treatments A, B C and D, that had ~~no zero~~ conception ranged from 2.5 to 15.0%, with a mean value of 4.5%. This observation agrees with that of [25] who documented that some mammals such as humans and cattle showed an X-chromatin incidence of 2% - 12% in a normal female. Also [26] reported an X-chromatin incidence of 0.5% per 200 polymorpho-nuclear neutrophils (pmn). These observations therefore suggests that the females in treatments D (F1-5), A (F6-9) and B (F10-11), ~~having no that had zero~~ conception (Table 2) may not have been affected by any form of health or management problems. According to this study, the experimental 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 the null 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 ~~suggest be suggestive~~ the existence of a fundamental genetic (sex-chromosomal) disorder with the consequence of reproductive failure [27,28,29,11,14].

Furthermore, the values of X-chromatin in of the males (ml-3) ~~had~~ (0% drumstick) was in line with [26] 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 coursed the observed differences in reproductive and productive performances.

Table 1: Mean values of rReproductive parameters performance of New Zealand white does in the first and second parity.

Reproductive Parameters	Parity 1				Parity 2			
	Experimental groups (mean + SEM)				Experimental groups (mean + SEM)			
	A	B	C	D	A	B	C	D
Av. Conception rate(%)	33.3±0.0 ^c	66.7±0.0 ^a	83.3±0.0 ^a		33.3±0.0 ^c	66.7±0.0 ^b	100.0±0.0 ^a	
Av. Gestation Length(days)	30.5±1.0	31.3±0.0	30.6±0.0		31.5±1.6	31±1.0	31.0±0.0	
KAv. kindling rate (%)	33.3±0.0 ^a	66.7±0.0 ^d	91.7±1.0 ^c		-	-	-	
Average litter size	3.00±0.0 ^a	4.67±1.0 ^c	5.8±1.0 ^c		3.0±1.0 ^a	4.325±0.0 ^{ab}	6.217±0.0 ^c	

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