SDI Paper Template Version 1.6 Date 11.10.2012

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

***[1]P.K. AJUOGU, [2]U. HERBERT and [3]M.A. YAHAYA**

[1] Department of Animal Science and Fisheries, Faculty of Agriculture, University of Port Harcourt, PMB 5323 Choba, Port Harcourt Nigeria.*

[2]Department of Animal Science, Micheal Okpara Federal University of Agriculture Umudike, Abia State Nigeria.

[3]Department of Animal Science, Faculty of Agriculture, Rivers State University of Science and Technology, Nkpolu Orowurukwu Diobu, Port Harcourt, Rivers State, Nigeria.

.

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 groups (A, B, C and D) in a Completely Randomized Design (CRD). Groups A to C had been subjected to different level of daily mating of once a day (treatment A-the control), twice daily (treatment B) and thrice daily (treatment C) mating pattern. Animals of group D

were subjected to artificial insemination. The experiment lasted for six months, during which two parities were obtained. The mean conception rate, kindling rate and litter size showed significant differences (p<0.001) among experimental groups according to increasing level of frequency of daily mating. Significant difference (p<0.05) was also observed on litter weight when decreasing the order of daily mating frequency. The frequency of daily mating had no influence (p>0.05) 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 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.

1 INTRODUCTION

Reproduction is a complex biological phenomenon which occurs in all living organisms. Its significance and manipulation 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].

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 neutral 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 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 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 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 on conception rate, gestation length, litter size, litter weight, survivability and weaning weight/age in rabbits.

2 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 breed were randomly assigned to four experimental groups A,B,C and D in a Completely Randomized Experimental design (CRD). Each experimental group was 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:

A = morning mating 8:00-9:00 am, (once mating per day) (control),

B = morning mating 8:00-9:00 am, afternoon mating 1:00-2:00 pm (twice mating per day),

C = 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 = artificial insemination (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 lager 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℃ for 10-1 5 minutes 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 prior to intromission of the penis in the AV, which elicited ejaculation within few seconds. The semen volume (in ml) 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 group were used to serve the females in that group. Females of groups A-C were naturally mated with their respective males while females of group D were artificially inseminated by the semen collected from the male of that group.

For the artificial insemination procedure, the female is placed into a restraining box, after this, the tail was lifted, and an insemination pipette with a bend approximately 8cm from the end, and inserted into the vagina at an angle of 45º in order progress beyond the pelvic rim according to [5]. Semen is deposited intra-vaginally.

Palpation for pregnancy was carried out on day 14 in accordance with the procedure described by [6]. Does must be relax and sitting naturally whereby fingers were gently run along the abdomen between the back legs, small bead-like lumps would be felt if the doe was pregnant. Blood samples were collected from the females that could not conceive after mating. Sterile syringes and hypodermic needles were used to collect blood from their ear veins. After collection, blood samples were immediately decanted into a sterile heparinized sample tubes 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 were fixed in 90% ethanol for 3 minutes and stained after drying for 5 minutes with Leishman's stain, then rinsed with tap water, and left to dry. Finally, blood slides were examined under oil immersion (x 100) for visualization of Barr bodies. The males belonging to the treatment groups that could not conceive were also screened for X-chromatin status.

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

$$\% \text{ incidence} = \frac{\text{Number of drumsticks observed}}{\text{Number of PMN observed}} \times \frac{100}{1}$$

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 of 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 parities 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 insemination. For

first parity group, treatments C and D recorded the highest and the 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 C and A (100% and 33.3%, respectively). The average gestation length had no significant ($p<0.05$) effect among experimental groups on both parties. Average kindling rate showed a significant increase ($p<0.05$), except for artificially inseminated group (33.3%, 66.7%, 91.7% and 8.4% for groups A, B, C, and D, respectively. Also, the results of the average litter size and litter weight showed significant difference among groups ($p<0.05$). Litter size 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, respectively). But the litter weight was negatively affected by the mating pattern, the higher the frequency of mating, the lower the litter weight. Pseudo-pregnancy was also observed among the 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 body) incidence are presented in table 2. The average drumstick incidence in female rabbits was 4.45%, while the average values in males was 0%. It was found no X-chromatin incidence in one of the females (F6) of group A.

Fairly high conception rates ranging from 33.3-100% were observed when 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 group recorded the lowest conception rates during the first parity (16.7%) and 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 assertion of 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; luteinizing 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 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 conception rate (16.7%) recorded in treatment D among the artificially bred does. The insemination may have been co-incidental on their estrus period since there was no tactile or physiological stimulation. The null conception recorded for the same does (group D) in the second parity supports this assertion. [20] similarly observed poor conception rate in artificially inseminated rabbits. He implicated lack of ovulation induction as being responsible.

However, since rabbits are induced ovulators [21] other than natural mating, stimuli, including direct vaginal stimulation with a vaginal swab or a glass rod [22] and administration of hCG [23; 24] or GnRH [25] could have helped to induced ovulation. Also [26; 27 and 28] have reported improvement of reproductive performance by the use of methods enabling the induction and synchronization of oestrus. Also [21], reported that hormonal treatments have been widely used in recent years, especially the use of prostaglandin F2 alpha (PGF2α) or its analogues to improve doe receptivity and improving reproductive parameters. The poor reproductive efficiency, observed in group D (artificially bred group), confirm the fact insemination should have preceded induction

The result showed that mating frequency and artificial insemination has no effect on gestation length ($p<0.05$). This result agrees with [29];[30] 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 kindling rate, which agrees with the assertion of [31] who implicated vaginal uterine stimuli (tactile stimulation through coitus) resulting in higher conception rate, kindling rate and greeter viability in-utero.

Also, litter size showed significant differences ($p<0.05$) among experimental groups, with the highest values recorded in groups B (5,4) and C (6,6). This fact could probably be due to increase in neural and endocrinal release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) during copulation, which act on the ovary causing more ova release. This agrees with [32] who observed that copulation in rabbits stimulates the immediate release of luteinizing 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 obviously will affect the number of litters and other reproductive parameters (conception rate, litter size and kindling rate).

The mean percent drumstick incidence obtained from animals of experimental groups that had no conception ranged from 2.5 to 15.0%, with a mean value of 4.5%. This observation agrees with that of [33] who documented that some mammals such as humans and cattle showed an X-chromatin incidence of 2% - 12% in a normal female. Also [34] reported an X-chromatin incidence of 0.5% per 200 polymorpho-nuclear neutrophils (pmn). These observations therefore suggest that the females in treatments D (F1 -5), A (F6-9) and B (F1O-11), having no conception (Table 2) may not have been affected by any form of health or management problems. According to this study, the experimental treatment 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 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 the existence of a genetic (sex-chromosomal) disorder with the consequence of reproductive failure [35, 36, 37, 11, 14].

Furthermore, the values of X-chromatin in the males (0% drumstick) was in line with [34] who reported an X-chromatin appendages of 0.0% -2.0% for normal mammalian males. The above observation therefore, suggests that genetic reproductive abnormalities may not have coursed the observed differences in reproductive and productive performances.

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

Reproductive	***Parity 1***				***Parity 2***			
Parameters	***Experimental groups (mean + SEM)***				*Experimental groups (mean + SEM)*			
	A	***B***	***C***	***D***	*A*	*B*	*C*	*D*
Conception rate(%)	***33.3±0.0^{c}***	***66.7±0.0^{a}***	***83.3 ±0.0^{a}***	***16.7±0.0^{d}***	*33.3±0.0^{c}*	*66.7±0.0^{b}*	*100.0±0.0^{a}*	-
Gestation 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± 0.0*	-
Kindling rate (%)	***33.3±0.0^{a}***	***66.7±0.0^{d}***	***91.7±1.0^{c}***	***8.4±0.0^{d}***	-	-	-	-
Litter size (Number)	***3.0±0.0^{a}***	***4.7±1.0^{c}***	***5.8±1.0^{c}***	***4.0±0.0^{b}***	*3.0±1.0^{a}*	*4.3±0.0^{ab}*	*6.2±0.0^{c}*	-
Litter weight (g)	***85.2±1.9^{a}***	***72.8±2.4^{b}***	***68.0±2.4^{b}***	***5.7±0.0^{ab}***	*81.7±57^{a}*	*71.3±31^{ab}*	*67.4±2.3^{b}*	-
Nº of pseudo-pregnancy	***2***	***2***	***0***	***-***	-	-	*3*	-
Nº of still births	***--***	***-***	***-***	***-***	-	-	-	-

Values in the same line with different lower scripts show significant differences among experimental groups (p<0.05).

Table 2. X-chromatin status of the animals used in the present study.

Animals	*Nº of Neutrophils*	*% incidence of Drumstick*
A1	*200*	*3.60*
A2	*200*	*6.67*
A3	*200*	*15.40*
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*	*2.50*
F1	*200*	*0.00*
F2	*200*	*0.00*
F3	*200*	*0.00*
Mean	*200*	*4.44*

4. CONCLUSION

It was demonstrated that increased natural mating frequency has better influence on reproductive parameters (conception rate, litter size and kindling rate) than artificial insemination 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 also be enhanced when the doe is properly induced physiologically (tactile induction).

REFERENCES

[1]. Berepubo NA, Nodu MB, Monsi A, Amadi EN, Reproductive response of pre-pubertal female rabbit to male presence and photoperiod. Int. J Anim Sci. 1993; 8:57-61

[2] Dalton DC. An Introduction to practical animal breeding. Granda Publishing Limited St. Abband and London. 1987; 12-16.

[3] Berepubo NA, Umanah AA. Preliminary investigations into primary anestrous/Infertility in a female rabbit (Dos). Int. J. Anim Sci . 1996; 11: 205-208.

[4] Ajuogu PK, Ajayi FO. Breeding responses of New Zealand white does to artificial insemination under humid tropical environment.Animal Production Research Advances. 2010; 6(1): 46-48.

[5] Fielding D. Rabbit: the Tropical Agriculturalist CTA/Macmillan Education Ltd. London. 1991; 21-25.

[6] Ajuogu PK. Influence of mating frequency and artificial insemination on fertility in rabbit. M.Sc. Thesis, Rivers State University of Science and Technology, 2002; 1 – 26.

[7] Pitkjanen IG. Ovulation, fertilization and first Stages of embryonic development in Pigs. Izv. Akad. Nauk SSR. Ser Blot. 1955; 3: 120-131.

[8] Reed HCB. Artificial Insemination. In Control of Pig Reproduction ed. DJ.A. Cole and G.R. Fox Croft. 1982; 65-90 Butteriorth, London.

[9] Randel RD, Short RE, Christensen DS, Below RA. Effect of various Mat ing Stimuli on the LH Surge and Ovulation time Following Synchronization of Estrus in the Bovine. J. Anim. Sci. 1973; 37:128- 130.

[10] Bhatia S, and V. Shanker V. Sex-chromatin test in the bovine: A cytodiagnostic tool for detection of poor breeders at birth. Proc. 10th Int. Congr. Anim. Reprod.1984; 10-14.

[11] Berepubo NA, Pinherio LE, Basrur PK. Biological significance of X-chromosomes inactivation pattern in sub fertile cows carrying an X- autosome translocation. Discov. Innov. . 1993b; 5(1): 57-62.

[12] Omeje SI, Berepubo NA Nwankwo PC. X-Chromatin Study of Native Breeds of Cattle and Small Ruminants in Nigeria, International J. Anim. Sci. 1994; 9: 181-184.

[13] Satbir K, Vasudha S, Anju S, Parminder K. Buccal. Mucosal X-Chromatin Frequency in Breast and Cervix Cancer, Anthropologist 2006; 8(4): 223-225.

[14] Wekhe SN, X-chromatin evaluation of reproductively abnormal Ruminants, pigs, rabbits and man in Rivers State of Nigeria. PhD thesis, p: 123. Rivers state university of science and technology, Nkpolu. Port Harcourt. 1998; 123

[15] Herbert U, Adejumo DO. Construction and evaluation of an Artificial vagina for collection of rabbit semen. Delta Agric. 1996; 2:99-108.

[16] Steel RGD, and IH Torrie. Principle and Procedures of Statistics. 2nd Ed. McGraw Hill Singapore. 1981.

[17] Duncan DB. Multiple Range and Multiple F-test Biometric ewe. Acta Vet. Scand. 1955; 16:145.

[18] Paufler S. A compendium of Rabbit Production Appropriate for Conditions in Developing Country. Eschborn, Germany. 1985;115-130.

[19] Murdoch WJ, Dunn TG.. Alterations of in follicular Stand Hormones During the Pre Ovulation Period in the Ewe. Bisit. Reprod. 1982;27:300.

[20] Rahajo YC, Cheekke PR, Patton NM. Growth and Reproductive performance of rabbits on a Moderately Low Grude Protein Diet with or without Methionine or Urea Supplementation. J. Anim. Sci. 1986; 63:795-803.

[21] Abdel-Azeem A S, Abdel-Azim AM, Hanan A. Hassan, Mona S. Ragab. Effects of prostaglandin F 2 alpha injection and rabbit does age on reproductive, hematological parameters and level of progesterone. Egypt. Poult. Sci. 2012; 32: I (189-199)

[22] Greulich WW. Artificially induced ovulation in the cat (*Felis domestica*). *The Anatomical Record 1934;* 58:217-224.

[23] Wildt DE, Kinney GM, Seager SWJ. Gonadotropin induced reproductive cyclicity in the domestic cat. *Laboratory Animal Science 1978;* 28:301-307.

[24] Cline EM, Jennings LL, Sojka NJ. Breeding laboratory cats during artificially induced estrus. *Laboratory Animal Science 1980;* 30: 1003-1005

[25] Chakraborty PK, Wildt DE , Seager SWJ. Serum luteinizing hormone and ovulatory response to luteinizing hormone releasing hormone in the estrous and anestrous cat. *Laboratory Animal Science 1979;* 29: 338-344.

[26] Boiti C. Besenfelder U. Brecchia G. Theau-Clément M. Zerani M. Reproductive physiopathology of the rabbit doe. In: Recent Advances in Rabbit Sciences (Maertens, L. and Coudert, P. Editors) 2006 ILVO. pp, 3-19.

[27] Theau-Clement ,M. . Facteurs de réussite de l'insémination chez la lapine et méthodes d'induction de l'oestrus. INRA Prod. Anim. 2008; 21: 221 – 230. (In French)

[28] Gogol, P. Effect of prostaglandin F2α on reproductive performance in rabbit does. Ann. Anim. Sci. 2009; 9: 395 – 400

[29] Omole TA, Sonaiya FO. Nutritional Report International. 1991;23(4): 729-739.

[30] Aduku A0, Olukosi BO. Rabbit Management in the tropics code living book series, Au publications Abuja Nigeria. 1990.

[31] Drugociu G. The Importance of Internal and External Stimuli for Sexual Function in Sows. Zerg Prod. Postep Nauh Rolu. 1966; 61:107-116

[32] Checke, PR Patton NM, Lukefohr SD, Mcnitt JI. Rabbit production. The interstate printers and Publishers, IIIinois 1987.

[33] Basrur PK. Test for nuclear dimorphism. In: A guide lo genetic counseling in veterinary medicine, P.K, Basrur (ed Ontario veterinary college. University of Guelph, Canada, 1984; 92.

[34] Davidson WM, Smith MD. A morphological sex difference in the Polymorph nuclear neutrophil. Brit. Med. J. 1954; 2: 67.

[35] Long S E, Williams CV. Frequency of chromosmal abnormalities in early embryos of the domestic sheep (*Ovis aries*) J. Reprod Fert . 1980; 58:197.

[36] Hare WCD, Sigh EL, Betteridge KJ, Eaglleson MD, Randall GCB, Mitchell D, Bilton TJ, Trounson AO. Chromosome analysis of 159 bovine embryos colleted 12 to 18 days after Oestrus. Can. J. Genet. Cytol. 1980; 22: 615-262.

[37] Bhatia S Shanker V. Practical Applications of Sex Chromatin Studies in the Investigation of distributed Fertility in Female Cattle. Proceeding of the second World Congress on Genetics Applied to Livestock Production held on October 1982 at Madrid Spain, 1982; 483.