MALES' NON-ENHANCEMENT OF BRUCE AND WHITTEN EFFECTS IN FEMALE ALBINO MICE – *Mus musculus*

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ABSTRACT

This study investigated whether strange males in Bruce and Whitten effects also include males placed in the same cage with females. Forty albino mice (25 females and 15 males) were used for the study. The mice were divided into five groups (A - E) with five females each, and 5, 4, 3, 2 and 1 male(s) respectively. During both first and second rounds of matings, all the females conceived and littered successfully. In both rounds of matings, there was no significant difference ($p > 0.05$) in the mean number of matings before conception, mean gestation length and mean weight of new-born among all the female groups. However, the second round of matings recorded a higher mean number of matings before conception, greater mean weight of new-born and slightly longer gestation length than the first round. In both rounds, group E females had a significantly ($p < 0.05$) longer mean length of time before conception than group C females, but did not differ from groups A, B and D. Generally all the groups in second round exhibited longer period before conception than in the first round. In the first round of matings, both the mean litter size and group weights of B and E were significantly ($p<0.05$) lower than that of group D, whereas in the second round, there was no significant difference ($p>0.05$) in both mean litter size and weight among all the five groups. Results showed that male mice placed in the same cage with female mice were not seen as strange by the females.

Keywords: Bruce and Whitten effects, Albino mice, Strange, Pheromones

INTRODUCTION

The reproductive success of many animals depends on factors more subtle than food availability and housing condition. Social stimuli have therefore, been discovered to be important contributors to regulation of reproductive events in all mammals, including humans (Vandenbergh, 1994). Two examples of such numerous factors that influence reproductive processes in mice are the Bruce and Whitten effects. Stimuli from the social environment have been shown to disrupt pregnancy as well as influence both the frequency and composition of oestrous cycle in the mouse (*Mus musculus*) (Bronson, 1971; Aron, 1979). Male mouse urine contains a complex mixture of chemosignals that exert powerful effect on the reproductive biology of female mice. For instance, exposure to male urine had been reported to hasten puberty in the pre-pubertal females (Vandenbergh, 1969), induce oestrous in group anoestrous females (Whitten, 1956) and block pregnancy of newly mated females (Bruce, 1959). Whitten effect has to do with induction and synchronization of oestrus among unisexual grouped females in the presence of a male (Whitten, 1959; 1966; Whitten *et al*., 1968), while Bruce effect has to do with the male-induced failure of implantation and return to the oestrous cycle (Bruce, 1960).

Studies have shown that the male signals responsible for inducing both the female's ovarian cycles and pregnancy-block involved pheromonal regulations (Bronson, 1971; Gangrade and Dominic, 1984), and that these pheromones are secreted or excreted via the urine (Marchlewska-Koj, 1977). Like hormones, pheromones are endogenous chemical signals secreted by multicellular organisms. However, whereas hormones affect the behaviour and development of the individual that produces them, pheromones trigger an innate response in another member of the same species (Schneider, 1992; Vandenbergh, 1994). It is only the urine from sexually mature male mice that produces these pheromonal regulations, and these activities are lost following castration (Bruce, 1965). The concerned pheromones are androgen-dependent (Bruce, 1965; Gangrade and Dominic, 1984) as testosterone injection into the castrated males or normal females produces these activities (Hoppe, 1975). Gangrade and Dominic (1984) were able to establish that two different pheromones, non-volatile and volatile, produce Bruce and Whitten effects respectively.

Female mice are able to recognize the chemosignal of the male with which they are mated, thereby preventing the abortion of his own offspring. This individuality of the pregnancy-blocking chemosignal is known to be influenced by genes of the major histocompatibility complex (Brennan and Peelet, 2003). However, the chemical nature of the signal remains unclear. In addition to the main olfactory system found in most mammals, mice possess a vomeronasal system that is specialized in detection and transmission of pheromonal information (Dominic, 1966; Brennan *et al*., 1990; Li *et al*., 1990). It is worthy of note that the most fascinating aspect of the pregnancy blocking effect is that the mating male also produces the pregnancy-blocking chemosignal and yet exposure to his urine does not block his mate's pregnancy (Parkes and Bruce, 1961). This is primarily because the mated female is well able to recognize and decode the pheromonal information of the male that mated her. The question now arises as to whether the other male or males in the same cage with male and female that mated are strange to the mated female. Seeking for the answer to this question was the objective of this study.

MATERIALS AND METHODS

Forty mature but maiden albino mice comprised of 15 males and 25 females, procured from and maintained at the Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. They were 14-15 weeks of age at the time of commencement of the study. All through the duration of the study they were kept in standard clean mice cages and fed *ad libitum* with standard feed (Grand Cereals and Oil Mills Limited, Jos-Nigeria) containing 16% crude protein. They were also provided freely with clean drinking water.

Initially, all the 25 females were kept in a single cage and all the 15 males kept in another single cage, both for a simultaneous period of 5 weeks. Both cages were kept at a distance from one another. After this period, the 25 females were randomly divided into 5 groups (A-E) of 5 female mice each. The 15 males were also randomly divided into 5 groups, but with graded numbers of 5, 4, 3, 2 and 1. These 5 groups of 5, 4, 3, 2 and 1 male mice were then respectively placed with groups A-E female mice in their cages, thereby making group A to have 5 females and 5 males, group B 5 females and 4 males, group C 5 females and 3 males, group D 5 females and 2 males, and group E 5 females and 1 male. After distribution, each of the mice was identified with an indelible marker. Starting from this time of grouping, the males were with the females till the end of the study except for the brief period surrounding parturition.

The mice were then allowed the first round of matings that resulted in the first pregnancy for each of the 25 female mice. The vaginal plug method of Bennett and Vickery (1970) and Voss (1979), modified by Ochiogu *et al.* (2006) was used in determining successful mating in the females. The checking of the evidence of successful mating using the vaginal plug method was done daily for each female mouse until each was obviously pregnant. The following parameters were determined: number of matings before conception, length of time before conception, gestation length, litter size, litter weight and average weight of the newborn. As each female mouse conceived and had their first litter and while they were still nursing their litter, the evidence for the second round of matings that resulted in the second pregnancy for each of the 25 females (using vaginal plug method) was also determined on daily basis starting from the day 1 postpartum till each of the mice was obviously pregnant for the second time. All the parameters that were determined during the first round of matings were also determined during the second round of matings.

Data generated from the study were subjected to Analysis of Variance (ANOVA) and variant means separated using least significant difference (LSD) (Okafor, 1992).

RESULTS

Effect of Number of Males on Number of Matings before Conception: The mean number of matings did not show any significant difference ($p>0.05$) among all the groups in both rounds of matings (Tables 1 and 2). However, the second round of matings recorded a higher number of matings before conception than the first round of matings (Tables 1 and 2).

Effect of Number of Males on Length of Time before Conception: For both first and second rounds of matings, the females of group E had a significantly ($p<0.05$) mean longer time before conception than the females of group C (Tables 1 and 2). However, the mean length of time before conception of the females of the remaining three other groups (5, 4 and 2 male groups) did not significantly ($p>0.05$) vary from those of groups C and E (Tables 1 and 2).

Effect of Number of Males on Gestation Length: During both the first and second rounds of matings, there was no significant difference ($p > 0.05$) in the mean gestation length among all the five groups (Tables 1 and 2). However, the mean gestation lengths during the second round of matings were slightly longer than those of the first round (Tables 1 and 2).

Table 1: The reproductive indices of the mice groups during the first round of matings

Reproductive indices	Group A	Group B	Group C	Group D	Group E
Number of matings before conception	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.60 ± 0.60
Length of time before conception (days)	2.20 ± 0.37^{ab}	2.40 ± 0.51^{ab}	2.00 ± 0.45^{a}	2.20 ± 0.58^{ab}	5.00 ± 2.00^{b}
Gestation length (days)	19.20 ± 0.20	19.20 ± 0.37	20.00 ± 0.32	19.40 ± 0.24	19.60 ± 0.24
Litter size at birth	6.60 ± 0.75^{ab}	5.00 ± 1.05^{a}	6.20 ± 0.66^{ab}	7.20 ± 0.37^{b}	5.00 ± 0.63^{a}
Litter weight at birth (g)	10.48 ± 1.35^{ab}	7.33 ± 1.24^{a}	10.00 ± 1.43^{ab}	11.28 ± 0.51^{b}	7.58 ± 0.80^{a}
Average weight of new-born (g)	1.57 ± 0.04	1.51 ± 0.06	1.58 ± 0.07	1.57 ± 0.02	1.52 ± 0.04

^{ab} *Means bearing different superscripts indicate significant difference: $p < 0.05$.*

Table 2: The reproductive indices of the mice groups during the second round of matings

Reproductive indices	Group A	Group B	Group C	Group D	Group E
Number of matings before conception	4.40 ± 0.75	3.20 ± 0.66	4.80 ± 1.32	3.80 ± 0.37	3.40 ± 0.93
Length of time before conception (Days)	36.60 ± 10.83^{ab}	16.80 ± 3.85^{ab}	13.00 ± 2.30^{a}	32.20 ± 6.52^{ab}	39.20 ± 13.05^{b}
Gestation length (Days)	20.40 ± 0.24	20.60 ± 0.24	20.40 ± 0.24	20.40 ± 0.24	20.40 ± 0.24
Litter size at birth	7.40 ± 0.51	7.00 ± 0.63	6.00 ± 0.77	6.40 ± 0.51	6.80 ± 0.58
Litter weight at birth (g)	13.39 ± 0.81	13.21 ± 0.97	11.32 ± 1.52	11.65 ± 0.84	12.24 ± 0.82
Average weight of new-born (g)	1.81 ± 0.02	1.90 ± 0.05	1.88 ± 0.03	1.82 ± 0.02	1.81 ± 0.07

^{ab} *Means bearing different superscripts indicate significant difference: $p < 0.05$.*

Effect of Number of Males on Litter Size and Weight: During the first round of matings, the females of groups B and E had a significantly ($p < 0.05$) lower mean litter size and weight than the females of group D (Table 1). Groups A and C females did not vary significantly ($p>0.05$) from groups B, D and E. During the second round of matings, there was no significant difference ($p>0.05$) in both the mean litter size and weight among all the five groups (Table 2).

Effect of Number of Males on the Average Weight of New-Born: During both the first and second rounds of matings, there was no significant difference ($p>0.05$) among all the five groups in the mean average weight of new-born (Tables 1 and 2). However, the mean average weights of new-born during the second round of matings were greater than those of the first round (Tables 1 and 2).

DISCUSSION

The 25 females were initially caged and housed as a dense group and kept at a distance from the 15 caged males for 5 weeks so as to induce disruption of oestrous cycle (anoestrus), hence Whitten effect to ensure that oestrous synchronization occurs within few days of introducing the males (Whitten, 1959; 1966). This feat was achieved as all the female mice groups in the first round of matings had, from the time of exposure to males, a mean length of time before conception ranging from 2.00 ± 0.45 days to 5.00 ± 2.00 days, which falls within the time frame of restoration of regular oestrous cycle in formerly anoestrous mice (Whitten, 1959). Also the fact that almost all the female mice groups in the first round of matings conceived out of a single mating and within a short time frame of 2.00 ± 0.45 days to 5.00 ± 2.00 days confirms the report of Scharmann and Wolff (1980) that when oestrous is synchronized in mice 50 - 70% of the females can conceive on a single night. Although the mean length of time before conception in group E females (5.00 ± 2.00 days) was significantly ($p<0.05$) longer than that of group C females (2.00 ± 0.45 days), both periods still fall within the time frame for return to regular oestrous. However, this is suggestive that the presence of more than one male mouse per five females (as was the case in this study) can help hasten a return to regular oestrous. Since pregnancy was achieved in most female mice groups in the first round of matings through a single mating and within a short time frame, it is then presumable that the presence of other male mice in groups A, B, C and D was not able to induce Bruce effect in the mated female mice (Bruce, 1959). If Bruce effect were the case, it would have been evidenced by at least two matings per female mouse, one at each of two separate oestrous intervals and of course a longer length of time before visible pregnancy, if such would occur at all.

During the second round of matings, both the mean number of mating before conception and the mean length of time before conception increased far more than what was obtained in the first round of matings with a range of 3.20 ± 0.66 to 4.80 ± 1.32 for the former and 13.00 ± 2.30 days to 39.20 ± 13.05 days for the latter. The number of matings experienced by these female mice is evidence that they were undergoing oestrous cycle after the first delivery. As a matter of fact, postpartum oestrous – a phenomenon in which a female ovulates and becomes sexually receptive immediately after parturition – occurs in mice (Enders, 1970). These high mean numbers of matings and length of time before conception may therefore not be attributable to Bruce effect. Rather, they may be attributable to large litters from the first pregnancy which the females were suckling at the time of these matings, which according to Enders (1970), causes implantation and pregnancy failure in mice. Moreover, all the mice eventually had sustained pregnancy at the end of three-week weaning period when the young ones were withdrawn from their mothers.

The mean gestation length in both rounds of matings did not vary significantly among all the groups. However, it was for the reason of parity that the mean gestation lengths of the second round of matings were slightly longer than those of the first round. The mean litter size and weight, and mean average weight of new-born in both rounds of matings were not actually affected in any significant and definite manner.

The most interesting aspect of the pregnancy-block (Bruce effect) is that the mating male also produces the pregnancy-blocking chemosignal and yet exposure to this does not block his mate's pregnancy (Parkes and Bruce, 1961). It is then conceivable that since all the males were introduced into the cages of the corresponding females at the same time, the females learnt of the pregnancy-blocking chemosignals from all the males in their cage. With this, the females were able to recognize as familiar all the chemosignals from all their male associates and this made it impossible for the chemosignals from the non-mating male associates to block a pregnancy sequel to a particular male (Brennan *et al.*, 1990).

The litter size at birth did not vary from the litter size at weaning period of three weeks. It has been proposed that the strange males will kill unrelated offspring and the Bruce effect could be a means of preventing this infanticide (Brennan *et al.*, 1990; Brennan and Peelet, 2003). If these males really saw themselves as strange to the females, they would have killed the offspring of the females that did not result from their mating with them. But because they did not see themselves as strange and the females recognized

them and their chemosignals as familiar, they were neither able to induce Bruce effect nor kill these unrelated offspring. In conclusion, the results obtained from this study show that male mice kept in the same cage with female mice were not recognized as being strange to them.

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