J. Reprod. Fertil. (1965) **10,** 469–472

BRIEF COMMUNICATION

THE ORIGIN OF THE PHEROMONES CAUSING PREGNANCY BLOCK IN MICE

C. J. DOMINIC

Physiological Laboratory, Cambridge

(*Received 2nd August* 1965)

Summary. In contrast to normal and spayed CBA females, spayed and androgenized CBA females are as effective as normal CBA males in blocking ovo-implantation in newly mated P mice. Hence it is concluded that the pheromones involved in producing the block to ovo-implantation are associated with androgens either directly, or indirectly through some androgen-dependent gland.

Recent reports (Dominic, 1964, 1965) suggest that urine is the immediate source of the pheromones produced by male mice which cause the olfactory block to pregnancy in newly mated females (Bruce, 1960; Parkes & Bruce, 1961). Urine of grouped prepuberally or post-puberally castrated male mice retains the pregnancy blocking capacity to some extent (Dominic, 1965) but in each individual this capacity may be too far diminished by castration to be effective when the female is exposed to a single male (Bruce, 1965).

The present report deals with experiments conducted to determine the capacity of normal, spayed or spayed and androgenized alien (CBA) females to block ovo-implantation in newly-mated albino mice belonging to the Parkes (P) strain. The spayed CBA female was androgenized by subcutaneous implantation of a pellet of testosterone (4-androsten-17-ol-3-one propionate, supplied by Steraloids, Ltd, Croydon) weighing about 50 mg. Tests were started about 2 weeks after implantation. The P female was separated from the stud P male after mating (i.e. vaginal plug found) and exposed on Days 1 to 3 *post coitum* to one of the following situations: (1) housed in the proximity of a normal CBA female, the latter confined in an expanded metal corral in the box housing the mated P female; (2) similarly housed in the proximity of a confined spayed CBA female; (3) housed in the proximity of a confined spayed and androgenized CBA female; (4) exposed to fresh urine from twelve spayed and androgenized CBA females; (5) exposed to the same situation as in (4) except that urine from androgenized CBA females was prevented from reaching the newly mated P females (urine controls); (6) housed in boxes with empty corrals; and (7) left undisturbed. Daily vaginal smears were examined from all test females up to Day 7 *post coitum* and a return of vaginal cornification within this period was taken to indicate a blocked pregnancy. The results are summarized in Table 1.

TABLE 1

Treatment days 1 to 3 post coitum	No. of females	No. and % of females returning to oestrus	No. of females returning to oestrus, days after stud coitus					No. and % of females pseudopregnant	No. and % of females pregnant
			3	4	5	6	7		
1. Proximity of CBA female	60	14 (23%)	3	6	3	2	–	3 (5%)	43 (72%)
2. Proximity of spayed CBA female	50	16 (32%)	1	6	9	–	–	1 (2%)	33 (66%)
3. Proximity of spayed and androgenized CBA female	57	49 (86%)	25	23	1	–	–	3 (5%)	5 (9%)
4. Urine of spayed and androgenized CBA females	54	44 (81%)	26	17	1	–	–	–	10 (19%)
5. Urine control	54	8 (15%)	–	5	3	–	–	8 (15%)	38 (70%)
6. Empty corral	146	19 (13%)	–	3	8	3	5	13 (9%)	114 (78%)
7. Undisturbed	80	8 (10%)	–	1	3	3	1	4 (5%)	68 (85%)

By contrast with the proximity of normal or spayed CBA females, more than 80% of pregnancies were blocked by the proximity of spayed androgenized females or exposure to their urine. In fact, androgenized CBA females are as effective as normal CBA males in blocking the pregnancy of newly mated P females.

The olfactory block to pregnancy exhibits characteristic time relationships (Bruce, 1961, 1963), and it is interesting to note that the acceleration of oestrus normally associated with the presence of a male (Whitten, 1958) is even more pronounced on exposure to androgenized females or their urine. (Table 2). Among P females exposed to CBA males or their urine about 80% of the females in which pregnancy was blocked returned to oestrus within 4 days *post coitum*, with a peak on Day 4. But among P females exposed to androgenized CBA females or their urine, 98% (91/93) of the females in which pregnancy

TABLE 2

COMPARISON OF THE PREGNANCY BLOCKING CAPACITY OF CBA MALES WITH THAT OF SPAYED AND ANDROGENIZED CBA FEMALES

Treatment days 1 *to* 3 post coitum	*Proportion and % of blocked pregnancy*	*Proportion of females returning to oestrus on Days* 3 *and* 4 *after stud coitus*	
		Day 3	*Day* 4
1. Proximity of CBA male	60/72 (83%)	13/60	32/60
2. Urine of CBA	43/51 (84%)	3/43	27/43
3. Proximity of spayed and androgenized CBA female	49/57 (86%)	25/49	23/49
4. Urine of spayed and androgenized CBA females	44/54 (81%)	26/44	17/44

was blocked returned to oestrus by Day 4, with a peak on Day 3. This implies that androgenized CBA females are more potent than normal CBA males in blocking the pregnancy of P females. It seems likely, therefore, that the pheromones involved in producing pregnancy block in newly-mated mice are associated with androgens directly (i.e. urinary excretion products of androgens) or indirectly through some androgen dependent gland.

My thanks are due to Professor A. S. Parkes and Miss H. M. Bruce for advice and encouragement and to Mrs Theo Moore and Mrs Kathleen McNicol for the care of animals and technical assistance. This investigation was supported by a Fellowship from the Population Council and a Gulbenkian Studentship from Churchill College. Working expenses were defrayed from a grant to Professor Parkes by the Ford Foundation.

REFERENCES

BRUCE, H. M. (1960) A block to pregnancy in the mouse caused by the proximity of strange males. *J. Reprod. Fertil.* **1**, 96.

BRUCE, H. M. (1961) Time relations in the pregnancy block induced in mice by strange males. *J. Reprod. Fertil.* **2**, 138.

Bruce, H. M. (1963) A comparison of olfactory stimulation and nutritional stress as pregnancy-blocking agents in mice. *J. Reprod. Fertil.* **6,** 221.

Bruce, H. M. (1965) Effect of castration on the reproductive pheromone of male mice. *J. Reprod. Fertil.* **10,** 141.

Dominic, C. J. (1964) Source of the male odour causing pregnancy block in mice. *J. Reprod. Fertil.* **8,** 266.

Dominic, C. J. (1965) *Olfactory effects on reproduction in the laboratory mouse.* Ph.D. thesis, University of Cambridge.

Parkes, A. S. & Bruce, H. M. (1961) Olfactory stimuli in mammalian reproduction. *Science, N.Y.* **134,** 1049.

Whitten, W. K. (1958) Modification of the oestrous cycle of the mouse by external stimuli associated with the male. Changes in the oestrous cycle determined by vaginal smears. *J. Endocr.* **17,** 307.