Notes on the breeding of the WHHL rabbit: an animal model of familial hypercholesterolemia

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Summary We describe in this study a reliable method for the breeding of the Watanabe heritable hyperlipidemic (WHHL) rabbit. Placing a male and a female WHHL rabbit in the same cage for the purpose of mating resulted in only two pregnancies out of a total of 227 mating attempts (0.9%). After manually assisting the rabbits, 15% of the matings resulted in pregnancies. When the female rabbits were injected with 40 I.U. of human chorionic gonadotropin within 1 hr of this procedure, 56% of the matings resulting in pregnancies. We feel that the inherent difficulty in breeding the WHHL rabbit, a model for the disease familial hypercholesterolemia, can be significantly overcome by the methods discussed in this report. —**Phelan, J. P., B. J. Van Lenten, A. M. Fogelman, C. Kean, M. E. Haberland, and P. A. Edwards.** Notes on the breeding of the WHHL rabbit: an animal model of familial hypercholesterolemia. *J. Lipid Res.* 1985. **26:** 776–778.

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The disorder, familial hypercholesterolemia (FH), is one of the most common genetic diseases among humans. The massive elevation in plasma low density lipoprotein (LDL) associated with the homozygous form of FH results from allelic mutations in the gene for the membrane receptor for LDL (1). Consequently, there is an impaired rate of LDL catabolism (2) causing the lipoprotein to accumulate in the plasma. Eventually the LDL deposits its cholesterol in the arterial wall causing atherosclerosis.

Watanabe and his co-workers (3, 4) have described an inbred strain of rabbit that exhibits hypercholesterolemia, elevated levels of LDL, skin and tendon xanthomatosis, and accelerated atherosclerosis. This strain has been designated Watanabe heritable hyperlipidemic (WHHL). Similar to their human counterparts, these rabbits demonstrate a single-gene mutation for the LDL receptor (5) and have been proposed as models for FH.

Since the WHHL rabbit is spontaneously hyperlipidemic, its dietary intake of fat is restricted. Possibly, as a result of this, the rabbits tend to be physically smaller than age-matched normal New Zealand white rabbits. The reduced growth and compromised nutritional status of WHHL rabbits may contribute to their generally less productive breeding habits compared with normal rabbits. In addition to our own laboratory there are a number of groups presently using the WHHL rabbit to study various aspects of atherosclerosis (3–7) and its use will doubtless increase. We report here our findings on the breeding characteristics of WHHL rabbits as well as simple routine methods we have employed to increase our colony.

MATERIALS AND METHODS

Management of colony

The WHHL rabbits used in this study were raised at UCLA from five rabbits (two males and three females) sent to us from Dr. Yoshio Watanabe at the Experimental Animal Laboratory of Kobe University.

All animals were fed 100 g/day of Purina Rabbit Chow (Ralston Purina Co., St. Louis, MO). The fat content of this diet is approximately 2.5% by weight. Water was supplied ad libitum. The temperature of the colony was maintained at 22 ± 2°C with a 12 hr light/dark cycle in effect. WHHL rabbits were housed in stainless steel cages in a clean quiet room separate from other animals.

Mating

Four groups of rabbits were compared. For the first group (Group A) the method was simply to put a male and female together in the same cage for at least 30 min, unassisted in any way. However, mating conducted in this way is often hindered by the non-receptivity of the female. Therefore, to ensure that copulation occurred in Groups B–D, we used a technique of manual restraint described by Hafez (8). The female was first taken to the male's cage. She was restrained by holding her ears and the loose skin over her shoulders in one hand and placing the other hand under her body and between her hind legs. The thumb was then placed on the right side of the vulva, the index finger of the same hand was placed on the left side of the vulva, and the skin was gently pushed upward until the tail was thrown up over the back. The body was then supported caudally by the hand to obtain the normal copulatory posture. After mounting, copulation occurs and is considered completed when the male's ears droop, he squeals, and abruptly falls on his side. Although two copulations are adequate for conception, three or four copulations are recommended (8). This is most easily accomplished by successively transferring the female to three or four separate male's cages.

Since Harper (9) has shown that the rabbit does not ovulate spontaneously, we induced ovulation in Group C by giving one injection of 40 I.U. of human chorionic gonadotropin (HCG) (Sigma Chemical Co., St. Louis, MO) in 0.5–0.7 ml of 0.85% sterile phosphate-buffered

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein; WHHL, Watanabe heritable hyperlipidemic; HCG, human chorionic gonadotropin.

TABLE 1. The effectiveness of various regimens in causing pregnancy in WHHL rabbits

Group	Treatment	Manipulation[a]	Number of Matings	Number of Pregnancies	Mean Litter Size	Efficiency[b]
A	None	–	227	2	5 ± 1	0.9%
B	None	+	41	6	5 ± 2	14.6%
C	HCG[c]	+	25	14	6 ± 2	56.0%
D	Saline[d]	+	5	0		0

[a] Animals were manually assisted in mating as described in Materials and Methods.
[b] Number of pregnancies/number of matings × 100.
[c] Each animal received an I.V. injection of 0.5–0.7 ml of sterile saline containing 40 I.U. human chorionic gonadotropin (HCG) within 1 hr of mating.
[d] Each animal received an I.V. injection of 0.5–0.7 ml of sterile saline within 1 hr of mating.

saline via an ear vein. The injection was made within 1 hr of mating. As a control for Group C, the rabbits in Group D received the same volume of saline without HCG.

Gestation and postnatal care

The period of gestation is 32 days. We have found that after 12–14 days of pregnancy, palpating the developing fetuses in utero is an accurate and quick method for documentation of pregnancy. The ears and loose skin over the shoulders are held in one hand, and the other hand is placed between the hind legs and slightly in front of the pelvis. The thumb is then placed on the one side of the abdomen and the fingers on the other side of the abdomen, drawing the skin inward towards the midline in order to brace the uterine horns for palpation. The marble-shaped fetuses can then be easily felt as they slip between the thumb and fingers when the latter are gently moved back and forth with slight pressure.

There is a tendency among WHHL rabbits to "scatter" the young outside the nest. Therefore, effort should be made to check periodically that all isolated bunnies are immediately returned to the nest. A female will occasionally divide the litter into two groups. If this occurs, the litter should be arranged into one group.

Another problem that can occur, particularly with first-time mothers, is the eating of the newborn bunnies. We routinely sedate the new mother by giving her 1 ml daily of 10 mg/ml Acepromazine Maleate (Ayerst Laboratories, Inc., New York, NY) for a week. No adverse effects on the nursing bunnies have been observed with the use of Acepromazine.

Females can be mated again 5–7 weeks after giving birth. The young are weaned when they are 8 weeks old and may be mated at 5 months of age.

RESULTS AND DISCUSSION

Table 1 shows the results of the various regimens used to cause pregnancy in the WHHL rabbit. When the colony was first established, the procedure was to put a male and female together in a cage for 30 min to 1 hr. (Group A). This procedure resulted in only two pregnancies out of a total of 227 mating attempts. By manually assisting the rabbits in mating, the efficiency increased so that almost 15% of the matings resulted in pregnancies (Group B). The most successful regimen required administering HCG I.V. to the rabbits and then mating them as above (Group C). The total amount of time required for the complete procedure of injection and matings was 10 min and resulted in an efficiency of 56%. In five cases where saline alone was injected before mating no pregnancies resulted. The mean litter sizes for each of the different treatment groups did not differ statistically.

Some have bred the FH gene into New Zealand white rabbits (10) in order to overcome the difficulty of breeding the original Japanese WHHL rabbits. This method requires larger numbers of animals and the differentiation of homozygotes from heterozygotes. Our results indicate that the use of HCG together with the manipulation method of Hafez (8) is an effective and simple method for breeding and developing a colony of homozygous WHHL rabbits.

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