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The effect of *H-2* region and genetic background on hormone-induced ovulation rate, puberty, and follicular number in mice

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Summary

We have examined the effects of major histocompatibility (*H-2*) haplotypes and genetic background (all loci other than the *H-2* region) on hormone-induced ovulation rate in congenic strains of mice. In comparison with the $H\text{-}2^a$ haplotype, the $H\text{-}2^b$ haplotype increased hormone-induced ovulation rate 92% on the A/J (A) genetic background. However, *H-2* haplotype did not affect hormone-induced ovulation rate on the C57BL/10J (C57) genetic background. The $H\text{-}2^b$-linked gene(s) increased hormone-induced ovulation rate on the A/J genetic background largely by (1) enhancing the maturation of follicles in response to pregnant mare's serum gonadotrophin (PMSG) and (2) altering the stages of follicular development which can be induced to ovulate in response to human chorionic gonadotrophin (hCG). The observed effects of *H-2* on hormone-induced ovulation rate were not explained by differences in the timing of puberty, the number of follicles present in untreated females, or the incidence of follicular atresia. The effect of genetic background on hormone-induced ovulation rate was much greater than was the effect of the *H-2* region. We found that hormone-induced ovulation rate was five- to six-fold higher on the C57 genetic background than on the A genetic background. The C57 genetic background increased hormone-induced ovulation rate by (1) enhancing the induction of follicular maturation in response to gonadotropins and (2) by reducing the incidence of follicular atresia.

1. Introduction

Previous studies in our laboratory have shown that six-fold differences between the hormone-induced ovulation rates of mouse strains A/J and C57BL/6J ($8{\cdot}8 \pm 0{\cdot}9$ and $53{\cdot}5 \pm 2{\cdot}2$ ova, respectively, segregate as though they were controlled by the action of approximately three to four loci (Spearow, 1988*a*). These strains of mice differed both in *H-2* haplotype and in *H-2* -independent genes (genetic background). The major histocompatibility region in the mouse (*H-2*) has been reported to affect a number of hormonal responses, including testosterone-stimulated vesicular gland weight (Ivanyi *et al.* 1972; Gregorova *et al.* 1977) and glucagon-stimulated cyclic AMP (cAMP) production (Lafuse & Edidin, 1980). *H-2* congenic strains were not available on the C57BL/6J genetic background; therefore, in the present study we examined the effect of *H-2* haplotype and genetic background on hormone-induced ovulation rate in *H-2* congenic strains of mice on the A/J (A) and C57BL/10J genetic backgrounds.

We also compared *H-2* congenics and genetic backgrounds to determine which physiological changes might explain the observed effect on *H-2* on hormone-induced ovulation rate. Ovulation rate increases for several cycles following puberty in swine (Anderson, 1980) and during the first few cycles of the breeding season of sheep. Thus, we tested whether the effects of *H-2* and genetic background on induced ovulation rate were due to high responding strains reaching puberty earlier. We also examined ovarian follicle populations in these strains of mice before and after PMSG treatment to determine whether the effects of *H-2* haplotype and genetic background on hormone-induced ovulation rate were due to differences in (1) the number of normal follicles prior to PMSG treatment; (2) the induction of follicle maturation by PMSG; (3) the incidence of follicular atresia; and/or (4) the induction of ovulation [i.e. the stages of follicular development which can respond to human chorionic gonadotrophin (hCG) and ovulate].

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2. Materials and methods

(i) *Strains and husbandry of mice*

Inbred strains and *H-2* congenic strains of mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Strains examined on the A/J genetic background included A/J ($H\text{-}2^a$), A.WY/SnJ ($H\text{-}2^a$), A.SW/SnJ ($H\text{-}2^s$), and A.BY/SnJ ($H\text{-}2^b$). Strains examined on the C57BL/10J background included C57BL/10J ($H\text{-}2^b$), B10.A/SgSnJ ($H\text{-}2^a$), and B10.BR/SgSnJ ($H\text{-}2^k$). Strain C57BL/6J ($H\text{-}2^b$) was compared to A.BY/SnJ ($H\text{-}2^b$) to determine the relative effects of *H-2* linked versus *H-2* independent loci. Mice were bred, housed and managed according to the procedures of Spearow (1988*a*), with lights on at 07.00 h and off at 21.00 h. After weaning at 3 weeks of age, females were housed in cages separate from males. Within each experiment, strains of mice were compared in overlapping contemporary groups as they were produced by mating cages.

(ii) *Hormones*

Pregnant mare serum gonadotrophin was from the National Hormone and Pituitary Program, NIADDK. The hCG used for injection in hormone-induced ovulation rate experiments was from Hypo Lab, Cousins, Switzerland and had an activity of 2700 IU/mg. Lyophilized aliquots of the same lot of hormones were used in each experiment. Purified hCG CR-123, used for iodination and injection in experiments characterizing follicle populations, was from the Center for Population Research, NICHD, and had a potency of 12780 IU/mg.

(iii) *Animal models*

Hormone-induced ovulation rate was measured after treatment with PMSG at 28 days and with hCG at 30 days of age (Fig. 1). Since this treatment protocol demonstrated genetic variation in induced ovulation rate, we then examined the development of strain differences in follicular characteristics both before the injection of PMSG, and in response to PMSG. As also shown in Fig. 1, follicular characteristics were examined in untreated female mice at 28 days of age, and in PMSG-treated females at the time of administration of hCG at 30 days of age.

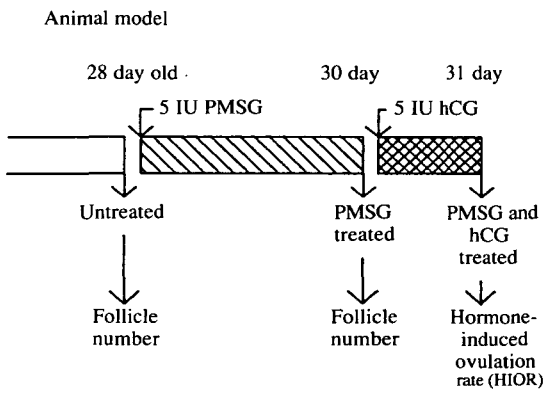

Fig. 1. Mouse model used in the present studies.

(iv) *Hormone-induced ovulation rate*

Female mice were injected subcutaneously with 5 IU PMSG in 50 μl 0·02 M-$NaPO_4$, 0·14 M-NaCl and 0·1% gelatin, pH 7·2 (PBS gel) at 28 days of age. On day 30 (48–52 h later), they were injected s.c. with 5 IU crude human chorionic gonadotrophin (hCG) in 50 μl PBS-gel. On day 31 (16–22 h later), mice were weighed, and then killed by cervical dislocation. The oviducts were examined with a dissecting microscope to determine the number of freshly ovulated ova in cumulus (ova) and the number of ova out of cumulus (old ova).

(v) *Follicular characteristics*

Untreated 28-day-old females were killed by cervical dislocation. Their ovaries were removed, fixed overnight in Bouin's fixative at 4 °C, rinsed in 70% EtOH and embedded in paraffin. Serial sections were cut at 6 μm, and stained with haematoxylin and eosin (H&E).

Twenty-eight-day-old females were injected s.c. with 5 IU PMSG in 50 μl of PBS gel. The mice were injected via the tail vein 48–52 h later with 20 ng [^{125}I]hCG per g body weight in PBS gel. Purified hCG was labelled with ^{125}I, to a low specific activity (Rao *et al.* 1977) for other purposes. Mice treated with PMSG and hCG were killed by decapitation 1 h after the hCG injection. Ovaries were immediately removed, fixed overnight in Bouin's fixative, and rinsed in 70% EtOH. Ovaries were then embedded in paraffin, and serially sectioned at 6 μm. Every tenth section was stained with H&E.

In both experiments, every tenth section was examined with the aid of a Nikon binocular microscope at 300×. Follicles were scored as normal (no pyknotic nuclei in cross section), in early atresia (1–5 pyknotic granulosa cells per cross section) and in mid-atresia (greater than 5 pyknotic) granulosa cells per largest cross section, but without the degeneration of the granulosa layer and of the oocyte characteristic of advanced atresia) (Lobel *et al.* 1961; Pedersen, 1970). Follicles greater than 70 μm in diameter were categorized into follicle types (FT) (Pedersen & Peters, 1968), according to the procedures of Spearow (1986) (Table 1). The number of follicles in each FT was estimated by (1) determining the number of follicles whose oocyte nucleus was in every tenth section and (2) correcting for section thickness, diameter of oocyte nucleus and proportion of sections examined (Abercrombie, 1946). While the maximum diameter of the oocyte nucleus averaged 20 μm, a comparison of adjacent sections revealed that sections containing

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Table 1. *General relationship between follicle type, follicular diameter, and number of granulosa cells in largest cross-section of a follicle*

Follicle type	Diameter (μm)	Number of cells in cross-section	General follicular characteristics
2	< 27	< 10	Primordial
3a	27–39	10–20	Smallest growing
3b	40–69	21–60	Preantral
4	70–96	61–99	Preantral
5a	97–112	100–199	Preantral – tiny antrum
5b	113–177	200–399	Small antrum
6	178–269	400–599	Medium antrum
7	> 269	> 600	Large antrum
8	> 269	> 600	Graafian Only seen after LH surge or hCG injection

2 μm or less of either edge of a oocyte nucleus could not be clearly distinguished as such. Thus, the diameter of the oocyte nucleus that was detectable was 16 μm. Rather than erring by scoring oocytes whose nucleus was not actually in the cross-section, we chose a more conservative approach of only scoring oocytes whose nucleus was clearly recognizable in the cross-section examined. Since every tenth section was examined, the

$$\text{correction factor} = \frac{10 \times 6\ \mu\text{m section thickness}}{\left(\begin{array}{l}16\ \mu\text{m nuclear diameter} \\ +6\ \mu\text{m section thickness}\end{array}\right)} = 2{\cdot}727.$$

(vi) *Statistical analysis*

Hormone-induced ovulation rate data were analysed with one-way analysis of variance and Scheffe's multiple range test. Hormone-induced ovulation rate data were also corrected for the effect of body weight within background genotypes with one-way analysis of covariance (Statistics Analysis System, SAS Institute Inc. Cary, NC). Puberty data and follicular number data were analysed using one-way analyses of variance and, where appropriate, with Tukey's multiple range test. Comparisons of hormone-induced ovulation rate with number of follicles in each FT were conducted with *t* tests using separate variances.

3. Results

(i) *The effects of* H-2 *haplotype and genetic background on hormone-induced ovulation rate*

As shown in Table 2, *H-2* haplotype had a significant effect upon hormone-induced ovulation rate in the A/J genetic background ($P < 0{\cdot}001$). The $H\text{-}2^b$ haplotype in strain A.BY increased induced ovulation rate by 51% over that of the $H\text{-}2^a$ haplotype in strain A.WY ($P < 0{\cdot}001$), 92% over that of the $H\text{-}2^a$ haplotype in strain A ($P < 0{\cdot}01$), and 55% over that of the $H\text{-}2^s$ haplotype in A.SW ($P < 0{\cdot}001$). In contrast, *H-2* haplotype did not significantly affect hormone-induced ovulation rate on the C57BL/10J background.

The largest genetic differences in hormone-induced ovulation rate were independent of *H-2*. Within $H\text{-}2^a$ haplotypes, the C57BL/10J genetic background increased hormone-induced ovulation rate about four-fold over that of the A genetic background ($P < 0{\cdot}001$). Within $H\text{-}2^b$ haplotypes, the C57BL/10J background increased hormone-induced ovulation rate 2·5-fold over that of the A genetic background ($P < 0{\cdot}001$).

(ii) *The effects of* H-2 *haplotype and genetic background on number of old ova*

Significant differences in the number of old ova were found among *H-2* congenic strains on the A/J genetic background by analysis of variance ($P < 0{\cdot}05$, Table 2). However, multiple range tests did not reveal any particular strain with a significantly greater number of old ova than those found in other strains on the A/J background. No significant differences in number of old ova were found among congenic strains on the C57BL/10 genetic background. Nevertheless, all mice on the C57BL/10 genetic background ovulated more eggs (e.g. old ova) in response to the initial dose of PMSG than did mice on the strain A background ($P < 0{\cdot}001$).

(iii) *The effects of* H-2 *haplotype and genetic background of body weight*

As shown in Table 2, on the A/J genetic background, congenic strains did not differ in body weight and the effect of body weight on hormone-induced ovulation rate was not significant. However, body weight did differ among *H-2* congenic strains on the C57BL/10J genetic background ($P < 0{\cdot}01$). Correction for the effect of body weight using the pooled among-strain regression of hormone-induced ovulation rate on body weight for congenics on the C57BL/10J background ($b = -2{\cdot}61 \pm 0{\cdot}66$ ova/g body weight) did not

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Table 2. *Number of fresh ova in cumulus (hormone-induced ovulation rate) and old ova[a] ovulated by* H-2 *congenic strains of mice in response to 5 IU PMSG and 5 IU hCG (mean* ± *S.E.M. for number (N) of individuals shown)*

Strain of mice	*H-2* haplotype	*N*	No. of fresh ova	No. of old ova	Body weight (g)
A/J background					
A/J[b]	*a*	20	8·8 ± 0·9 a[b]	1·4 ± 0·5 a	14·9 ± 0·5 a b
A.WY/SnJ	*a*	21	11·2 ± 1·2 a	0·9 ± 0·4 a	14·6 ± 0·4 a b
A.BY/SnJ	*b*	20	16·9 ± 1·6 b	0·2 ± 0·1 a	14·1 ± 0·3 a
A.SW/SnJ	*s*	23	10·9 ± 1·2 a	0·8 ± 0·4 a	13·5 ± 0·4 a
C57BL/10J background					
B10.A/SnJ	*a*	23	41·8 ± 1·6 c	6·2 ± 0·9 b	15·7 ± 0·2 b
C57BL/10J	*b*	31	43·3 ± 2·1 c	5·8 ± 0·9 b	17·0 ± 0·3 c
B10.BR/SnJ	*k*	27	45·6 ± 2·8 c	6·9 ± 1·1 b	15·3 ± 0·4 b
C57BL/6J background					
C57BL/6J[b]	*b*	44	53·5 ± 2·2 d	7·8 ± 0·7 b	15·3 ± 0·4 b

a, b, c, d: Data within columns differing by a single letter differ significantly according to Scheffe multiple range test ($P < 0·05$).
[a] 'Old ova' are eggs found out of cumulus 16–22 h after hCG treatment, and 66–70 h after PMSG treatment. Previous studies (Spearow, 1988*a*) indicate that these 'old ova' ovulated in response to the initial dose of PMSG, while the ova in cumulus at this time were matured by the PMSG and ovulated by the hCG.
[b] Data on these strains originally reported in Spearow (1988*a*).

Table 3. *The effect of* H-2 *and genetic background on the timing of puberty in the presence of a male. Age and body weight of vaginal opening and first vaginal plug (mean* ± *S.E.M. for number of animals shown)*

Strain	No. of mice	Vaginal opening		First vaginal plug	
		Age (days)	Body wt (g)	Age (days)	Body wt (g)
A/J[a]	35	24·7 ± 0·8 a	12·5 ± 0·3 a	27·7 ± 1·0 a	14·1 ± 0·4 a
A.BY/SnJ	40	23·7 ± 0·4 a	13·2 ± 0·2 a b	27·0 ± 0·6 a	14·9 ± 0·4 a b
C57BL/6J[a]	32	27·4 ± 0·6 b	14·0 ± 0·4 b	32·4 ± 1·3 b	15·7 ± 0·6 b

a, b: Data within columns with different letters differ significantly according to Tukey multiple range test ($P < 0·05$).
[a] Data on these strains originally reported in Spearow (1988*b*).

significantly alter the performance of *H-2* congenic strains. In other words, the differences in body weight did not significantly increase the number of fresh ova.

(iv) *The effects of* H-2 *and background genotype on the timing of puberty*

The lack of differences between strains A/J and A.BY/SnJ shown in Table 3 indicates that *H-2* haplotype does not affect the age or body weight at vaginal opening or at first vaginal plug. A comparison of A.BY/SnJ and C57BL/6J reveals that loci independent of *H-2* significantly affect the age but not the weight at vaginal opening and puberty. These results show that the effect of *H-2* on hormone-induced ovulation rate cannot be explained by strain differences in the age at puberty.

(v) *Follicular populations*

We then examined the number of normal and atretic follicles immediately before and again 2 days after PMSG treatment, to determine whether genetic differences in hormone-induced ovulation rate were explainable by strain differences in the characteristics of the follicular population. The number of normal healthy follicles in each follicle type at 28 days of age in untreated females is shown in Table 4. *H-2* haplotype had little effect on the number of normal follicles in untreated females at 28 days of age. Strains

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Table 4. *Number of normal follicles in 28-day-old untreated A/J, A.BY/SnJ and C57BL/6J mice (mean ± S.E.M. per mouse for 6–7 females per strain)*

	Follicle type (size) classification						
Strain	4	5a	5b	6	7	Large (5b–7)	Total (4–7)
A/J	65 ± 6	22 ± 5	45 ± 6	10 ± 3	4 ± 2	59 ± 7	145 ± 13
A.BY/SnJ	54 ± 9	8 ± 3	30 ± 5	12 ± 3	5 ± 1	46 ± 6	109 ± 11
C57BL/6J	125 ± 11	35 ± 4	38 ± 7	24 ± 5	4 ± 1	65 ± 12	226 ± 16
Sign.[a]	N.S.	$P < 0.05$	N.S.	N.S.	N.S.	N.S.	N.S.
Sign.[b]	$P < 0.05$	$P < 0.05$	N.S.	N.S.	N.S.	N.S.	$P < 0.05$

[a] Significance of difference between strains A/J and A.BY.
[b] Significance of difference between strains A.BY and C57BL/6J.

Table 5. *Number of atretic follicles in 28-day-old untreated A/J, A.BY/SnJ and C57BL/6J mice (mean ± S.E.M. per mouse for 6–7 females per strain)*

	Large follicles				All follicles	
Strain	Early atresia	Mid-atresia	Total atretic	Percent atretic	Total atretic	Percent atretic
A/J	27 ± 2	45 ± 6	73 ± 7	55 ± 4	84 ± 8	37 ± 3
A.BY/SnJ	25 ± 4	44 ± 8	75 ± 5	62 ± 4	86 ± 7	45 ± 3
C57BL/6J	52 ± 6	85 ± 7	136 ± 9	68 ± 5	175 ± 16	43 ± 4
Sign.[a]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sign.[b]	$P < 0.05$	$P < 0.05$	$P < 0.05$	N.S.	$P < 0.05$	N.S.

[a] Significance of differences between strains A/J and A.BY.
[b] Significance of differences between strains A.BY and C57BL/6J.

A/J and A.BY/SnJ differed significantly only in FT 5a for number of normal follicles per mouse, with A.BY having fewer than A/J. No significant differences were found between A/J and A.BY/SnJ in the incidence of atresia either before or after PMSG treatment.

A comparison of strains A.BY and C57BL/6J revealed that the C57 genetic background contains more normal healthy type 4, 5a follicles and total follicles (FT 4–7) ($P < 0.05$) than does the A genetic background. However, genetic background does not significantly affect the number of healthy normal type 6 and 7 follicles or large (FT 5b–7) follicles

The number of atretic follicles in untreated 28-day-old females does not differ significantly between strains A/J and A.BY for any follicle type (Table 5). Thus, *H-2* haplotype has no significant effect on the incidence of atresia in untreated females. In contrast, a comparison of strains A.BY and C57BL/6J revealed that the C57 genetic background has more large atretic follicles ($P < 0.05$), and total number of atretic follicles ($P < 0.05$), but does not differ in the percent of large or total follicles undergoing atresia in untreated females.

No significant differences were found 2 days after PMSG treatment between *H-2* congenic strains on the A/J genetic background for the number of follicles in any FT (Table 6). A.BY mice showed a non-significant decrease in the number of type 6 follicles and a non-significant increase in the number of type 7 follicles. In contrast, a comparison of Strains A.BY and C57BL/6J revealed highly significant effects of background genotypes on the number of type 5a, 5b, 6 and 7 follicles, total number of large normal follicles and the total number of normal follicles ($P < 0.01$). Thus, genetic background has a much larger effect on the number of normal follicles after gonadotrophin treatment than does *H-2* haplotype.

A comparison of the number of normal follicles at 28 days of age in Table 4, and the number of large normal follicles at 30 days of age in Table 6 shows genetic differences in the induction of follicular maturation. While *H-2* showed a marginally significant effect on the number of follicles matured to FT 7 by PMSG, it did not affect the number of large or the total number of follicles matured by PMSG. In contrast, genetic background has a dramatic effect on the induction of follicular maturation. Within the first

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Table 6. *Number of normal follicles in both ovaries of 30-day-old A/J A.BY/SnJ and C57BL/6J strain mice 2 days after treatment with 5 IU PMSG (mean ± S.E.M. for 8 mice per strain)*

	Follicle type (size) classification						
Strain	4	5a	5b	6	7	Large (5b–7)	Total (4–7)
A/J	11 ± 3	47 ± 5	11 ± 3	2 ± 1	6 ± 2	19 ± 4	77 ± 7
A.BY/SnJ	7 ± 2	47 ± 4	8 ± 2	0·4 ± 0·4	11 ± 3	19 ± 3	74 ± 5
C57BL/6J	12 ± 2	167 ± 11	50 ± 4	13 ± 3	55 ± 7	118 ± 8	298 ± 9
Sign.[a]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sign.[b]	N.S.	$P < 0·1$	$P < 0·01$	$P < 0·01$	$P < 0·01$	$P < 0·01$	$P < 0·01$

[a] Significance of differences between strains A/J and A.BY/SnJ.
[b] Significance of differences between strains A.BY and C57BL/6J.

Table 7. *Number of atretic follicles in 30-day-old A/J, A.BY/SnJ and C57BL/6J strain ovaries 2 days after treatment with 5 IU PMSG (mean ± S.E.M. for 8 mice per strain)*

	Early atresia		Large follicles						All FT	
Strain	FT 6	FT 7	Early atresia	(%)	Medium atresia	(%)	Total atretic	(%)	total atretic	(%)
A/J	2 ± 1	3 ± 1	14 ± 3	(18)	42 ± 6	(55)	55 ± 7	(73)	70 ± 10	(47)
A.BY/SnJ	1 ± 1	4 ± 2	16 ± 3	(22)	38 ± 4	(52)	55 ± 5	(74)	74 ± 5	(49)
C57BL/6J	15 ± 1	8 ± 3	40 ± 4	(18)	67 ± 10	(29)	107 ± 13	(47)	121 ± 15	(28)
$P <$[a]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
$P <$[b]	< 0·01	N.S.	< 0·01	N.S.	< 0·05	< 0·01	< 0·01	< 0·01	< 0·05	< 0·01

[a] Significance of differences between strains A/J and A.BY/SnJ.
[b] Significance of differences between strains A.BY and C57BL/6J.

2 days of PMSG treatment, the number of normal large follicles decreased by 59–68 % on the A genetic background and increased by 80 % on the C57BL/6J genetic background. Even the total number of healthy follicles decreased by 33–47 % on the A genetic background and increased 32 % on the C57BL/6J genetic background.

Table 7 shows the number of atretic follicles 2 days after treatment with 5 IU PMSG. No significant differences were found between strains A/J and A.BY/SnJ in the number of atretic follicles in any follicular size class or the percentage of follicles undergoing atresia. A comparison of A.BY and C57BL/6J shows that the C57BL/6 genetic background had more follicles undergoing atresia ($P < 0·05$), but a lower percentage of follicles undergoing atresia ($P < 0·01$) than were found in females on the A/J genetic background.

4. Discussion

Our data demonstrate that one of the major loci by which A/J and C57BL/6J differ with respect to hormone-induced ovulation rate is closely linked to *H-2*. This genetic difference may be an effect of *H-2* haplotype *per se*, or of a closely linked locus such as Hormone-2 (Ivanyi *et al.* 1972*a*, *b*). We chose to compare A.BY/SnJ to A.WY/SnJ and A/J since A.BY/SnJ was the only *H-2* congenic strain available on the A/J genetic background which is $H\text{-}2^b$. Even though the *H-2* region of A.BY came from Brachyury, strains A.BY and C57BL/6, as well as strains C57BL/10, C57L, 129 and LP all have the same $H\text{-}2^b$ haplotype, i.e. the same *H-2* region (Altman & Katz, 1979). Regardless of whether the *H-2* region of C57BL/6J is perfectly identical to that of A.BY/SnJ, the fact remains that the substitution of the $H\text{-}2^b$ haplotype on to the A.WY/SnJ genetic background increased hormone-induced ovulation rate by 51 %. This clearly shows that the *H-2* region of mouse chromosome 17 has a major effect on ovarian responsiveness to gonadotropins.

The *H-2* region influences intracellular cAMP levels and glucagon binding to hepatic membranes (Lafuse *et al.* 1979; Lafuse & Edidin, 1980). Associations of Class I antigen complexes with insulin receptors (Due, Simonson & Olsson, 1986), extend the concept of MHC effects on hormone responses. Furthermore, both LH and the T-lymphocyte factor, interferon gamma, modulate the expression of Class I and Class II antigens in bovine luteal cells (Fairchild & Pate, 1989). These effects may not be limited to the cAMP-second message pathway. The genomic region encoding *H-2* also influences steroid 21-hydroxylase, which is involved in the synthesis of mineralocorticoids and glucocorticoids (Chaplin *et al.* 1986) and mem-

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brane methyltransferase I, which synthesizes phosphatidylmonoethylethanolamine from phosphatidylethanolamine (Markovac & Erickson, 1986).

H-2 haplotype demonstrates a significant effect on hormone-induced ovulation rate on the A/J genetic background. However, we found no *H-2* effect on the C57BL/10J genetic background. This is probably due to an already high cAMP production and hormone-induced ovulation rate on the C57 genetic background (Spearow *et al.* 1983), which is likely to hide the effects of additional cAMP.

The present study showed that *H-2* haplotype did not significantly effect age or weight at puberty. Thus, we must reject the hypothesis that the observed effect of *H-2* on induced ovulation rate was mediated by changing the timing of puberty.

Since the number of follicles matured by large doses of PMSG and hCG varied between *H-2* haplotypes, we examined the effect of *H-2* haplotype on follicle populations. The only statistically significant difference that we observed between untreated A/J and A.BY/SnJ mice was in the number of normal type 5a follicles. However, this difference was inversely related to the induced ovulation rate of these congenic strains and may be due to genetic differences in follicular growth rate (Cahill & Mauleon, 1980; Spearow, 1986), with faster growth in A.BY/SnJ mice. Thus, we must also reject the hypothesis that *H-2*-linked differences in follicular number in untreated females mediates the *H-2* effect on hormone-induced ovulation rate.

The present study did not find significant differences between *H-2* haplotypes in the incidence of atresia either before or after PMSG treatment. Thus, the $H\text{-}2^b$ haplotype does not alter induced ovulation rate by changing the incidence of atresia.

If the observed effect of *H-2* haplotype on induced ovulation rate is clearly not due to a difference in follicle population prior to PMSG, or to a difference in atresia, then *H-2* must have increased induced ovulation rate by increasing the induction of follicle maturation in response to PMSG, or increasing the induction of ovulation by hCG. While the 5 follicle effect of $H\text{-}2^b$ haplotype on the number of normal type 7 follicles following PMSG (6 follicles in A/J *vs.* 11 follicles in A.BY/SnJ) was only significant at the $P < 0{\cdot}2$ level, this difference in induction of follicle maturation through FT 7, does explain much of the *H-2* effect on induced ovulation rate. To fully account for their observed hormone-induced ovulation rate, A/J mice would have to ovulate their full complement of normal type 7 follicles plus an additional three follicles, while A.BY/SnJ mice would have to ovulate the full complement of normal type 7 follicles plus about six more follicles from other size classes or stages of atresia. Thus, while the evidence based on the limited number of animals in the follicle studies is not compelling, we cannot reject the hypothesis that the observed effects of *H-2* on induced ovulation rate were due to differences in the induction of follicle maturation by PMSG and the induction of ovulation by hCG.

The effect of genetic background on hormone-induced ovulation rate is clearly greater than that of *H-2*. Since the hormone-induced ovulation rate of A/J and C57BL/6J differs due to the effect of approximately 3–4 loci (Spearow, 1988*a*), and since one of these loci is tightly linked to *H-2*, approximately 2–3 loci mediate the differences in induced ovulation rate between genetic backgrounds with the same *H-2* haplotype (i.e. A.BY/SnJ *vs.* C57BL/6J).

The present study clearly shows that the increased induced ovulation rate of the C57 genetic background cannot be due to an earlier timing of puberty. Mice on the C57 genetic background reached puberty later than did mice on the A genetic background. Furthermore, untreated 28-day-old C57BL/6J females did not have significantly more large follicles (FT 5b–7) than those on the A genetic background.

We also determined if strain differences in the number of healthy follicles at 28 days mediated the effect of genetic background on induced ovulation rate. At first glance, the increased number of type 4 follicles in untreated 28-day-old C57BL/6J ovaries seemed sufficient to account for the increased hormone-induced ovulation rate of this strain over A/J or A.BY/SnJ. However, barring a major strain difference in granulosa cell growth rate, it seems highly unlikely that type 4 follicles could respond to PMSG and mature to type 7 within 50 h of PMSG treatment. Based on the follicular responses of rats to PMSG (Hirshfield, 1985), and the kinetics of follicular growth in untreated mice (Pedersen, 1970; Spearow, 1986), it should require 110–131, 58–67 and 22–28 h for the smallest untreated type 4, 5a and 5b follicles, respectively, to grow and enter FT 7. Thus, depending on their stage of development and PMSG growth response, no type 3b or 4 follicles, some of the larger type 5a, and all type 5b follicles at the time of PMSG injection would be able to mature to FT 7 within 50 h and be ready to respond to hCG and ovulate.

On both genetic backgrounds, the sum of the healthy type 6 and 7 follicles and large early atretic follicles at 28 days closely approximated the sum of medium atretic follicles and old ova at 30 days. This strongly suggests that the normal type 6 and 7 follicles at 28 days either ovulated in response to the PMSG to form 'old ova', or underwent atresia. Cumulatively, these data suggest that follicles destined to ovulate were recruited from FT 5a and/or 5b.

For follicle types larger than FT 4 as well, the differences in follicle numbers at 28 days were clearly insufficient to account for the differences in induced ovulation rate among strains. For example, the number of type 5a and 5b follicles at 28 days in A/J and C57BL/6J differed by 9%, while their induced ovulation rate differed by 600%. While the present data does not definitively establish which follicle

type(s) in 28-day-old mice respond to PMSG and eventually ovulate in response to hCG, the observed differences in the number of follicles at 28 days clearly fails to explain the effect of genetic background on hormone-induced ovulation rate.

The number of normal type 7 follicles present 2 days after PMSG treatment accounts for essentially all of the differences in the effects of background genotype on induced ovulation rate. Thus, the main effect of the alleles on the C57BL/6 genetic background, relative to those of the A genetic background, is to enhance the induction of follicular maturation to FT 7 in response to PMSG. The present data clearly shows that the C57BL/6 genetic background increases the number of follicles maturing to FT 7, and thus hormone-induced ovulation rate, at least in part, by decreasing the incidence of follicular atresia following gonadotrophin treatment.

We discovered both similarities and differences between the mechanisms by which the *H-2*^{b} linked and independent loci control hormone-induced ovulation and by which the Booroola *F* gene controls natural and hormone-induced ovulation rate in Merino sheep (Kelly *et al.* 1983; Piper & Bindon, 1985). On the Merino genetic background, the *F* gene increases the number of preantral follicles without affecting the total number of antral follicles (Draincourt *et al.* 1984; McNatty *et al.* 1986). The Booroola *F* gene alters the induction of ovulation so that much smaller follicles are capable of responding to LH and ovulating (Bindon *et al.* 1985; McNatty *et al.* 1986). In a similar fashion, the mouse *H-2*^{b} linked locus is a single locus or tightly linked loci which increases induced ovulation rate without increasing the total number of antral follicles. In contrast to the Booroola *F* gene, genes on the C57 background decrease the incidence of atresia, and dramatically increase the number of normal graafian follicles in response to gonadotropins.

We conclude that the 6-fold difference in hormone-induced ovulation rate between C57BL/6J and A/J is controlled by the action of a *H-2* linked locus, as well as the action of about two to three *H-2* independent loci. The effect of the *H-2*^{b} linked locus on hormone-induced ovulation rate is clearly not due to a difference in (1) the timing of puberty; (2) the number of follicles prior to gonadotropin treatment; or (3) the incidence of atresia. The results also suggest, but do not prove, that the *H-2*^{b} linked locus increases ovulation rate by increasing the maturation of follicles to type 7 in response to PMSG, and/or by allowing a wider range of follicles to ovulate in response to hCG.

In contrast, the *H-2* independent loci on the C57BL/6J genetic background, relative to that of the A/J background, clearly increase hormone-induced ovulation rate by increasing the number of type 7 follicles matured by PMSG. The increased number of normal type 7 follicles in C57BL/6J were due to an increase in the induction of follicular maturation and a decrease in the incidence of follicular atresia in response to PMSG.

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