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Genetic determination of coat color affects testicular steroidogenesis in the *Mustela vison*

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Coat color genes in mammals are known to be developmental genes with wide pleiotropic effects. The present study was undertaken to study testicular steroidogenesis in American Mink (*Mustela vison*) of various coat color phenotypes. No differences in testicular steroid levels were observed between fertile and infertile mink with the standard phenotype and genotype (*BB jj MM PP*). Mink with the opaline phenotype and genotype (*bb mm pp*), were found to have in their testes, 20-40 % higher levels of progesterone, five times higher levels of 17-hydroxyprogesterone, and eight times higher levels of testosterone, than the corresponding values in other mink. No other differences were observed among the different types of mink. Since the genotype of the opaline mink differs from the other mink studied, only in their combination at the *pastel* (*b*) and *moyle* (*m*) loci, their *bb mm* genotype could be assumed to be responsible for the increase in testicular steroids.

Key words: Testes, Testosterone, Progesterone, Hydroxyprogesterone, Mink, Steroidogenesis, Genotype, Coat color.

The American mink is an economically important animal in many parts of the world with some pelts with specific coat colors and/or color patterns having greater commercial value, which leads mink farmers to produce animals with such fur patterns. However, some of those coat colors are the result of genotypes associated with reduced or poor reproductive performance (13).

Since most, if not all, of the genes determining coat color are important pleiotropic developmental genes, it is understandable that manipulation of these

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genotypes might have repercussions beyond the color of the fur. In fact, mutations in coat color genes have been shown to greatly alter testicular steroidogenic function in house mice (1, 5), Norway rats (2), and Syrian hamsters (4). Significant variations in steroid levels have also been observed among normal mice (6) and rats (7).

Furthermore, the dark mink phenotype is associated with high incidence of primary infertility, presumably due to deficient GnRH secretion (15, 16), and with high incidence of secondary infertility due to autoimmune orchitis (9, 14, 16). Therefore, the present study was conducted to determine the impact of different coat color phenotypes on testicular steroidogenic function.

Materials and Methods

Adult American mink (*Mustela vison*) were obtained at Northwood Fur Farms, Inc. (Cary, Illinois). The phenotypes and genotypes of the animals are listed in table I. The animals were sacrificed by cervical dislocation, and testes were obtained. The testes were placed in cryovials and instantly frozen in liquid nitrogen and they were stored frozen at –70 °C. They were later thawed, decapsulated, weighed, and testis fragments of about 500 mg were obtained. These were homogenized in 5 ml double distilled H_2O for 1 min at 10,500 rpm with a Tekmar Tissuemizer. Homogenates were then stored frozen at –20 °C until assayed for steroids.

Table I. *Phenotype and pertinent genotypical information on the different types of American mink used (11).*
The gene symbols are: *b* = pastel; *J* = Jet black (dark); *m* = Moyle buff; *p* = platinum; – = either the dominant or recessive allele may occur.

Phenotypes	Genotypes			
Fertile standard	BB	jj	MM	PP
Infertile standard	BB	jj	MM	PP
Fertile pastel	B–	jj	M–	pp
Fertile jet	B–	Jj	M–	P–
Fertile opaline	bb	jj	mm	pp

Testicular steroid levels were determined using solid-phase radioimmunoassay procedures. Since these kits (Diagnostic Products Corporation, Los Angeles) use standard curves based on human serum, parallelism between the standard curves for progesterone, 17-hydroxyprogesterone and testosterone, and curves made with pooled aliquots from the mink testis homogenates was first established (table II).

Data from the RIA were obtained using the RIAPLOT and RIADOSE programs (3). Data were evaluated by one-way analysis of variance using the SPSS-X software on an IBM mainframe (10). Data were tested for normality of distribution by the Kolmogorov-Smirnov test and for homogeneity of variance by Barlett's test, and log- or square-root transformed as needed (10, 12).

Results

No differences in testicular steroid concentrations were observed between fertile and infertile standard mink (fig. 1). Opaline mink (*bb mm pp*) had significantly higher testicular progesterone concentrations than standard (*BB jj MM PP*), pastel (*bb*) or jet (*Jj*) mink. This difference was of about 20-40 % (fig. 1 a). However, the differences in testicular 17-hydroxyprogesterone and testosterone concentrations were much greater. Opaline mink had a 5-fold higher concentration of testicular 17-OH-progesterone, and an 8-fold greater concentration of testicular testosterone, when compared with the other types of mink (fig. 1 b,c). No signif-

Table II. *Characterization of solid-phase radio-immunoassays.* Comparison between the statistical parameters of the curves derived from American mink testis homogenate pools and those of the standard curves with which they were assayed. *m* = slope, *YI* = *Y* intercept, and *r* = correlation coefficient.

Hormonal Sample	m	YI	r
a) Testicular progesterone	−1.720	0.352	−1.000
Standard curve	−1.724	0.352	−0.998
b) Testicular 17-OH-progesterone	−1.496	−0.609	−1.000
Standard curve	−1.498	−0.607	−0.999
c) Testicular testosterone	−1.734	0.573	−1.000
Standard curve	−1.732	0.566	−0.999

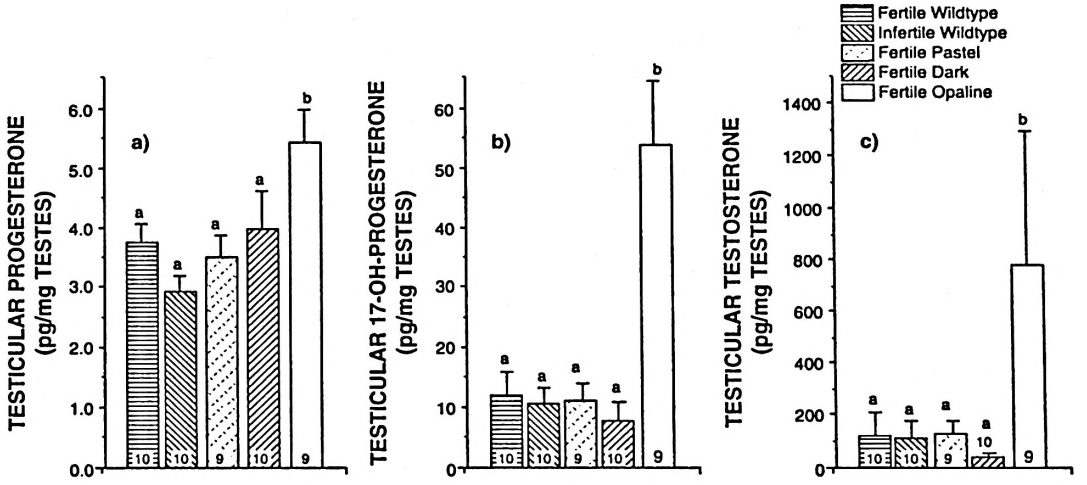

Fig. 1. *Testicular progesterone (a), 17-OH-progesterone (b), and testosterone (c) concentrations in mink as a function of coat color phenotype.*
The values are represented as mean ± sem for the number of animals indicated. Within each panel, points with the same letter are not significantly different (P > 0.05; Student-Newman-Keuls procedure of the multiple range test).

icant differences in testicular weight were recorded (data not shown).

Discussion

Opaline mink had higher testicular steroid levels than any of the other types of mink studied. Circulating testosterone levels in opaline mink had also been reported to be higher than in pastel and jet mink (16). The differences in circulating levels were not as marked as those reported here for testicular levels. The effect of the opaline phenotype appears to be on the conversion of progesterone to androgens. The effect would include stimulation of the 17-hydroxylase/C17-20-lyase

complex and of the 17-hydroxysteroid dehydrogenase.

TUNG *et al.* (16) compared fertile with infertile jet mink. They found circulating testosterone levels in infertile jet mink to be about half those measured in fertile jet mink. Since the infertile jet mink had low LH levels, but responded well to both hCG and GnRH stimulations, the etiology of the infertility in jet mink with primary infertility was proposed to be a deficiency in the secretion of GnRH in these animals. In the present study the comparison is between fertile and infertile standard mink, and no differences in steroid levels were detected. Since autoimmune orchitis has been found to be the cause of secondary infertility in jet mink, we wondered if this could be the cause of infertility in standard mink. This is highly unlikely, since although anti-sperm antibodies have been detected in several phenotypes, autoimmune orchitis has only been reported in jet mink (13). This would indicate that the infertility in standard mink is due to a different, yet to be determined, etiology.

Analyzing the current results, it becomes apparent that, from the known differences in genomic composition among the types of mink studied, the differences in testicular steroidogenesis could be attributed to homozygosity for the *b* allele at the pastel locus and/or for the *m* allele at the moyle locus. Mink with the opaline phenotype are known to differ from the ones with the standard phenotype at the pastel, moyle and platinum loci. Thus, any of these three could be responsible for the differences observed in opaline mink. However, pastel mink have the same genotype at the platinum locus as opaline mink. Since these two types of mink also differ in steroidogenic characteristics, the platinum locus can be eliminated. Both the pastel and the jet (dark) mink may be homozygous for the *bb* and/or *mm* genotype. Yet both have similar steroidogenic profile, and both differ from opaline mink. It appears then, that the *bb mm* genotype may be responsible for the increased testicular steroidogenic activity observed in opaline mink. Since having an homozygous recessive genotype at only one of these loci does not appear to affect steroidogenesis, it could be assumed that the products of these loci have complementary metabolic actions, and thus may rescue each others' deficiency. Therefore, the *b* and *m* loci must be encoding proteins from the same family. Mutations in the genes coding for the stem cell factor receptor and its ligand have been shown to produce similar changes in testicular steroidogenesis to those reported here (1, 5). Furthermore, the stem cell factor regulatory axis is complemented in many tissues by axes involving other tyrosine kinase III/IV/V receptors (8, 17). In the case of the opaline mink, it may be said that these animals have mutations involving both the genes coding for the stem cell factor receptor and the platelet-derived growth factor receptor, or that both genes are coding for the stem cell factor and the platelet-derived growth factor.

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Los genes que determinan el color del pelaje están, en su mayoría, encargados del desarrollo y por lo tanto poseen una multitud de funciones. Por esto se estudian los efectos del color del pelaje sobre la esteroidogénesis testi-

cular del visón americano (*Mustela vison*). No hay diferencias en los niveles testiculares de esteroides entre los visones fértiles e infértiles de fenotipo y genotipo estándar (*BB jj MM PP*). Los visones con fenotipo y genotipo opalino (*bb mm pp*), tienen más elevados (20 - 40 %) los niveles de progesterona, cinco veces mayores los de 17-hidroxiprogesterona, y ocho veces más los de testosterona, en comparación con los demás visones. No hay diferencias entre los otros tipos de visones. Ya que el visón con el fenotipo opalino sólo difiere de los otros visones en la combinacion genotípica de los loci *pastel* (*b*) y *moyle* (*m*), se sugiere que su combinacion genotípica, *bb mm*, podría ser la responsable del aumento de los niveles de esteroides testiculares de estos animales.

Palabras clave: Testículos, Testosterona, Progesterona, Hidroxiprogesterona, Visón, Esteroidogénesis, Genotipo, Color de pelaje.

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