ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (4): 787-792, 2008
ISSN 1682-296X

Variant Molecular Marker in MHC Effect Fertility Trait in Sheep

[1]M.R. Mir and [2]H. Geldermann
[1]Division of Veterinary Biochemistry, Faculty of Veterinary Sciences and A.H. Sher-e Kashmir,
University of Agricultural Sciences and Technology of Kashmir,
Shuhama, Srinagar, 190006, J and K State, India
[2]Department of Animal Breeding and Biotechnology, University of Hohenheim, Stuttgart, Germany

Abstract: Present study was aimed at comparative analysis of microsatellite polymorphism at DRB_1 intron 2 locus of Ovine MHC in German Merino sheep. Experiment was conducted in four consecutive lambings comprising flock of adult males, females and offspring's totaling 639 individuals. A total of 16 DRB_1 microsatellite alleles, ranging between 353-857 bp were detected associated with variable reproductive performances among males and females. Ewes carrying allele 386 bp had a higher ($p<0.01$) number of lambs born, carriers of allele 389 bp had a lower ($p<0.01$) number of lambs weaned and allele 411 bp occurred together with higher values of all recorded fertility traits. The associations of different alleles with variable reproductive traits in sheep could be individual variability to humoral immune response, cell recognition or tissue differentiation between carriers of various MHC genotypes. The observed associations within DRB_1 intron 2 locus of Ovine MHC in German Merino sheep may be used as a molecular marker for identifying QTL in genetic improvement of the sheep

Key words: Sheep, DRB_1, microsatellite, fertility, QTL

INTRODUCTION

The ovine Major Histocompatibility Complex (Ovar) is a group of linked genes, playing a significant role in development of immunity, metabolism, endocrines and general vitality (Rupp *et al*., 2007). The MHC genes could be divided in three classes: class I, class II and class III, out of which loci of the MHC class I and II genes encode membrane-bound proteins that play a key role in the initiation of the immune response (Gruszczynska *et al*., 2002). Genes of class II are the most variable genes of vertebrates (Stear *et al*., 2005). There are 76 described alleleic sequences for the expressed DRB genes in cattle and 54 sequences for this locus are stored in GenBank for sheep and even inside a population, the number of alleles reaches 10 or more, which is maintained by a selective advantage of heterozygote individuals (Griesinger *et al*., 1999).

Inside exon 2 of DRB_1 loci a microsatellite is found, which is used to investigate the genetic variation of the DRB gene within MHC (Ammer *et al*., 1992) and is expected to play an important role in fertility of sheep (Duarte *et al*., 2005). A relationship of MHC genes and reproductive traits can be expected because of many immunological processes involved during the implantation of embryos (Jin *et al*., 1995; Ober *et al*., 1998; Van der Ven *et al*., 2000). The genetic fundamentals of this phenomenon remain unclear but hypothesis based on the influences of genes linked to the MHC like pre-implantation embryo development gene (PED) (Warner *et al*., 1987), T/t associated genes (Ho *et al*., 1994) or the maternal fetal interactions caused by MHC antigens (Beer *et al*., 1985; Roy *et al*., 1999) has been proposed. A positive association of fertility measured as non-return after artificial insemination and BoLA-A alleles was observed in Norweigian cattle (Mejdell *et al*., 1994) and in contrast other studies in cattle (Arriens *et al*., 1996) show no significant associations between MHC genes and reproductive traits. Associations between MHC genotypes and several reproductive traits including testicular size of males respectively ovulation rate, litter size, number of piglets born alive in females were found in pigs (Warner *et al*., 1991).

Very little information is presently available with respect to the influence of MHC on the fertility in sheep. The aim of this study include to identification of Ovar-

Corresponding Author: Manzoor R. Mir, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences and A.H. Sher-e Kashmir, University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alustang, Post Bag No. 135, Srinagar, PIN: 190006, J and K State, India

DRB_1 microsatellite polymorphism in German Merino sheep and to associate the variability in Ovar-DRB_1 microsatellite polymorphism with the fertility trait in German Merino sheep. The Ovar-DRB_1 microsatellite polymorphism in this experiment may help in identification of the QTL associated with fertility and the genetic improvement in sheep.

MATERIALS AND METHODS

A total of 7 rams, 249 ewes and 381 lambs were used for the study at research station Oberer Lindonhof, University of Hohenheim, Stuttgart Germany. The experiment includes four mating periods. Young, virgin female sheep were mated with the same ram for two consecutive periods in order to establish feto-maternal immune response. After two pregnancies ewes were taken out of the experiment.

Ten milliliter blood or spleen samples were collected from live and dead animals respectively. DNA was extracted from the respective material using a standard phenol-chloroform extraction protocol. The microsatellite in exon 2 of the DRB_1 gene was amplified by the polymerase chain reaction (PCR) in a thermocycler (Biometra) using the following primers: 5'GGGGGATCCGCTTCGACAGCGACTGGGGCG3' and 5'CTGACCCAGAKTGAGTGAAAGTATC3' (K = G or T) (Griesinger *et al*., 1999). Two hundred nanogram of genomic DNA as template in a volume of 25 μL. First cycle of the PCR were 3 min at 94°C, 1 min at 60°C and 1 min at 72°C followed by 30 cycles with 30 sec at 94°C, 1 min at 60°C and 1 min at 60°C and finally one cycle with 30 sec at 94°C, 1 min at 60°C and 5 min at 72°C. Separation of the PCR product for fragment length analysis was carried on Automated Laser Fluorescent (ALF) sequencer (Pharmacia, Germany). One microliter of the PCR product and 3 μL of ALF loading buffer was mixed, denatured at 90°C for 2 min and chilled on ice before loading on the ALF gel. Electrophoresis was performed in 0.6% TBE at 1500 V, 45 mA, 34 W and 50°C for 7 h. Analysis of the data for fragment lengths was carried with the ALF-Fragment Manager Evaluation software in relation to external standards.

During the research period, fertility parameters were recorded for rams and ewes and fed to a data base. Complete particulars with respect to fertility trait and genotype information of DRB_1 microsatellite from all animals were recorded and were analyzed for association between the DRB_1 microsatellite alleles of rams and ewe with fertility trait. ANOVA analysis was done with General Linear Models (GLM) of the statistical package SAS (1994). To correct the variation due to environment, important environmental factors were considered to each trait in ANOVA model.

RESULTS AND DISCUSSION

Sixteen alleles based on the number of base pairs were detected in the experimental flock at this locus (Table 1). The most frequent alleles were 411, 405, 394 and 383 and accounted for 63.3% of the allele frequency in the entire flock. Allele 411 was the most common allele with 22.5% of the frequency, followed by the allele 405 (14%), 394 (14.1%) and 383 (12.2%). The number of alleles reported in this study corresponds to similar findings of previous studies in the same breed (Griesinger *et al*., 1999) as well for other sheep breeds (Schwaiger *et al*., 1993). A total of 92 genotypes were found for the DRB_1 microsatellite locus with genotypes 394/411 (7.1%), 411/411 (6.0%), 383/405 (6.0%) and 383/411 (6.0%) most frequently reported in parents. In offspring's the most frequent genotypes were 405/411 (9.8%), 394/405 (6.8%) and 394/411 (5.4%) (Table 1).

Significant difference ($p<0.05$) was found in the pregnancy status for ram1 (389/411) {low value} in comparison to ram 6 (405/420) {high value}. However, for

Table 1: Allele/Genotype frequencies of DRB_1 microsatellite locus

	Parents		Offspring		Total	
(bp)	(n)	%	(n)	%	(n)	%
Allele frequencies						
353	15	3.0	12	2.3	27	2.6
374	16	3.2	7	13.0	23	22.0
380	21	4.2	14	27.0	35	34.0
383	69	13.7	57	108.0	126	122.0
386	28	5.6	14	27.0	42	41.0
389	26	5.2	20	38.0	46	45.0
394	72	14.3	74	140.0	146	141.0
400	18	3.6	5	09.0	23	22.0
405	65	12.9	85	161.0	150	145.0
411	102	20.2	130	246.0	232	225.0
420	2	0.4	11	21.0	13	13.0
430	3	0.6	7	13.0	10	10.0
443	7	1.4	4	0.8	11	11.0
455	26	5.2	36	68.0	62	60.0
803	13	2.6	46	87.0	59	57.0
857	21	4.2	6	11.0	27	26.0
Genotype frequencies						
383/383	10	4.0	6	2.3	16	3.1
383/394	3	1.2	8	3.0	11	2.1
383/405	15	6.0	10	3.8	25	4.8
383/411	15	6.0	8	3.0	23	4.5
389/411	8	3.2	5	1.9	13	2.5
394/405	11	4.4	18	6.8	29	5.6
394/411	18	7.1	18	6.8	36	7.0
405/411	2	0.8	26	9.8	28	5.4
405/803	3	1.2	8	3.0	11	2.1
411/411	15	6.0	16	6.1	31	6.0
411/455	3	1.2	12	4.5	15	2.9
411/803	2	0.8	12	4.5	14	2.7

Table 2: Association between the rams DRB_1 microsatellite genotype and fertility traits

	Source of variance						
	Observations on mated ewes (LS-Means±SE)				Observations on pregnant ewes (LS-Means±SE)		
Ram genotype	n	Pregnancy status	Lambs born	Lambs weaned	n	Lambs born	Lambs weaned
389/411	97	0.508±0.045[a]	0.823±0.068	0.552±0.062	50	1.577±0.069[a]	1.013±0.087
383/383	54	0.602±0.065	0.772±0.098	0.701±0.088	39	1.286±0.082[b]	1.092±0.103
394/411	101	0.542±0.044	0.689±0.067	0.498±0.060	55	1.252±0.065[b]	0.888±0.081
455/803	93	0.609±0.046	0.798±0.070	0.634±0.063	61	1.301±0.062[b]	0.993±0.077
405/803	59	0.536±0.061	0.647±0.092	0.492±0.083	29	1.195±0.094[b]	0.890±0.118
405/420	51	0.663±0.065[b]	0.818±0.098	0.542±0.089	32	1.231±0.089[b]	0.792±0.111
394/430	40	0.478±0.074	0.659±0.112	0.493±0.101	16	1.307±0.124[ab]	0.900±0.155
Sign (p)		ns	ns	ns		0.004**	ns
r^2		0.224	0.258	0.232		0.163	0.123

Probability at **: p<0.01, ns: not significant, LS-Means within columns with different characters differ at p<0.05

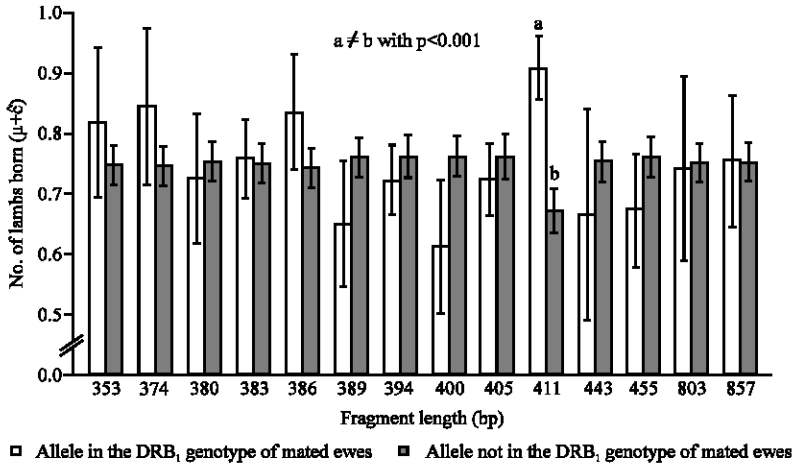

Fig. 1: Number of lambs born in groups of ewes or with or without DRB_1 specific microsatellite allele

data on pregnant ewes, ram 1 differed positively (p<0.01) in lambs born comparing to all other rams except the ram 7 (Table 2).

DRB_1 microsatellite genotypes of the ewes were highly associated with the fertility traits of the ewes (p<0.01 to p<0.08). The genotype classes of 380/411 and 386/405 were associated with high numbers of lambs born and the genotype classes 383/383, 394/405 and 405/455 were negatively associated with pregnancy status and lambs weaned. In pregnant ewes genotype classes 374/411 and 380/411 of ewes showed positive effects on number of lambs weaned, whereas genotype classes 383/383 and 389/411 were observed with negative effects. The genotype class 383/383 of ewes had considerable negative effects in all fertility traits. The effects of genotypes with allele 394, allele 411 and the residual allele have been analyzed in ewes and it was shown that genotype with above mentioned alleles have strong effects on the fertility traits in ewes as well as in rams, with slight superior fertility associated with allele 411 than allele 394. Within mated ewes, the classes of genotypes 411/411 and 411/Rest were superior to the residual class in pregnancy status, lambs weaned and lambs born. The genotype classes containing the allele 411 (394/411, 411/Rest, 411/411) were associated with higher values of lambs born and lambs weaned than the class 394/Rest and the residual genotype class (Fig. 1, 2).

Significant association between some DRB_1 microsatellite alleles and fertility traits in German Merino sheep has been observed in this study, as reproduction is governed by heterogeneous immunological cycles including synergistic effects from disease resistance, partly controlled by MHC genes.

The effects of the rams are taken as overall flock effects. Development of the accessory sex organs was found to be associated with MHC alleles in boars with high fertility. Contrary to this study, Conley *et al.* (1988) found no difference in conception rates among the different Swine Leukocyte Antigen (SLA) genotypes of boars. Renard and Vaiman (1989) observed two positive SLA haplotypes and two negative haplotypes which affect the development of the reproductive organ in boars.

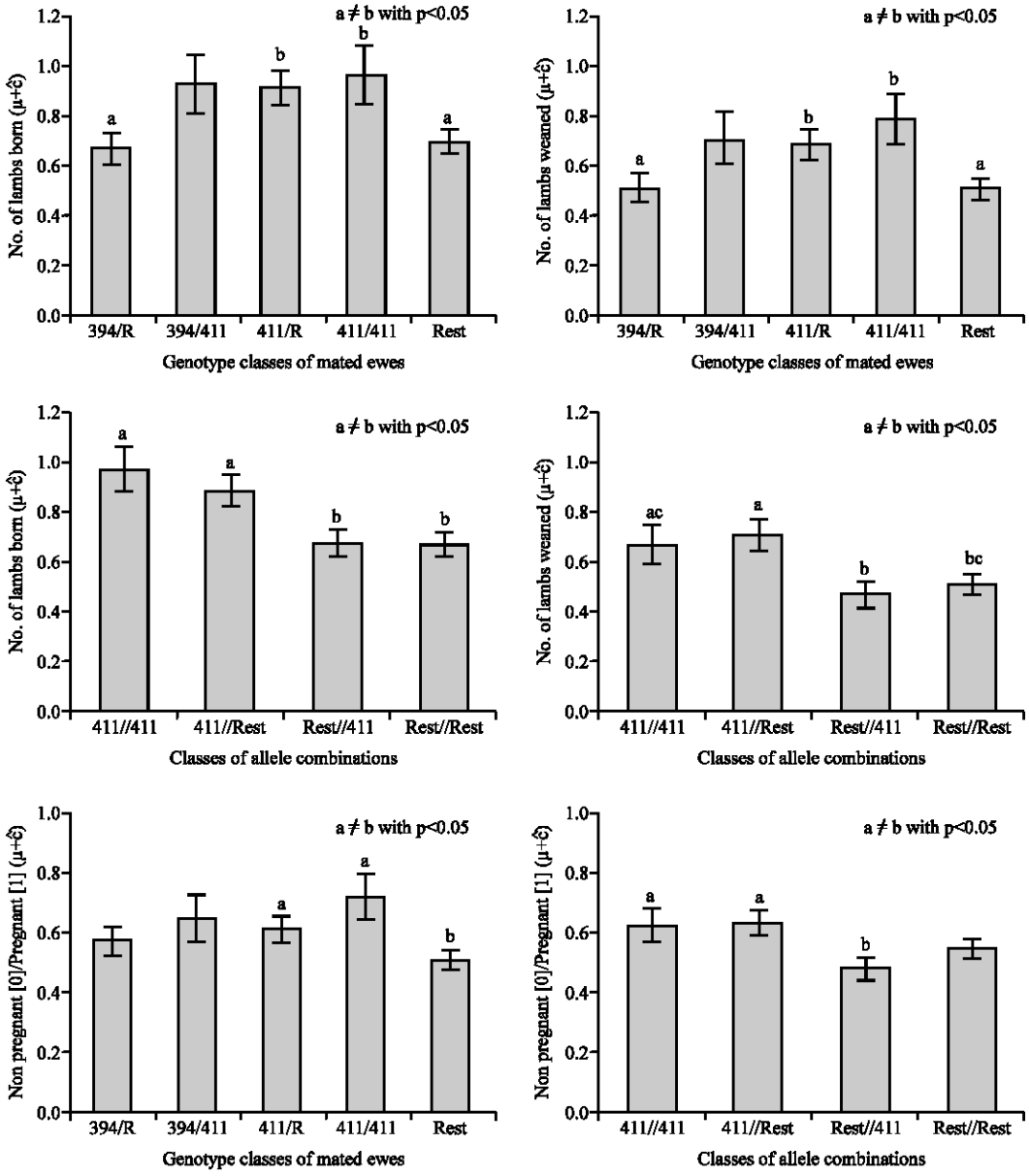

Fig. 2: Fertility trait values within ewes grouped for specific genotypes

In our study association between DRB_1 microsatellite genotypes of the rams and fertility traits may be due to the variant spermatogenesis controlled by MHC genes as has been also reported by Van der Ven *et al.* (2000).

DRB_1 microsatellite alleles and respective genotypes in ewes have found to be highly associated positively as well as negatively with fertility traits, due to linked genes within MHC. *CYP21* gene within MHC governs ovulation and prolectin gene which is important for mothering instinct, is linked to MHC (Lewin *et al.*, 1992). Increased fertility associated with certain DRB_1 microsatellite alleles may also be due to the certain other linked genes conferring resistance to various sub-clinical infections and worm infestations (Buitkamp *et al.*, 1996). Besides, certain haplotypes, linked negatively with fertility trait in this study, may be due to presence of t-complex haplotypes (Browning *et al.*, 2002) which cause segregation distortion leading to abortion and related fertility complications.

The true effects of DRB_1 microsatellite analyzed in this study, although are difficult to evaluate the direct effects on gene function. However, it is possible that

DRB_1 microsatellite could influence gene expression (Coming, 1998) and the effect being dependent on repeat size. It is possible that the microsatellite size could interfere with gene action, altering follicular size, ovulation and mothering instinct associated with genes within MHC (Roy *et al*., 1999). On the other hand, it is possible that DRB_1 microsatellite could be in linkage disequilibrium with some gene mutation influencing fertility that may be influencing its biological role (Duarte *et al*., 2005). The positive/negative associations observed needs to be confirmed in other breeds to determine if it is a general phenomenon or is a phenomenon specific to this herd, so that QTL with fertility is identified and Marker Assisted Selection (MAS) is undertaken so as to improve the reproductive performance of this breed.

ACKNOWLEDGMENTS

Authors are thankful to H. Bartenschlager for statistical analysis and valuable suggestions. The first author is grateful to German Academic Exchange Service (DAAD) for the financial support during the doctorate studies.

REFERENCES

Ammer, H., F.W. Schwaiger, C. Kammerbauer, M. Gomolka, A. Arriens, S. Lazary and J.T. Epplen, 1992. Exonic polymorphism vs intronic simple repeat hypervariability in MHC-DRB genes. Immunogenetics, 35: 332-340.

Arriens, M.A., A. Hofer, G. Obexer-Ruff and S. Lazary, 1996. Lack of association of bovine MHC class I alleles with carcass and reproductive traits. Anim. Genet., 27: 429-431.

Beer, A.E., J.F. Quebbeman, J.W.T. Ayers and R.F. Haines, 1985. Major histocombability complex antigens, maternal and paternal immune responses and chronic habitual abortions in humans. Am. J. Obst. Gyne., 141: 987-999.

Browning, V.L., R.A. Berstrom, S. Daigle and J.C. Schimenti, 2002. A haplotype locus uncovered by deletion in the mouse T complex. Genetics, 160: 675-682.

Buitkamp, J., P. Filmether, M.J. Stear and J.T. Epplen, 1996. Class I and class II major histocompatibility complex alleles are associated with fecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. Parasit. Rev., 82: 693-696.

Coming, D.E., 1998. Polygenic inheritance of micro/minisatellites. Mol. Psychiatry, 3: 21-31.

Conley, A.J., Y.C. Jung, N.K. Schwartz, C.M. Warner, M.F. Rothschild and S.P. Ford, 1988. Influence of SLA haplotype on ovulation rate and litter size in miniature pigs. J. Reprod. Fertil., 82: 595-601.

Duarte, L.B.M., J.C.F. Moraes and T.A Weimer, 2005. Diversity of microsatellites linked to FSHβ gene, their usefulness for individual identification and association with reproductive performance. Cienc. Rural., 35: 145A-149.

Griesinger, I., F. Pruser, K. Siemienski and H. Geldermann, 1999. Extreme fragment lengths differences of the microsatellite in the expressed MHC-DRB gene of Merinoland sheep. Anim. Genet., 30: 77-78.

Gruszczynska, J., K.M. Charon, W. Swiderek and M. Sawera, 2002. Microsatellite polymorphism in locus OMHCI (MHC class I) in Polish Heath sheep and Polish Lowland sheep (*Zelazna variety*). J. Applied Genet., 43: 217-222.

Ho, H.N., Y.S. Yang and R.P. Hsieh *et al*., 1994. Sharing of human leukocyte antigen (HLA) in couples with unexplained infertility affects the success of *in vitro* fertilization and tubal embryo transfer. Am. J. Obstet. Gynecol., 170: 63-71.

Jin, K., H.N. Ho, T.P. Speed and T.J Gill, 1995. Reproductive failure and the major histocompatibility complex. Am. J. Hum. Genet., 56: 1456-1467.

Lewin, H.A., K. Schmitt, R. Hubert, M.J.T. Van Ejik and N. Arnheim, 1992. Close linkage between bovine prolactin and BoLA-DRB3 genes: Genetic mapping in cattle by single sperm typing. Genomics, 13: 44-48.

Mejdell, C.M., O. Lie, H. Solbu, E.F. Arnet and R.L. Spooner, 1994. Association of major histocompatibility complex antigen (BoLA) with AI bull progeny test results for mastitis, ketosis and fertility in Norweigian cattle. Anim. Genet., 25: 99-104.

Ober, C., T. Hyslop and S. Elias *et al*., 1998. Human leukocyte antigen matching and fetal loss, results of a 10 year prospective study. Hum. Reprod., 13: 33-38.

Renard, C. and M. Vaiman, 1989. Possible relationship between SLA and porcine reproduction. Genet. Pol., 29: 569-576.

Roy, F., B. Combes and D. Vaiman *et al*., 1999. Humoral immune response to equine chorionic gonadotropin in ewes: Association with major histocompatibility complex and interference with subsequent fertility. Biol. Reprod., 61: 209-218.

Rupp, R., A. Hernandez and B.A. Mallard, 2007. Association of bovine leukocyte antigen (BoLA) DRB 3.2 with immune response, mastitis and production and type traits in Canadian Holsteins. J. Dairy Sci., 90: 1029-1038.

Schwaiger, F.W., E. Weyers, C. Epplen, J. Brun, G. Ruff, A. Crawford and J.T. Epplen, 1993. The paradox of MHC-DRB exon/intron evolution:α-helix and β-sheet encoding regions diverge while hypervariable intronic simple repeats coevolve with β-sheet codons. J. Mol. Evol., 37: 260-272.

Stear, M.J., G.T. Innocent and J. Buitkamp, 2005. The evolution and maintenance of polymorphism in the major histocompatibility complex. Vet. Immunol. Immunopathol., 108: 53-57.

Van der Ven, K., R. Fimmers and G. Engels *et al*., 2000. Evidence for major histocompatibility complex-mediated effects on spermatogenesis in humans. Hum. Reprod., 15: 189-196.

Warner, C.M., S.O. Gollnick and S.B. Goldbard, 1987. Linkage of the pre-implantation-embryo-development (Ped) gene to the mouse major histocombatibility complex. Biol. Reprod., 36: 606-610.

Warner, C.M., M.S. Brownell and M.F. Rothschild, 1991. Analysis of litter size and weight in mice differing in Ped gene phenotype and the Q region of the H-2 complex. J. Reprod. Immunol., 19: 303-313.