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July 19, 2001

Richard N.Sifers,Ph.D.
Research Director
The Moran Foundation
Department of Pathology
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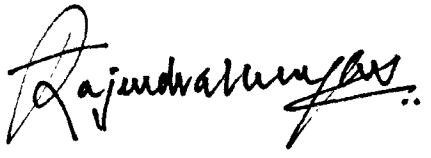
Dear Dr. Sifers:

Enclosed please find the annual report of The Moran Foundation supported project entitled "Regulation of the Hypothalamus-Pituitary-Testis Axis in the Atrichosis Mutant Mouse" which was awarded to me on August 01, 2000. I sincerely thank The Moran Foundation for their support with which we have initiated this important project. Enclosed please find copies of two of our publications in which support from The Moran Foundation has been acknowledged.

Sincerely,



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Annual Report of the project:

"Regulation of the Hypothalamus-Pituitary-Testis Axis in the Atrichosis Mutant Mouse"

August 01, 2000-July 31, 2001

T. Rajendra Kumar, Ph.D.
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Summary of the Project

The project consists of 3 Specific Aims that were all initiated in one year. We have made substantial progress on two of the three Specific Aims and completed Specific Aim 3.

Specific Aim 1: Characterize the testicular phenotypes in *at* mutant mice. We have successfully generated multiple breeding lines of at/+ male and female mice. The hairless phenotype is clearly obvious in homozygosity. We have performed morphological and histological analyses of the testes samples obtained from WT and mutant mice. Further, we have developed methods to isolate total RNA from single testis (6-9 mg) isolated from the mutant mice at both 7 days and 42 days. These RNA pools are currently being tested for functional differences in gene expression patterns in the testis of *at* mice. We have also performed cardiac bleedings from the mutant mice at 42 days and collected serum samples. These will be used for steroid and gonadotropin assays.

Specific Aim 2: Examine the pituitary gonadotropin gene expression in at mutant mice. Pituitary glands were isolated form the same mice from which blood and testes were collected. We have successfully developed dot blot methods to quantify RNA expression from single pituitary glands (< 1mg). We can pick up messages for LH beta, FSH beta gonadotropin sub unit genes from as small as half a pituitary gland. We are waiting to collect few more pituitaries from *at* mice to complete these studies.

Specific Aim 3: Analyze the hypothalamic monoamine content in *at* mutant mice by biochemical methods using RP-HPLC analysis. We have completed these studies. First, we have standardized the hypothalamic extraction methods and standardized all the conditions for a complete and quick separation of monoamines, dopamine, serotonin, and metabolic products of dopamines. The entire hypothalamus was used for each extraction (500 µl) using perchloric acid method. Approximately 50 µl were injected on to the column. Between retention time 2-14 minutes, all the above mentioned hypothalamic components are separated with no background/base line problems. There were no differences between wt and *at* mutant mice hypothalami. Therefore, the testicular defects in at mice are not due to changes in hypothalamic neurotransmitters related to reproduction.

We believe that our further analyses will establish whether the defects are due to changes in gonadotropin gene expression (i.e. extra-gonadal) or inherent to testis itself (i.e. intra-gonadal) in *at* mutant mice. **Clearly, the studies initiated in this project through the support from The Moran Foundation have immense potential and form the basis for all our future investigations of this project.**

Publications and Meetings attended during the project period:

1) Invited Speaker, European Congress of Endocrinology, Turin, Italy. June 07-June 14, 2001

2) Borrelli E, **Kumar TR** and Sassone-Corsi P. (2001) Mouse models to study the pituitary-testis interplay leading to regulated gene expression. In: " Transgenics in Endocrinology" Ed: Matzuk MM, Brown CE and **Kumar TR**, Humana Press, Totowa, NJ, PP: 91-114.

3) **Kumar TR,** Varani SA, Wreford N, Telfer N, de Kretser D, Matzuk MM. (2001) Male reproductive phenotypes in double mutant mice lacking FSHβ and activin receptor IIA. **Endocrinology** 142:3512-3518.

4) Huang, S, Dass B and Kumar TR (2001) Characterization of the male reproductive phenotypes in the atrichosis (*at*) mutant mouse. (**Manuscript in Preparation**)