Some Cytological Effects of Sex Stimulating Hormones on Abnormal Mature Ova of Mice

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(Tables 1-3; Text-fig. 1)

(I) INTRODUCTION

A considerable amount of the literature has been published pertaining to the mechanism of ovulation in mammals by many authors. ASAMI (1920), ALLEN (1923), BRANCA (1925), ENGLE (1927), MAKINO (1941) and SIGEMORO (1947) have dealt with morphological features of the Graafian follicle and atresia in mature ovaries of rabbits, rats and mice. In one of the previous reports, NAKAMURA (1957) has concerned with some cytological studies on abnormal mature ova in mouse ovaries during different phases of oestrous cycle. It seems probable that the disintegration may take place in both the egg body and the granulosa cells under the influence of pituitary gonadtrophic hormones on the follicular apparatus.

In the present study, the effects of sex stimulating hormones derived from the anterior pituitary gland, placenta gland and pregnant mare serum of horses, on abnormal mature ova were investigated cytologically in the sexual cycle of mice.

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(II) MATERIAL AND METHOD

Unmated mature female mice (*Mus musculus*) at 70～120 days of age furnished the material for the present study. There are Swiss-albinos purely bred in our laboratory and showed a normal sexual cycle with the vaginal smear method. On the first experiment, 25 individuals received injections of anterior pituitary sex gland stimulating hormones and the sex gland stimulating hormones of placenta "Synaphorin" (Teikoku Hormone MFG., Co.) 2 R.U., every day for 3 successive days in a period of the estrus stage. On the second experiment, starting 5 days befor the estrus stage, 25 individuals received injection of "Synaphorin" 2 R.U. per day for 3 successive days and again an injection of pregnant mare serum of horses, "Serotropin" (Teikoku Hormone MFG., Co.) 50 I.U. Twenty-four hours after injection, the ovaries were fixed with Bouin's solution. The sections were made according to the usual paraffin method and stained with Heidenhain's iron-haematoxylin.

(III) OBSERVATIONS

(1) Mature mice received injections of mixed preparation of anterior pituitary sex gland stimulating hormone and the sex gland stimulating hormones of placenta "Synaphorin" (A.P.H. treated experiment): From the anatomical view-point, the ovaries of 25 treated mice were more heavily weighed than normal ovaries. Microscopical observations revealed that the abnormal mature ova proceeding further than the first maturation stage are very smaller in number in hormone treated mice than in the untreated mice: twenty-five mice which received the injection of Synaphorin showed in their ovaries 202 normal ova, 45 abnormal ova, and 48 polyovular in total (Tables 1 & 3). In the former study, the author (1957) has observed in untreated ovaries from thirty mice 157 normal, 85 abnormal and 80 polyovular ova in total (Table 3).

Abnormal mature ova showed the chromosomes lying on the equatorial plate of the

Table 1. Number of normal mature ova and abnormal mature ova in A.P.H. treated mouse ovaries

Mouth Number	Body weight in gram	Phase of oestrous cycle	Ovary weight in mg.		Normal mature ova			Abnormal mature ova			Polyovular ova		
			Ovary		Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left	Right	Left		Right	Left		Right	Left	
No. 8	21.0	Proestrus	19.0	12.5	10	6	16	0	1	1	4	0	4
No. 11	23.5	//	16.5	25.0	2	3	5	0	0	0	0	1	1
No. 14	24.0	//	20.0	19.0	3	6	9	1	2	3	1	0	1
No. 23	22.5	//	18.5	12.5	8	4	12	0	0	0	1	1	2
No. 25	23.0	//	17.5	17.0	8	1	9	0	0	0	0	0	0
No. 1	21.5	Oestrus	13.5	16.9	4	7	11	1	1	2	3	1	4
No. 24	24.0	//	17.5	21.0	5	3	8	0	0	0	0	1	1
No. 6	19.0	Metoestrus	11.0	12.5	1	2	3	0	0	0	1	1	2
No. 18	23.0	//	20.0	15.0	3	2	5	6	1	7	2	0	2
No. 19	23.5	//	21.0	19.0	5	2	7	1	0	1	0	0	0
No. 21	25.0	//	17.0	24.0	0	5	5	1	1	2	0	1	1
No. 3	17.0	Dioestrus	9.5	9.0	1	2	3	0	1	1	3	1	4
No. 10	18.0	//	21.5	18.0	0	2	2	2	0	2	0	1	1
No. 22	22.0	//	17.0	16.0	2	0	2	0	0	0	1	3	4
No. 2	21.0	Late Dioestrus	22.0	17.5	9	6	15	1	3	4	0	2	2
No. 4	24.0	//	18.1	14.0	5	3	8	0	2	2	1	2	3
No. 5	23.0	//	21.0	6.8	6	5	11	1	1	2	1	2	3
No. 7	24.0	//	16.8	17.9	6	9	15	2	5	7	2	2	4
No. 9	23.0	//	13.5	16.0	7	8	15	0	0	0	0	0	0
No. 12	24.0	//	17.0	15.0	5	4	9	1	0	1	1	1	2
No. 13	24.0	//	20.5	21.5	4	3	7	1	0	1	1	0	1
No. 15	22.0	//	21.0	20.5	5	2	7	4	1	5	1	0	1
N0. 16	24.5	//	18.5	19.5	2	5	7	0	2	2	2	0	2
No. 17	18.0	//	15.0	17.0	3	2	5	0	1	1	2	0	2
No. 20	24.5	//	18.7	18.0	6	0	6	0	1	1	1	1	2

Table 2. Number of normal mature ova and abnormal mature ova in P.M.S. treated mouse ovaries

Mouth Number	Body weight in gram	Phase of Oestrous cycle	Ovary weight in mg.		Normal mature ova			Abnormal mature ova			Polyovular ova		
			Ovary		Ovary		Total	Ovary		Total	Ovary		Total
			Right	Left	Right	Left		Right	Left		Right	Left	
No. 4	22.5	Proestrus	28.0	26.0	10	8	18	1	3	4	1	2	3
No. 7	21.5	〃	23.0	24.0	9	7	16	2	1	3	1	3	4
No. 11	30.0	〃	37.0	26.5	11	7	18	0	1	1	3	0	3
No. 12	27.5	〃	24.5	34.5	10	7	17	3	1	4	1	0	1
No. 16	21.0	〃	27.0	20.5	9	4	13	1	1	2	0	1	1
No. 18	22.0	〃	24.0	23.5	14	8	22	2	2	4	0	0	0
No. 19	24.0	〃	23.0	22.5	7	9	16	0	0	0	2	1	3
N0. 20	24.5	〃	29.5	30.5	9	9	18	1	1	2	0	1	1
No. 22	24.0	〃	14.0	22.0	9	15	24	1	1	2	0	0	0
No. 1	25.0	Oestrous	27.5	25.0	8	2	10	0	0	0	0	0	0
No. 8	23.5	〃	27.0	24.0	7	9	16	0	0	0	1	1	2
No. 10	23.0	〃	18.5	19.0	6	7	13	1	2	3	1	1	2
No. 13	27.5	〃	22.5	31.5	7	10	17	2	0	2	1	0	1
No. 14	27.5	〃	23.0	24.0	3	7	10	1	1	2	0	0	0
No. 21	28.0	〃	17.5	20.0	9	7	16	1	1	2	1	1	2
No. 24	23.0	〃	15.0	23.0	5	6	11	1	1	2	1	0	1
No. 5	23.0	Metoestrus	28.5	18.0	5	2	7	2	2	4	1	0	1
No. 6	23.0	〃	24.5	21.0	2	3	5	2	0	2	1	0	1
No. 15	29.0	〃	27.5	22.5	2	5	7	1	1	2	0	1	1
No. 23	25.5	〃	16.5	23.5	3	3	6	1	1	2	0	0	0
No. 2	23.5	Late Dioestrus	29.5	35.0	2	4	6	0	0	0	0	0	0
No. 3	23.0	〃	27.0	25.0	4	6	10	1	1	2	1	2	3
No. 9	20.5	〃	16.5	16.0	1	1	2	0	0	0	0	0	0
No. 17	24.0	〃	17.0	11.0	4	0	4	0	0	0	0	1	1
No. 25	22.0	〃	12.5	10.5	1	5	6	0	1	1	0	1	1

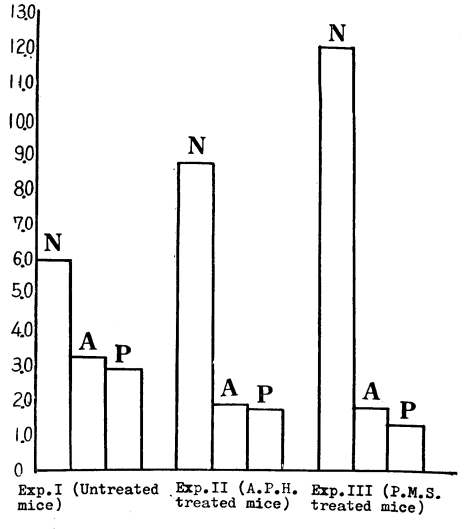
first spindle, having no nuclear membrane and antrum. Some ova showed the second polar spindle at metaphase accompanied by the first polar body in the follicle with a few antra. Some others showed the completion of the second division offering the second polar body, though no antrum follicle has been formed. There occur some chromosome abnormalities and the aberrant first division showing a tetra-polar spindle. In the extreme case, the ovum contained many vacuoles in its ooplasm. The abnormal mature ova were abundant in number in untreated ovaries. The detailed descriptions regarding the abnormal ova showing further advance toward the first maturation division are referred to the author's former papers (1957, '59).

The evidence here presented indicates that the abnormal ova decrease in number in treated ovaries in comparison with those in untreated ovaries, probably under the influence of sex stimulating hormones.

(2) Mature mice received injections of Synaphorin and mare serum of horses

Table 3. Total number of normal mature ova and abnormal mature ova in the mouse ovaries at three experiments

Method	Oestrous cycle	Number of individual	Normal mature ova	Abnormal mature ova	Polyovular ova
Exp. I (Untreated mice)	Proestrus	6	45	32	18
	Oestrus	5	20	15	9
	Metoestrus	7	28	8	18
	Dioestrus	4	0	4	9
	Late Dioestrus	8	64	26	26
	Total	30	157	85	80
Exp. II (A.P.H. treated mice)	Proestrus	5	51	4	8
	Oestrus	2	19	2	5
	Metoestrus	4	20	10	5
	Dioestrus	3	7	3	9
	Late Dioestrus	11	105	26	21
	Total	25	202	45	48
Exp. III (P.M.S. treated mice)	Proestrus	9	162	22	16
	Oestrus	7	93	11	8
	Metoestrus	4	25	10	3
	Late Dioestrus	5	28	3	5
	Total	25	308	46	32



N. Normal mature ova; A. Abnormal mature ova; P. Polyovular ova.

Text-fig 1. Average number of normal mature ova, abnormal normal mature ova and polyovular ova in the mouse ovaries at three experinemts

"Serotropin" (P.M.S. treated experiment): Microscopical observations have revealed that abnormal mature ova preceding further than the first maturation stage are very few in number in P.M.S. treated mice than in the untreated mice. Twenty-five treated mice showed in their ovaries 308 normal, 46 abnormal and 32 polyovular ova in total (Tables 2 & 3).

The data here obtained indicate the remarkable reduction in number of abnormal ova in P.M.S. treated mice than in A.P.H. treated mice. Particularly, the decrease in number of abnormal ova showing an immature condition of granulosa cells is remarkable.

Based on the findings in this study, the conclusion is possible that the reduction in number of abnormal ova may be explicable by the effect of sex stimulating hormones. Also, the evidence presented in the two experiments has indicated that the abnormal ova significantly decrease in number in P.M.S. treated ovaries in comparison with those in A.P.H. treated mice (Text-fig. 1). It is evident further that the sex stimulating hormones are not effective on polyovular follicles.

(IV) DISCUSSION

A good deal of work have been published so far on the mechanism on the follicular atresia in mammals (ASAMI 1920, ENGLE 1927, LANE 1938, PLISKE 1940, SIGEMORO 1947, etc.). In one of the previous papers, NAKAMURA (1957) dealt with a cytological study on abnormal mature ova in ovaries of mice obtained at different phases of oestrous cycle. The abnormal ova showing a further advance towards the first maturation division were very abundant in number in the mature ovary, being 35% in total. It seems probable that the disintegration takes place in either in the egg body or in the granulosa cells. From the findings obtained it is presumable that there is a relation between the maturation of the Graafian follicle and the effect of sex stimulating hormones.

The present study deals with a cytological effect of sex stimulating hormones on abnormal mature ova of mice. Microscopical observations have revealed that abnormal mature ova preceding further than the first maturation stage are very few in number in hormone treated mice than in the untreated mice. Especially, the P.M.S. treated mice showed a remarkable reduction in number of the abnormal mature ova. Recently, many papers have appeared regarding polyovulation under the effect of anterior pituitary hormone or pregnant mare serum in certain vertebrates (PARKES 1943, ROBINSON 1949, GATES 1956, GREEN 1956, RAESIDA & LAMOND 1956, OKIGAKI 1958, etc.). Based on the author's experiments, it is presumed that abnormal mature ova which failed to ovulate may undergo normal ovulation under the influence of sex stimulating hormones.

(V) SUMMARY

The effects of sex stimulating hormones on abnormal mature ova of mice were investigated cytologically during the sexual cycle. The results indicate that abnormal mature ova preceding further than the first maturation stage are very few in number in hormone

treated mice than in the untreated mice. Especially, the P.M.S. treated mice indicate the remarkable decrease in number of abnormal ova. Further the decrease in number of abnormal ova showing an immature condition of granulosa cells is remarkable. It seems probable that the sex stimulating hormones are not effective on polyovular follicles. Based on the results of the present study, the assumption is possibly made that abnormal mature ova which failed to ovulate may undergo ovulation under the effect of sex stimulating hormones.

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