

## RESTORATION OF FERTILITY IN SUCCESSIVELY OLDER E-LOW FEMALE RATS <sup>1</sup>

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Several years ago it was noted in this laboratory that the usual 'curative' dose of wheat germ oil did not invariably lead to the birth of living young when administered later than the first half year of life to E-low female rats of proved sterility. This led to standardization of the test animal as a female under 6 months of age whose trial resorption gestation was begun at 60 days.

In the study herein presented, nearly 300 rats, ranging in age from 3 to 18 months, have been given graded doses of wheat germ oil. All rats were reared on the standard E-low diet 427 <sup>2</sup> and were bred for their trial gestation at the first

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<sup>2</sup>Diet 427:

	%
Casein (commercial)	27
Cornstarch (cooked)	35
Lard	22
Cod liver oil	2
Yeast	10
Salt mixture no. 185	4

(The mixed diet without the cod liver oil was allowed to stand for 2 weeks at room temperature to permit the rancid substances of the lard to destroy incipient traces of vitamin E. The cod liver oil was added just before feeding.)

pro-estrus after the sixtieth day of age. The wheat germ oil was administered in a single dose by stomach tube on the day vaginal spermatozoa were encountered in the case of the groups receiving the lower levels of oil. In the group receiving 4 gm. of wheat germ oil, a 2-gm. dose of the oil was given on the first day and the remaining 2 gm. on the following day. In the case of the group on the 10-gm. level, five doses of 2 gm. each were given on alternate days.

Groups were bred at monthly intervals but in table 1, age groups which showed similar responses are combined. The

TABLE 1  
*Capacity of wheat germ oil to restore fertility to successively older female rats of proved sterility*

WHEAT GERM OIL	AGE IN MONTHS	NUMBER OF COPU- LATIONS	PLACENTAL SIGN		NUMBER OF LITTERS	PER CENT LITTERING AFTER SHOWING PLACENTAL SIGN	AVERAGE NUMBER LIVING YOUNG PER LITTER
			Number	%			
gm.							
0.5	3-6	21	21	100	21	100	7.3
0.5	7-9	34	28	82	15	54	4.3
0.5	10-11	19	16	84	1	6	5.0
1.0	7-9	31	22	71	13	59	6.5
1.0	10-11	30	21	70	1	5	3.0
2.0	8-10	32	23	72	10	43	5.4
2.0	11-12	30	16	53	1	6	3.0
3.0	9-12	30	20	67	5	25	2.4
4.0	11-12	32	20	63	6	30	3.0
10.0	15-18	24	8	33	0	0	0.0

table shows that during the first half year of life, a single dose of 0.5 gm. of wheat germ oil invariably led to the birth of living young. An inferior outcome when 0.25 gm. wheat germ oil was fed has led us to designate 0.5 gm. as the minimal effective single dose (MED) of wheat germ oil for the restoration of fertility. The same dose, when administered from the seventh to the end of the ninth month of life, led to the birth of living young in only half the cases, and when given from the tenth to the eleventh month, was almost entirely inefficient. A considerable elevation of wheat germ oil dosage

(two to four times) in the period elapsing from the seventh to the eleventh month does not substantially increase the instances of the birth of living young. Yet pronounced elevation of dosage does have an effect. From the eleventh to the twelfth month (a period, as has been mentioned, of virtually complete ineffectiveness of the MED of wheat germ oil) an eightfold elevation of the dose (4 gm.) led to the birth of living young in approximately a third of the cases (although the litter size remained subnormal).<sup>3</sup> From the fifteenth to the eighteenth month of life the administration of as much as 10 gm. of wheat germ oil did not lead to the birth of living young.

Until the end of the first half year of life, interference with implantation was not observed. In a total of 1211 test animals 3 to 6 months of age and of proved sterility, 94.7% have shown the placental sign following the next positive mating. This figure happens to be actually somewhat above the average for our stock colony (93%). This is not in agreement with the reports of Bacharach et al. ('37, '38) who state that they have found a clearly subnormal proportion of implantations in animals which had undergone a resorption gestation. Nevertheless, a comparison of the instances of copulation and the implantations following shows that there is evidence of increasing failure in implantation as age progresses. Established occlusion of the oviducts from infection explained about

<sup>3</sup>The possibility of increasing the number of instances of the birth of living young by fourfold dose increase of wheat germ oil was demonstrated by another group of twenty-two females on the E-low regimen but allowed to attain the ninth month of life without a trial gestation. Half of these received 1 gm., half of them 4 gm., of wheat germ oil on the inauguration of a test gestation. The results are subjoined. Here also the litter size was not improved by quadrupling the dose.

WHEAT GERM OIL	AGE IN MONTHS	NUMBER OF COPU- LATIONS	PLACENTAL SIGN		NUMBER OF LITTERS	PER CENT LITTERING AFTER SHOWING PLACENTAL SIGN	AVERAGE NUMBER LIVING YOUNG PER LITTER
			Number	%			
gm. 1.0	9-10	11	10	91	4	40	4.5
4.0	9-10	11	10	91	7	70	4.3

half of the implantation failures, but we are not yet ready to state to what extent actual failure of the implantation mechanism accounts for the remaining half of such cases. In animals 15 to 18 months old no implantation sites were ever encountered, although uterine bleeding had been observed.

At autopsies, the uteri were invariably dark brown in color and were usually stiff and hard. Martin and Moore ('36, '38) found that rats (whether virgins or with the history of a single resorption gestation) when maintained for long periods on a vitamin E-low diet, developed a brown uterus which could not be restored to normal color either by treatment with a vitamin E concentrate or by shift to a diet consisting of natural food. Barrie ('38) found that the uterine pigmentation progressed with age. After 4 months on the diet there was a slight increase in the size of the uterus and definite, although slight, discoloration; after 5 months the discoloration and enlargement<sup>4</sup> were more marked and the condition was markedly expressed after 6 months. A resorption accelerated the appearance of the pigmentation. Barrie stated that the vitamin E requirement for restoring fertility increased as these changes advanced and postulated that pregnancy would be impossible if the uterine changes had progressed to an advanced stage.

We have given some attention to the phenomenon of uterine pigmentation. Twenty-seven E-low rats approximately 6 months of age were divided into three groups. Group 1 was maintained on the vitamin E-low diet throughout the experiment. Group 2 received 4 drops of wheat germ oil in addition to the vitamin E-low diet. Group 3 received 4 drops of wheat germ oil and were bred at the beginning of the experiment. All individuals were sacrificed at the end of 20 days. In agreement with the findings of Martin and Moore ('36, '38), the uteri were as darkly pigmented after the administration of wheat germ oil as in the control group, if the animals were

<sup>4</sup> The enlargement is not invariable. The average weight of the uteri and oviducts of fourteen of our E-low females 19 to 20 months old, with a history of two pregnancies, was 0.78 gm.; for eight natural-food virgin animals of the same age, the average figure was 1.31 gm.

not bred, but in the case of pregnant animals given wheat germ oil (in all cases living fetuses were found) the uterus was normal in color.<sup>5</sup>

In the present communication, we have contented ourselves with statements of fact and do not venture an explanation of the increasing reproductive disability of increasingly older vitamin E-low females. The need for elevated dosage with E shown by older E-low females can, we feel, hardly be taken to indicate that the embryos require more E with advancing age of the mother, but is preferably interpreted as due to: (1) increasing incapacity of the placental mechanism to deliver adequate vitamin E to the embryos, and (2) final failure of the implantation mechanism.

#### SUMMARY

The reproductive performance of successively older vitamin E-low female rats permits us to recognize three periods:

1. From the age of 3 to 6 months, at which time the response to a single dose of 0.5 gm. of wheat germ oil invariably leads to the birth of normal sized litters of living young. Only this period can be recommended for assay studies.

2. From the seventh to the twelfth month, at which time the response is variable; in the earlier part of this period only half the animals give birth to living young with this dosage (0.5 gm.) and these are undersized litters; moderate elevation of the dosage does not greatly change the result. Toward the eleventh month practically no animals respond to 0.5 gm. but even in this period great elevation of dosage shows a definite effect, for almost a third of such animals respond to 4.0 gm. of wheat germ oil, with living young but small litters.

3. After the fifteenth month of life no response can be secured with a twentyfold increase of dosage.

<sup>5</sup>Barrie ('38) observed a normal uterus in one instance of a female that had cast young.

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