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Congenital Malformations in Mice After Gonadotropin-Induced Ovulation (38811)

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For the preovulatory growth of the follicle, as well as for the induction of ovulation in mammals and man, the hypophyseal gonadotropic hormones FSH and LH, and the ovarian steroid hormones related to them, play an important role. It is well known that the process of ovulation can be induced and regulated by exogenous administration of gonadotropins.

Recent papers of both experimental animal and clinical content deal with limb malformations in connection with a shift of sex ratio in mice (1), and malformations of the central nervous system in man (2–4) due to hormonally stimulated ovulation. As yet it is unknown how much the use of hormones for inducing ovulation before pregnancy can be considered responsible for malformations during embryogenesis.

In the present paper, malformations of the central nervous system and of limbs, as a consequence of stimulated ovulation in mice, are described.

Materials and Methods. Randomly bred virgin Swiss albino mice (353), 6–9 wk of age were given ip injections with an FSH–LH mixture (PMS, "Gestyl," Organon, Holland) and LH (HCG, "Pregnyl," Organon, Holland). PMS was injected at noon, and after 48 hr HCG was given. Two dosages were used: (a) 5 IU PMS and 5 IU HCG dissolved in 0.15 ml and 0.1 ml of 0.9% saline, respectively; (b) 10 IU PMS and 10 IU HCG each in 0.1 ml of 0.9% saline. The solutions were freshly prepared just before injection. After the injection of HCG the animals were mated overnight with untreated males of the same strain. Mating was detected next morning by the occurrence of a vaginal plug. This day was counted as the first day of gestation. Because of blocked delivery in most of the females, cesarean section was performed on the 19th or 20th day of gestation. The sex of the young was determined by inspection of the spatial relation of the genital papilla and anus.

Three experiments were performed: (a) Ninety females were injected with 5 IU PMS and 5 IU HCG between February and April (5 IU-spring group); (b) 100 females with 5 IU PMS and 5 IU HCG between September and November (5 IU-autumn group); and (c) 163 females received 10 IU PMS and 10 IU HCG in October and November (10 IU-autumn group).

Results. General observations. Although the animals were kept in air-conditioned rooms under constant light–dark periodicity (12 hr light and 12 hr dark), differences in frequency of vaginal plugs and pregnancy between spring and autumn groups have been observed. In untreated mice, the vaginal plug frequency was three times, and the pregnancy frequency three to five times higher in the February–April group than in the October–November group (Table I). Stimulation of the cycle with gonadotropic hormones increased mating frequencies and pregnancies 1.5 times in the 5 IU-spring group, two times in the 5 IU-autumn group, and three times in the 10 IU-autumn group (Table I).

Outcome of pregnancy. The influence of PMS and HCG treatment on pregnancies is given in Table I. In five of the 32 litters of the IU-spring group nine abnormal embryos were found, whereas in the 5 IU-autumn group the progeny was normal. In the 10 IU-autumn group, however, eight of the 27 litters contained 10 abnormal embryos. A shift in the sex ratio occurred only in those groups in which abnormal embryos were observed (Table I).

Malformations observed. Malformations were observed in the 5 IU-spring and in the 10 IU-autumn group. In both groups abnormal development of limbs and of the

TABLE I. EFFECTS OF THE INDUCTION OF OVULATION BY GONADOTROPIC HORMONES ON THE PROGENY IN MICE.

Group	5 IU-Spring		5 IU-Autumn		10 IU-Autumn	
	Treated	Control	Treated	Control	Treated	Control
No. of females	90	180	100	330	163	360
Vaginal plug	34	46	24	35	38	27
No. pregnant	32	44	22	32	27	18
Average litter size	14.3±6.5	12.4±2.3	16.9±6.9	11.8±2.1	9.3±7.5	10.8±2.4
Abnormal litters	5[a]	ϕ	ϕ	ϕ	8[b]	ϕ
Total progeny	458	545	372	378	251	195
Embryos abnormal	9[b]	ϕ	ϕ	ϕ	10[b]	ϕ
Sexed progeny	336	521	372	375	251	195
Sex ratio						
Males: Females	1:1.8[c]	1:0.96	1:1.1	1:0.88	1:2.0[c]	1:0.86

[a] P values computed by Fischer–Yates "exact test" ($P < 0.05$).
[b] P values computed by Fischer–Yates "exact test" ($P < 0.01$).
[c] P values computed by chi-square test ($P < 0.001$).

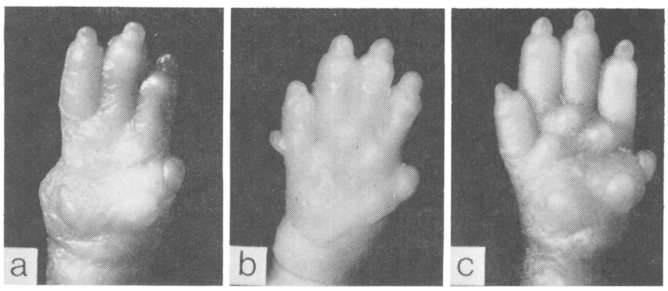

FIG. 1. Defects of the right forelimbs (ventral view, thumb right) in the offspring of mice whose ovulation was stimulated with gonadotropins. (a) Oligodactylism after administration of 5 IU PMS and 5 IU HCG. (b) Polydactylism after treatment with 10 IU PMS and 10 IU HCG. (c) Control.

central nervous system occurred. Two different types of digital anomalies were prominent within these groups: (1) oligodactylism (absence of the 5th digit; Fig. 1a) in the 5 IU-spring group and (2) polydactylism (rudiment of a digit, with or without a nail, present on the outside of the 5th digit; Fig. 1b) in the 10 IU-autumn group. The malformations were exclusively observed in the forelimbs, and in most cases the right side was affected. Only one left-side oligodactylism (male) and one bilateral polydactylism (female) were observed. A total of 16 embryos had limb malformations, among these were 12 females. Anomalies of the nervous system were observed only in female embryos: a spina bifida occulta was found in the 5 IU-spring group, one case of exencephaly (Fig. 2), and one animal with the left eye opened in the 10 IU-autumn group.

Discussion. Effects of gonadotropin-induced ovulation in the offspring of mice were examined.

In humans, after induced ovulation, multiple gestations, as well as a risk of abortion, premature labor, placental insufficiency, and intrauterine death are not uncommon (5). Reports of malformed infants are more sporadic, and the anomalies (anencephaly) are not directly correlated with such hormone treatment (2, 6, 7), even though chromosomal examinations after induced ovulation have shown that such a treatment increases the risk of chromosomal anomalies in blastocysts (8) and conceptus (9). Re-

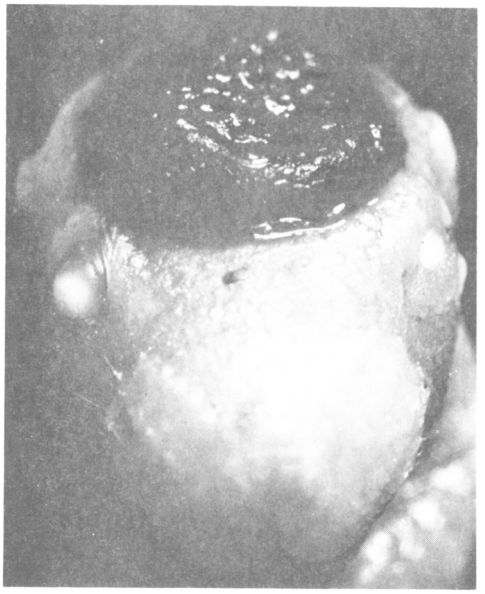

FIG. 2. Exencephaly after gonadotropin-stimulated ovulation (10 IU PMS and 10 IU HCG). Female embryo obtained by cesarean section on the 20th day of gestation.

sults of the experiments presented here, show unequivocally that there is a tendency of administered gonadotropins to lead to anomalies in sex distribution and to malformations of embryos. The observed dose dependency of these defects can be explained by seasonal influences on the endogenous level of gonadotropic hormones. (The occurrence of abnormal litters (abnormal embryos) in the 5 IU-spring group in comparison to the 5 IU-autumn group is statistically significant at $P < 0.01$ ($P < 0.001$) and the shift of sex ratio at $P < 0.001$.) Diurnal influences, associated with fluctuations in light–dark cycle, can be excluded because of strict, automatically controlled conditions.

The etiology of the effects after ovulation stimulation should be considered as genetic, because of the well-known fact that induced teratogenesis is dependent on embryonic stage. The known critical period of organogenesis for malformation-induction of the forelimbs and the central nervous system (10–13) excludes a role for gonadotropic hormones that are administered before mating, in causing such malformations. Since ovarian hyperfunction, as a response to gonadotropic hyperstimulation, manifests itself by an elevated level of sex steroids, this hormonal imbalance at the time of ovum maturation, which is the sensitive phase for inducing genetic defects (14), could increase the tendency of defective genes to be expressed. Consequently, since the shift of the sex ratio to an increased proportion of females was found only in those groups of mice in which anomalies were observed, this can be interpreted as an additional x-chromosomal lethal mutation (15). This is in contrast to previous suggestions (16).

Although extrapolations from animals to man must be guarded, one must not overlook the occurrence of analogous birth defects observed in both infants and mice after hormonally stimulated ovulation.

Summary. Virgin mice were treated with gonadotropic hormones in order to induce superovulation; at term the embryos were removed by cesarean section.

This treatment induced malformations (mainly forelimb defects and to a smaller extent central nervous system anomalies), as well as an altered sex ratio in the offspring. Both phenomena were statistically significant. These hormone-induced effects on the progeny were significantly dependent on both the dosage of hormones and the time (season) of administration.

Because the time of administration (before mating) of gonadotropic hormones does not coincide with the critical period during embryogenic development for the teratogenic induction of malformations in the limbs and in the central nervous system (ca. 8th–12th day of gestation), the investigated defects are interpreted as of mutagenic origin.

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