BIOLOGY OF REPRODUCTION 16, 216–227 (1977)

Effects of Fetal or Maternal Hypophysectomy on Endocrine Organs and Body Weight in Infant Rhesus Monkeys (Macaca mulatta): With Particular Emphasis on Oogenesis

BELA J. GULYAS, GARY D. HODGEN, WILLIAM W. TULLNER and GRIFF T. ROSS

Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Auburn Building 203, Bethesda, Maryland 20014

ABSTRACT

The effects of fetal and maternal hypophysectomy on the development of the fetal endocrine glands in the rhesus monkey (*Macaca mulatta*), with particular emphasis on the progress of oogenesis is reported. Maternal hypophysectomy was performed between 58 and 71 days of gestation. Fetal hypophysectomy was performed between 114 and 117 days of pregnancy. Gravimetric data on body and organ weights were compiled on term infants. Completeness of hypophysectomy was confirmed by histological examination of the sella turcica from each operated animal. Morphological and quantitative studies were performed on serially sectioned ovaries of term infants. Maternal, but not fetal, hypophysectomy produced a generalized fetal growth retardation. Term infant mean adrenal, but not thyroid weight was elevated significantly ($P<0.05$) following maternal hypophysectomy. Conversely, mean ovarian, adrenal and thyroid weights were markedly reduced ($P<0.05$) in term infants following fetal hypophysectomy. Maternal hypophysectomy had no adverse effects on the morphology of term infant ovaries. In contrast, within ovaries of infants following ablation of the fetal hypophysis, the course of oogenesis was disordered and oocytes had undergone atresia. Quantitative data indicate that fetal hypophysectomy resulted in a reduction in total numbers of germ cells and a significant increase in the percentage of germ cells undergoing atresia. Although the total numbers of germ cells in ovaries of term infants in the absence of the maternal hypophysis was half that in intact controls, the percentage of atretic germ cells was similar to the controls. We conclude that a dynamic interaction of fetal and maternal hypothalamic-pituitary axes governs the development of the fetus and its endocrine organs. Survival of gametes within the fetal ovaries of these primates is dependent primarily on secretions of the fetal pituitary.

INTRODUCTION

Although the sequential morphological development of the fetal human and monkey ovary has been described in detail (Baker, 1963, 1966), it remains unclear whether or not fetal ovarian differentiation, particularly oogenesis, is dependent on extraovarian influences. In the human fetus, the sources of gonadotropin secretion that may potentially affect fetal organogenesis may be the placenta, or fetal and maternal pituitary glands. In contrast, in the rhesus monkey placental secretion of macaque chorionic gonadotropin (MCG) ceases by the 42nd day of gestation (Hodgen et al., 1974, 1975b; Hobson et al., 1975; Atkinson et al., 1975), leaving only the fetal and maternal pituitary glands as sources of gonadotropic stimulation. To determine whether or not gonadotropins from either of these two alternative sources affect the development of the fetal ovary, we have examined the morphology of infant ovaries at term following surgical ablation of the maternal pituitary during the period from the 58th to 71st day of gestation or alternatively, of the fetal pituitary at the 114th to 117th day of gestation. To facilitate comparisons, gravimetric data on body weights and of other endocrine organs have been included.

Accepted September 20, 1976.
Received August 17, 1976.

MATERIALS AND METHODS

Monkeys were housed and maintained as described previously (Hodgen et al., 1974). Diagnoses of pregnancy and estimations of the time of fertilization were done as reported earlier (Hodgen and Ross, 1974). Maintenance of pregnancy was checked periodically (4 to 6 weeks) by rectal palpation.

Following abdominal hysterotomy under either

sodium pentobarbital or ketamine hydrochloride anesthesia, fetal hypophysectomy was performed between 114 and 117 days of pregnancy, using a transbuccal approach. Nearly all of the amniotic fluid was withdrawn into syringes before orienting the head of the fetus through the uterine incision (about 4 cm). No other parts of the fetus were exposed and no attempt was made to prevent the fetus from breathing. After clearing the fetal mouth of amniotic fluid and mucus, an 18 gauge (bevel-tipped, stainless steel) needle was used to probe progressively downward along and perpendicular to the midline of the palate at approximately one mm intervals and at an angle nearly 130 degrees below the vertical plane aligning the fetal cranium and spine. Upon reaching the soft palate, the needle was inserted with slight pressure until meeting the sella turcica. After ensuring that placement (angle and depth) of the needle was satisfactory, the sella turcica was punctured to a depth of about 2 mm. The bevel tipped needle was replaced by a blunt one of the same size. A 5 ml syringe was attached to permit aspiration of blood and tissue fragments. In a few instances the entire hypophysis was withdrawn by this aspiration procedure, but more often other efforts were needed to loosen pituitary tissue. In these cases, the blunt needle was removed and a dental scoop measuring 2 mm in diameter was inserted through the transbuccal canal and into the cavity of the sella. Using a rotating motion to loosen attached pituitary tissue, the contents of the sella were prepared for evacuation through the blunt needle. Bleeding, often associated with this scraping procedure, was controlled effectively by applying pressure onto the opening of the wound using a sterile cotton tipped applicator. In order to decrease the chance of survival of any remaining hypophyseal cells, 0.05 ml of 4 percent chromic acid solution was injected into the cavity of the sella and aspirated after 15 seconds. Upon completion of this procedure the head of the fetus was placed back inside the uterus, and the opening in the amnion and uterine musculature was closed. In some instances, all the amniotic fluid was injected back into the amniotic sac, but in other cases only a portion or none was returned. The presence or absence of amniotic fluid had no apparent influence on subsequent development of the fetus, but immediate postoperative convalescence of the mothers was improved when the intrauterine pressure was less than that of preoperative state.

Maternal hypophysectomy between 58 and 71 days in pregnancy was performed as described earlier (Tullner and Hodgen, 1974; Hodgen et al., 1975a; Tullner et al., 1975) using a parapharyngeal technique.

Pregnancies were terminated either by natural delivery or elective cesarean section near the expected term date (151 to 168 days). In all instances, the term infant was alive when delivered, but the vigor of some (infants) from ablated groups appeared to be much below that of infants from normal, intact pregnancies. Despite some obvious differences in real degree of maturity, these infant monkeys will be referred to as *term infants* in this report, on the basis of gestational age.

In this study we present findings from 4 fetal and 3 maternal hypophysectomies in rhesus monkeys, as well as 3 pregnancies in which no ablations were performed, and one sham operated animal as a control for fetal hypophysectomy.

Histology

In order to check the completeness of hypophysectomy of both mothers and fetuses, the sella turcica from each operated animal was removed either on the day of cesarean section or a day after natural delivery, fixed in Bouin's solution, decalcified in 5 percent formic acid, sectioned serially and stained with H & E. The sections were evaluated for completeness of hypophysectomy as described previously (Hodgen et al., 1975a).

In addition to the fetal gonads, maternal and term infant thyroids and adrenals were also removed, weighed and prepared for histological examinations at the time of autopsy. The weight and histological appearance of the adrenals and thyroids were used as additional evidence for determining completeness of hypophysectomy. Infant ovaries embedded in paraffin wax were serially sectioned (5 μm) parallel to the midsagittal plane.

Quantitative Histological Procedures

The objective of the quantitative histological studies was to determine: 1) the total number (N) of germ cells per ovary, and 2) the proportion of oogonia, oocytes and atretic germ cells in ovaries of all three groups of term infants. In order to obtain these two sets of information, several intermediate quantitative parameters had to be determined. For this purpose we adapted the method of Beaumont and Mandl (1962) as applied by Baker (1966), and briefly described below.

Ovarian volume (Vo) was determined for each organ. Because all microscopic measurements were made on fixed and embedded tissue, we redetermined the ovarian volumes after dehydration and the adjusted ovarian volumes were used in all calculations.

The proportion (P) of somatic versus germinal tissue was estimated by Chalkley's (1943) method. Between 300 and 400 cells were scored for each of the 17 ovaries[1]. In addition, a separate differential (d) count was made for each ovary, classifying an average of 770 germ cells per ovary. The germ cells in the differential count, as well as in the measurement of germ cell sizes, were classified into six categories: 1) normal oogonia; 2) normal oocytes between leptotene and diplotene, but without attenuated granulosa cells; 3) normal primordial follicles with attenuated granulosa cells; 4) normal primary follicles; 5) atretic oogonia and atretic early prophase of meiosis ('Z' cells) combined; and 6) atretic primordial and primary follicles. The criteria used to identify atresia were those used by Beaumont and Mandl (1962) and Baker (1966).

The mean cell volume (Vc) of the six categories of

[1] A small portion of one ovary from 2 intact, 2 maternal hypophysectomized and one fetal hypophysectomized animals was used for fine structural studies. Although gravimetric data were collected from the remainder of these infant ovaries, they were not included in any of the data presented here, except in Table 1 on fetal ovarian weights.

TABLE 1. Effects of fetal or maternal hypophysectomy on ovarian, adrenal and thyroid growth in term infant rhesus monkeys.

Group	Day of pregnancy		Term infant	Ovarian weight (mg)		Adrenal weight (mg)		Thyroid weight (mg)	
Intact term	Surgery	Delivery	body wt (g)	Total	per 100g body wt	Total	per 100g body wt	Total	per 100g body wt
1174	...	162 (N)	482	84	17	452	94	158	33
P-178[a]	(117 sham)	158 (N)	424	157	37	437	103	187	44
W-801	...	164 (N)	550	75	14	483	88	195	35
P-178[a]	...	164 (N)	470	99	21	327	65	171	41
$\bar{X}$			482	104	22	427	88	178	38
± SD			±52	±37	±10	±68	±16	±17	± 5
Maternal-hypophysectomy									
C-195	58	161 (N)	320	51	16	780	244	120	38
O-590	61	166 (C)	236	69	29	898	381	173	73
L-842	71	168 (C)	324	34	10	908	280	191	59
$\bar{X}$			293*	51	18	862**	302**	161	57
± SD			±50	±18	±10	±71	±71	±37	±18
Fetal-hypophysectomy									
9890	114	159 (N)	320	22	7	51	16	87	27
Q-837	117	151 (N)	291	28	10	41	14	58	20
52	114	161 (C)	498	61	12	59	12	84	17
W-534	114	155 (N)	432	48	11	37	9	61	14
$\bar{X}$			385	40*	10*	47*	13*	73*	20*
± SD			±97	±18	± 2	±10	± 3	±15	± 6

[a]Monkey P-178 was used during 2 consecutive pregnancies. During the first one, a sham fetal hypophysectomy was performed.

N = natural delivery; C = cesarean section delivery.

*Significantly less than Intact-term group ($P<0.05$), F statistics.

**Significantly more than Intact-term group ($P<0.05$), F statistics.

germ cells was calculated from formula $Vc = 4/3\pi r^3$, where r is the mean radius of the cells. The diameter of 40 cells was measured for each category of germ cells.

Before the population of germ cells could be calculated for each ovary, the mean volume of germ cells of all three groups had to be determined. These numbers were obtained from the equation:

$$M = (d_1v_1 + d_2v_2 \cdots d_xv_x)/X',$$

where M is the mean volume of germ cells, d is the percentage of each cell category (obtained from the differential count); v is the mean volume of the respective germ cell categories; and X' is the number of germ cell categories (six). The total volume of ovarian tissue occupied by germ cells (V) was determined for each ovary from the product of ovarian volume (Vo) and the proportion (percentage) (P) of the ovary occupied by germinal tissue,

$$V = VoP$$

The total number of germ cells/ovary was obtained from the equation:

$$n = V/M$$

Lastly, the numbers of oogonia, leptotene to diplotene oocytes, primordial follicles, etc, were calculated by multiplying the mean total population of germ cells (n) by the respective percentage of differential counts (d).

RESULTS

Body and Organ Weights

The data in Table 1 summarize the effects of fetal or maternal hypophysectomy and sham surgical procedures on ovarian, adrenal and thyroid wet weights, and total body weights, of term infant rhesus monkeys. Maternal hypophysectomy produced a generalized growth retardation ($P<0.05$) as shown by the body weights of this group in comparison with those of normal, intact pregnancies. Although the body weights of hypophysectomized term infants were somewhat lower than those of the intact controls, these differences were not significant ($P>0.05$). The body weights of infant monkeys may have reflected more the vigor of the mother and thus her ability to nourish the fetus *in utero*, than direct effects of the ablations themselves. In addition to these differences in body weights, infants resulting from pregnancies in which either ablation was performed obviously lacked the strength and vigor of comparable intact control monkeys. Since each infant was autopsied within 24 h after delivery, the survival rate of these hypophysectomized infants for more than 24 h was not evaluated. Following maternal hypophysectomy, mean infant ovarian weight was only half that of the comparable controls, but this difference was not significant and was primarily a function of reduced body weight (Table 1). In contrast, irrespective of body weight changes, the wet weight of newborn infant monkey ovaries was reduced significantly ($P<0.05$) following ablation of the fetal hypophysis.

As shown in Table 1, term infant mean adrenal weight was elevated significantly ($P<0.05$) following removal of the maternal hypophysis. Conversely, depriving the fetal monkey of its own pituitary gland caused a striking reduction ($P<0.05$) in weights of adrenals at term. In addition, the effects of both ablations were sustained when adrenal weights were corrected for total body weight.

Enlargement of the thyroid glands of infant monkeys from hypophysectomized mothers was not significant, but surgical removal of the fetal hypophysis resulted in a marked reduction ($P<0.05$) in mean thyroid weight, irrespective of changes in the body weights (Table 1).

Completeness of Hypophysectomy

From the histological examinations of the

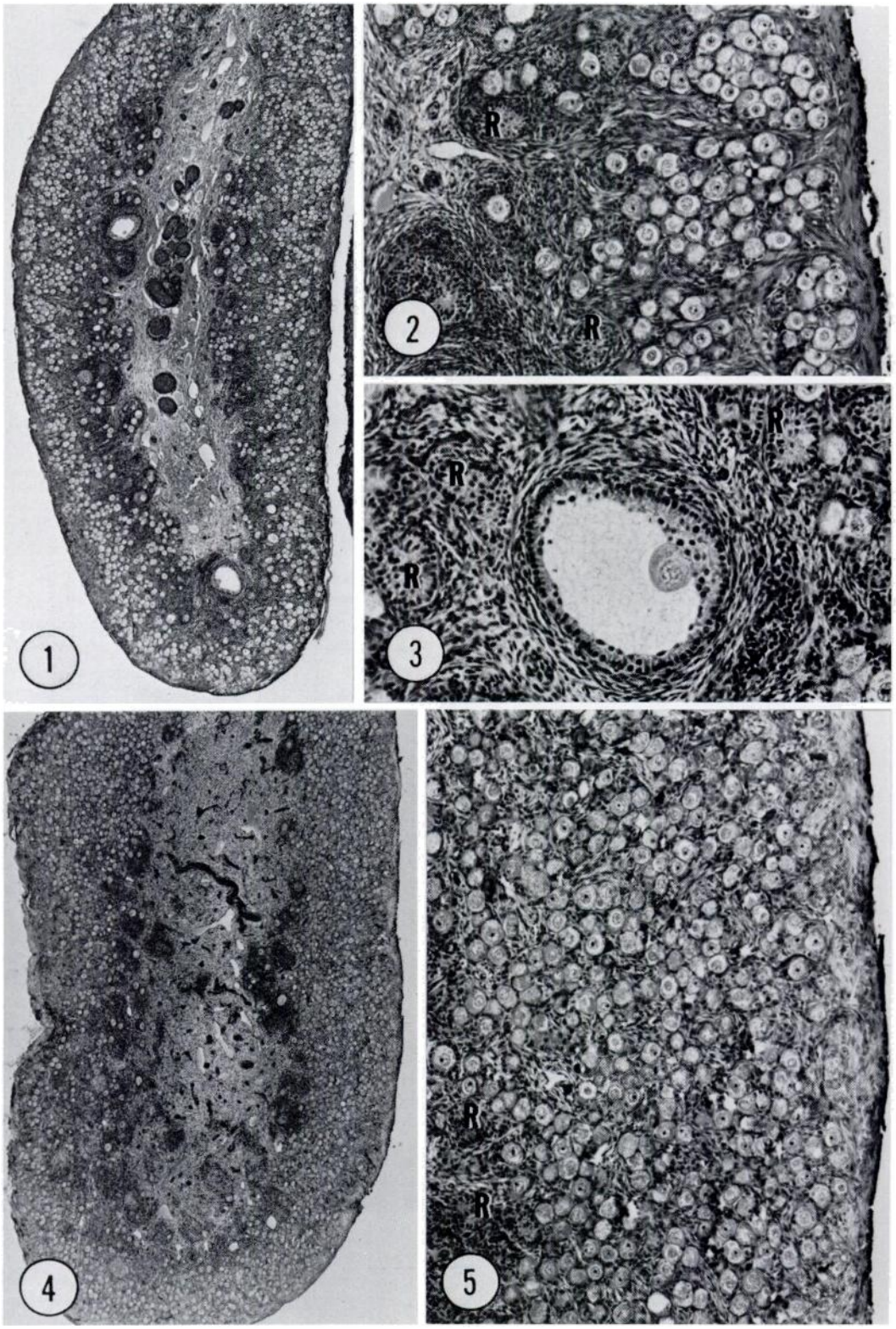
FIG. 1. Sagittal section of ovary from intact term infant. A normal progression of oogenesis is shown from the outer cortex toward the medulla. ×255.

FIG. 2. Higher magnification of a region of the cortex from the ovary illustrated in Fig. 1. Oogonia and primordial follicles occupy the outermost region of the cortex. Primordial follicles and primary follicles make up the inner portion of the cortex which is divided into lobules by the connective tissue. Rete ovarii (R) are well developed and involved in follicle formation. ×107.

FIG. 3. Intact infant ovary at higher magnification. Numerous rete tubules (R) are present. Two-to-three layers of cuboidal cells comprise the granulosa region of the antral follicle located centrally. The cuboidal cells do not surround the oocytes. Four-to-five layers of theca-like cells make up the outermost border of the follicle. ×150.

FIG. 4. Low magnification view of term infant ovary following maternal hypophysectomy. For the most part, it appears normal. ×25.5.

FIG. 5. Higher magnification of a portion of the ovarian cortex of a term infant whose mother was hypophysectomized. Lobulation is less distinct than in intact term infant ovaries. Rete ovarii (R) are present and are involved in follicle formation. ×107.



sella turcica it was confirmed that maternal hypophysectomy was complete in two of three adult monkeys. A few cells of the pars tuberalis were found in one animal (C-195); however, atrophy of her adrenal glands was comparable to that of the other hypophysectomized mothers, suggesting that the ACTH secretory activity of this remnant of pituitary stalk was minimal (Hodgen et al., 1975a). Histological examinations of the sella turcica from the operated infants showed that hypophysectomy was complete in all 4 monkeys.

Infant Ovarian Morphology

Normal intact term infants: In the ovaries of the intact control infants, the cortex occupied about two-thirds of the cross-sectional area (Fig. 1). Under the surface epithelium there was a wide layer of connective tissue. The amount of undifferentiated germ cells in the outer cortex differed considerably from animal to animal, confirming earlier reports of van Wagenen and Simpson (1965). Although occasional primitive egg masses were observed in the outermost portion of the cortex, this region of the ovaries consisted mostly of oogonia and leptotene to diplotene oocytes (Fig. 2). The central and the inner regions of the cortex consisted of primordial follicles, which made up the largest portion (37 percent of the total germ cells – Fig. 10). At the cortico-medullary border several primary follicles, consisting of a single layer of cuboidal cells, were present (van Wagenen and Simpson, 1965). In other primary follicles which contained 3 to 4 layers of cuboidal cells and reached 1.2 mm in diameter, the outer limit of the follicle was delineated by two to three layers of thecal-like cells. On the average 9 antral follicles were counted per ovary (Table 5). Most of the oocytes in the antral follicles lacked corona radiata cells (Fig. 3). In addition to the normal germ cells, numerous atretic oocytes and oogonia were counted. The rete ovarii system was developed, and it appeared to be involved in follicle organization (Figs. 2, 3). In summary, an orderly progression of oogenesis was observed from the inner to the outermost portion of the cortex, much like that described by van Wagenen and Simpson (1965).

After maternal hypophysectomy: Histologically the ovaries of infants from hypophysectomized mothers closely resembled those of the intact controls (Figs. 4, 5). Although individual variations existed, neither the Chalkley count (Table 4) nor the differential count (Fig. 10) discriminated between these two groups of ovaries in any of the categories examined. The only apparent difference was in the paucity of antral follicles (Table 5) in ovaries of infants born after maternal hypophysectomy. The thickness of medulla varied, but no gross differences were noted. The rete ovarii system appeared normal, although cross-sections of tubules were less numerous than in the intact ovaries (Fig. 5).

After fetal hypophysectomy: In all ovaries of the fetally hypophysectomized infants the cortex was narrower than the medulla (Fig. 6). In the ovaries of two fetuses, very few germ cells were present in the outer cortex (Fig. 7). Instead, interstitial cells formed disorganized aggregates without enclosing a germ cell. The middle to inner portion of the cortex in these ovaries showed early follicular organization, although the number of normal primordial, and especially primary follicles, was low (15 percent and 2 percent respectively—Fig. 10). In the ovaries of the other two animals numerous primitive nest cells were present in the outer cortex (Figs. 8, 9). Half the width of the cortex was occupied by oogonia, most of which were atretic (Fig. 9). Atretic primordial follicles made up a large portion of the inner cortex in these two animals. In general, an orderly progression of oogenesis could not be reconstructed in these ovaries. Normal primary follicles were few, and approximately 4 antral follicles were found per ovary (Table 5).

Invasion of the outer cortex by connective tissue cells varied considerably among the infants of this group. In all ovaries lobulation of the cortex was incomplete, and in some instances it was absent. Their rete cord system had undergone considerable degeneration or was absent. In summary, in ovaries of all infants born following ablation of the fetal hypophysis, the course of oogenesis was disordered, and most of the oocytes were undergoing atresia.

Ovarian Quantitative Data

Ovarian volumes: If it be assumed that the density of the term infant ovaries is uniform in all cases, then ovarian volume (*Vo*) would be a direct function of the weight of the organ. Accordingly, newborn infant ovarian volumes (Table 2) of intact monkeys and of infants following maternal or fetal hypophysectomy

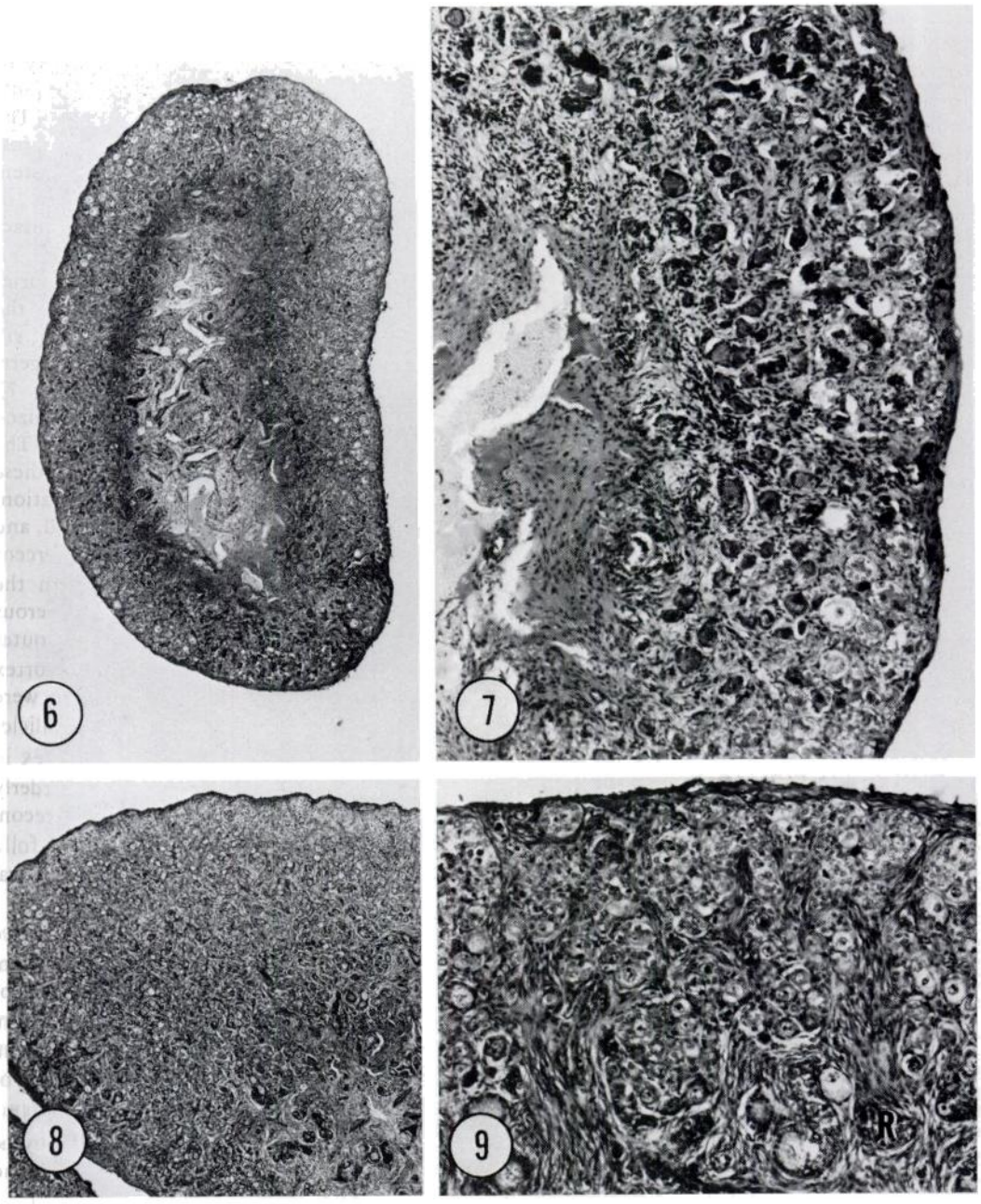


FIG. 6. Sagittal section of term infant ovary after ablation of the fetal hypophysis. Cortical zone is narrower than in intact, control monkeys. X25.5.

FIG. 7. Higher magnification view of term infant ovary after fetal hypophysectomy. Most of the cortex is disorganized. Lobulation and progression of oogenesis are absent. Rete cords are degenerated or they are not present. Most of the germ cells are atretic. X107.

FIGS. 8 and 9. Ovary from another hypophysectomized infant. Most of the cortex is disorganized although at selected areas the cortex is lobulated and some organized progression of oogenesis is present. Large numbers of atretic oogonia are in the outer cortex. Rete ovarii (*R*) are present near the cortico-medullary junction, however, disorganization and degeneration is noticeable. X25.5; X107.

TABLE 2. Actual (wet) and adjusted volumes of infant ovaries.

	No. of animals	No. of ovaries	Ovarian wet volume (mm^3)[a]	Wet ovarian volume/ 100 g body wt (mm^3)[a]	Adjusted volume after fixation (mm^3)[a]
Intact	4	6	46.7 ± 16.6 (33.8 – 70.7)	10.0 ± 4.6 (6.1 – 16.7)	25.8 ± 9.1 (18.7 – 38.9)
Maternal hypophysectomy	3	4	23.1 ± 7.9 (15.3 – 31.1)	8.3 ± 4.4 (5.2 – 14.5)	12.7 ± 4.3 (8.4 – 17.1)
Fetal hypophysectomy	4	7	17.7 ± 7.9* (9.9 – 27.0)	4.5 ± 1.0* (3.4 – 6.0)	9.8 ± 4.4* (5.4 – 14.9)

[a]Values are given as means ± standard deviation.
Numbers in parentheses represent range.
*Significantly less than Intact-term group ($P<0.05$), F statistics.

reflect their respective ovarian weights. Among all groups the volume of the ovaries after fixation and dehydration was 55 percent of the original wet volume. Although infant ovarian volume after maternal hypophysectomy was half that of the intact controls the difference was negligible when ovarian volume was normalized to body weight (Table 2).

Mean volume of germ cells: The mean diameters and volumes (*Vc*) of germ cells are presented in Table 3. Normal germ cells were divided into four groups according to the progression of their development. There was a progressive increase in size of germ cells as they developed. Under the heading of atretic oocytes, are included oocytes from the leptotene stage of meiosis to primary follicles.

Proportion of germinal vs. somatic cells (Chalkley counts): The proportion (*P*) of germinal tissue to somatic tissue in term infant ovaries is presented in Table 4. The proportion of the total germ cells to the somatic cells of the ovaries was essentially the same in all three groups of monkeys. The percentage of atretic germ cells in ovaries of infants after maternal hypophysectomy was seven-fold less than that in ovaries from intact ones.

Differential count and total number of germ cells: The results of the differential (*d*) germ cell counts are graphically presented in Fig. 10. Oogonia, leptotene to diplotene oocytes and primordial follicles constituted 29 percent, 11 percent and 37 percent respectively, of the total germ cell population in the ovaries of intact term infants having intact mothers. Primary follicles made up only 5 percent of all germ cells.

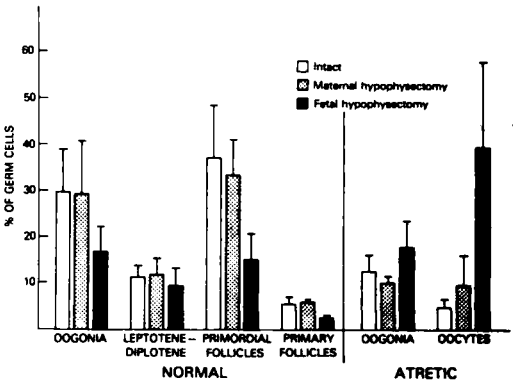


FIG. 10. Graphic presentation of the differential germ cell count in the ovaries of the three different groups of term infants. Results expressed as mean ± S.E.

TABLE 3. Diameter and volume of oogonia and oocytes.

	No. of cells measured	Mean cell diameter and range (μm)	Mean cell volume (μm³)
Normal germ cells			
Oogonia	40	13.1 (8.0 – 16.3)	1,165
Oocytes			
Leptotene to diplotene	40	16.5 (11.3 – 22.5)	2,350
In primordial follicles	40	35.3 (28.7 – 42.5)	23,117
In primary follicles	40	52.7 (45.0 – 62.5)	76,815
Atretic germ cells			
Oogonia	40	10.5 (5.0 – 15.0)	611
Oocytes	40	27.5 (18.3 – 53.7)	10.919

Atretic oogonia and oocytes constituted 17 percent of all germ cells. Of these, 12 percent were atretic oogonia and degenerating meiotic oocytes or 'Z' cells (Beaumont and Mandl, 1962); and, 5 percent were diplotene oocytes of degenerating primordial and primary follicles. Although minor variations occurred, the proportion of the different categories of germ cells after maternal hypophysectomy was similar to that in intact controls. However, after ablation of the fetal pituitary the percentages of normal oogonia, primordial follicles and primary follicles were 16, 15 and 2, respectively. These values were significantly different from those of intact, control infants ($P<0.05$). Leptotene to diplotene oocytes constituted 9 percent of the germ cells after fetal hypophysectomy, a percentage similar to that in both the intact control and maternal hypophysectomy groups.

The percentage (17 percent) of atretic oogonia after fetal hypophysectomy was not significantly higher than that of the intact controls. Atretic oocytes (leptotene to diplotene, primordial follicles and primary follicles combined) made up 39 percent of the total germ cell population, significantly ($P<0.01$) higher than from the intact controls.

The calculated total population of germ cells in the ovaries of the three groups of infants is presented in Table 5. Our data obtained from the term control infants of intact mothers compare favorably with those of Baker (1966). When compared to intact controls, the total number of normal germ cells declined to approximately one-half after maternal and one-third after fetal hypophysectomy. The total number of atretic germ cells following removal of the maternal pituitary declined to less than one-half of the intact controls. However, the total number of atretic germ cells after fetal hypophysectomy did not differ appreciably from that of intact controls.

DISCUSSION

Although surgical removal of the maternal or fetal hypophysis affected fetal growth and development profoundly in the rhesus monkeys studied here, ablation of these two separate sources of metabolic support led to markedly different effects on term infant body weight and on the development of fetal endocrine organs. Infants of hypophysectomized mothers were consistently underweight, implying either a deficiency of trophic substances or failure of tissue response to these. When normalized for differences in body weight, weights of the fetal ovaries and thyroids following maternal hypophysectomy were similar to those of normal, control pregnancies, suggesting that endocrine organs were susceptible to factors resulting in intrauterine growth retardation of other tissues.

One striking exception was found to this otherwise widespread growth retardation in fetuses of mothers lacking hypophyseal support: fetal adrenals were enlarged significantly following maternal hypophysectomy. This finding suggests that the fetal hypothalamus and pituitary may be competent to respond to diminished maternal corticosteroidogenesis, perhaps manifest to the fetus as a decrease in the quantity of corticosteroids derived from the maternal circulation (Kittinger, 1974). Although we have not measured ACTH levels in fetal circulation, we postulate that the fetal

TABLE 4. Proportion of cell types in infant ovaries as estimated by the Chalkley method.

	No. of animals	No. of ovaries	Percent of cell types[a]			
			Normal germ cells	Atretic germ cells	Total germ cells	Somatic cells
Intact	4	6	10.1 ± 2.4 (7.8 – 13.2)	3.4 ± 1.0 (2.0 – 4.3)	13.6 ± 2.7 (11.1 – 17.5)	86.4 ± 2.7 (82.4 – 88.8)
Maternal hypophysectomy	3	4	14.5 ± 4.7 (9.0 – 17.7)	0.5 ± 0.1 (0.3 – 0.6)	15.0 ± 4.6 (9.6 – 18.0)	85.0 ± 4.6 (81.9 – 90.3)
Fetal hypophysectomy	4	7	10.7 ± 8.8 (1.9 – 19.9)	4.2 ± 1.6 (1.8 – 5.5)	15.2 ± 7.6 (7.5 – 21.7)	85.0 ± 7.6 (78.3 – 92.5)

[a]Values are given as means ± standard deviation.
Numbers in parentheses represent range.

pituitary responds to this perceived deficiency with enhanced ACTH secretion. The hypertrophy of infant adrenal cortical zones, particularly the zona reticularis, is consistent with this hypothesis. However, the hypothesis is complicated by our observations suggesting that the rhesus placenta secretes an adrenocorticotropin which stimulates cortisol secretion by the maternal adrenals of hypophysectomized-fetectomized monkeys (Hodgen et al., 1975a), thus limiting the cortisol deficit in maternal blood. It is tempting to speculate that the intrauterine growth retardation may have been due in part to excessive fetal adrenal cortical secretion of glucocorticoids. Hypoplasia of thymus, spleen and other lymphoid tissues observed in these fetuses (unpublished) is consistent with this concept of the origin of intrauterine growth retardation. Furthermore, concomitant excessive adrenal cortical secretion of androgens might either suppress fetal pituitary gonadotropin secretion or alternatively, act directly on the fetal ovary to reduce the number of ooctyes seen at term.

Unlike maternal hypophysectomy, fetal hypophysectomy did not lead to generalized retardation in growth and development. However, after ablation of the fetal hypophysis, development of the fetal ovaries, thyroids, and adrenals was impaired even when normalized for body weight. This leads us to propose that hormones of fetal pituitary origin are an essential requirement for cell proliferation and differentiation in these endocrine organs. Results obtained following fetal decapitation of rabbits (Jost, 1956, 1966) and monkey fetuses (Kittinger et al., 1972; McNulty et al., 1973), as well as destruction of the fetal monkey pituitary by ionizing radiation (Chez et al., 1970), led to similar conclusions regarding the dependence of fetal endocrine tissues on secretions of the fetal pituitary gland.

To appreciate more fully the effects of fetal and maternal hypophysectomy on fetal ovarian growth and development, brief consideration of normal fetal ovarian development is relevant. The establishment of the cortex and the medulla of the monkey fetal ovaries occurs between 50 and 65 days in gestation. The lobulation of the cortex and enlargement of the oogonia at the cortico-medullary border begins 70 days postconception, and these changes progress gradually toward the outer portion of the cortex (van Wagenen and Simpson, 1965).

Meiosis starts between 70 and 100 days in

TABLE 5. Calculated germ cell population per infant ovary.

	Intact	Maternal hypophysectomy	Fetal hypophysectomy
Oogonia	142,098	52,466	39,330
Oocytes			
Leptotene to diplotene	51,991	20,791	22,119
Primordial follicles	88,859	47,225	35,031
Primary follicles	13,646	8,452	5,340
(Antral follicles)	(9)	(<1)	(4)
Atretic oogonia	60,789	17,954	30,562
Atretic oocytes	12,672	12,733	33,297
Total normal germ cells	296,594	128,934	101,820
Total atretic germ cells	73,461	30,687	63,859
Total germ cells	370,055	159,621	165,679

gestation among the largest oogonia (thereafter called oocytes) while the cortical lobules become subdivided by connective tissue. Although the layer of spindle-shaped elongated cells that surrounds these oocytes is incomplete at first, by the time meiosis reaches the diplotene stage, the oocytes are completely enclosed. These units, consisting of an oocyte surrounded by these progenitors of granulosa cells, are called primordial follicles. By 110 days in pregnancy, these primordial follicles begin to separate from the cortico-medullary border (van Wagenen and Simpson, 1965). At this stage of development, 35 to 50 percent of the total germ cells are still oogonia (Baker, 1966) in which meiosis has not begun.

Maternal hypophysectomy was performed at 58 to 71 days in pregnancy, that is, prior to onset of progressive meiotic activities. The present observations show that meiotic activities progressed in an orderly manner, and on a normal time schedule after maternal hypophysectomy. We noticed no gross cytological indications of abnormal development and proportions of atretic and normal germ cells in these ovaries were similar to the intact term infants. The Chalkley count showed no differences in the ratios of somatic cells to total germ cells when ovaries of infants from hypophysectomized mothers were compared with those from intact controls. Also, the differential count showed that the proportions of the six categories of germ cells, were similar to the intact controls.

The decline in ovarian weight following maternal hypophysectomy was accompanied by a decrease in total ovarian volume occupied by germ cells, and therefore, a reduction in the total number of germ cells in these ovaries. However, when ovarian weights were normalized for body weight, the decline in ovarian weight (therefore total ovarian volume), after maternal hypophysectomy was negated. We are unaware that either ovarian weight or the number of oocytes per ovary have been shown to correlate with total body weight of primate infants born at term. As noted above, either direct or indirect effects of putative excessive adrenal androgen secretion provide a more plausible hypothesis to account for these changes in fetal ovarian morphology.

After ablation of the fetal pituitary, the ratios of somatic cells to total germ cells (Chalkley count) in infant ovaries were not different from those in ovaries of intact, control monkeys. However, the wide range of these counts after fetal hypophysectomy indicates considerable between-animal variation. Reduction in ovarian weight, and thus volume as well, was accompanied by a reduction in total numbers of germ cells in operated fetuses, as in infants born of hypophysectomized mothers. Although the total number of germ cells after fetal hypophysectomy was comparable to those after maternal hypophysectomy, the numbers of atretic germ cells were twice that following ablation of the maternal pituitary. These results suggest that products of the fetal monkey pituitary are essential to an orderly progression of early oogenesis. Byskov (1974) recently demonstrated that in fetal mice the onset of oogenesis, as well as follicle formation is dependent on the presence of well organized rete ovarii. In the present study, rete ovarii were absent or were degenerated markedly subsequent to fetal hypophysectomy. Perhaps the massive atresia of follicles after fetal hypophysectomy was due to the lack of support from

the rete system rather than a direct dependence of the follicles on fetal pituitary secretions.

In summary, our findings indicate a dynamic interaction of fetal and maternal hypothalamic-pituitary axes in the growth and development of the monkey fetus and its endocrine organs. Within this group, enlarged fetal adrenals may indicate that the fetus responded to decreased levels of maternal corticosteroids reaching the fetal circulation. However, it was apparent that fetal endocrine organs (ovaries, adrenals, and thyroids) were dependent specifically on secretions of the fetal hypophysis. Indeed, the fetal pituitary was required to sustain the rete ovarii and/or the normal progression of early events in oogenesis and follicular maturation. Insufficient support of the fetal ovaries by the fetal pituitary during mid- and late pregnancy was manifest most profoundly by the marked rise in the incidence of oogonia and oocytes undergoing atresia near term.

ACKNOWLEDGMENTS

We thank Dr. James Bosma for his ideas on developing a surgical procedure for hypophysectomy of fetal rhesus monkeys by approaching the sella turcica through the soft palate. We are grateful for the technical assistance of Mr. Donald Barber and Mr. Charles Turner and animal care provided by Mr. Donnie Cromartie and Mr. James Lewis. We thank Dr. Hannah Peters of The Finsen Institute, Copenhagen, for examining our specimens and independently confirming our histological findings; and for providing specially stained slides for the identification of the rete system.

REFERENCES

Atkinson, L. E., Hotchkiss, J., Fritz, G. R., Surve, A. H., Neill, J. D. and Knobil, E. (1975). Circulating levels of steroids and chorionic gonadotropin during pregnancy in the rhesus monkey, with special attention to the rescue of the corpus luteum in early pregnancy, Biol. Reprod. 12, 335-345.

Baker, T. G. (1963). A quantitative and cytological study of germ cells in human ovaries. Proc. Roy. Soc. B. 158, 417-433.

Beaumont, H. M. and Mandl, A. M. (1962). A quantitative and cytological study of oogonia and oocytes in the foetal and neonatal rat. Proc. Roy. Soc. B. 155, 557-579.

Byskov, A. G. (1974). Does the rete ovarii act as a trigger for the onset of meiosis? Nature 252, 396-397.

Chalkley, H. W. (1943). Method for the quantitative morphologic analysis of tissues. J. Nat'l. Cancer Inst. 4, 47-53.

Chez, R. A., Hutchinson, D. L., Salazar, H. and Mintz, D. H. (1970). Some effects of fetal and maternal hypophysectomy in pregnancy. Amer. J. Obstet. Gynec. 108, 643-650.

Hobson, W., Faiman, C., Dougherty, W. J., Reyes, F. I. and Winters, J. S. D. (1975). Radioimmunoassay of rhesus monkey chorionic gonadotropin. Fertil. and Steril. 26, 93-97.

Hodgen, G. D., Gulyas, B. J. and Tullner, W. W. (1975a). Role of the primate placenta in cortisol secretion by the maternal adrenals. Steroids 26, 233-240.

Hodgen, G. D., Niemann, W. H. and Tullner, W. W. (1975b). Duration of chorionic gonadotropin production by the placenta of the rhesus monkey. Endocrinology 96, 789-791.

Hodgen, G. D. and Ross, G. T. (1974). Pregnancy diagnosis by a hemagglutination inhibition test for urinary macaque chorionic gonadotropin (mCG). J. Clin. Endocrinol. Metab. 38, 927-930.

Hodgen, G. D., Tullner, W. W., Vaitukaitis, J. L., Ward, D. N. and Ross, G. T. (1974). Specific radioimmunoassay of chorionic gonadotropin during implantation in rhesus monkeys. J. Clin. Endocrinol. Metab. 39, 457-464.

Jost, A. (1956). The secretory activities of fetal endocrine glands and their effect upon target organs. Conf. on Gestation (ed. C. A. Villee) Josiah Macy Jr. Found. 129-171.

Jost, A. (1966). Problems of fetal endocrinology: The adrenal glands. Rec. Prog. Hormone Res. 22, 541-569.

Kittinger, G. W. (1974). Feto-maternal production and transfer of cortisol in the rhesus (*Macaca mulatta*). Steroids 17, 229-243.

Kittinger, G. W., Beamer, N. B., Hagemenas, F., Hill, J. D., Baughman, W. L. and Ochsner, A. J. (1972). Evidence for autonomous pituitary-adrenal function in the near-term fetal rhesus (*Macaca mulatta*). Endocrinology 91, 1037-1044.

McNulty, W., Hagemenas, F., Beamer, N. and Kittinger, G. (1973). The effect of chronic decapitation on the development of the rhesus fetus (*Macaca mulatta*). The Endocrine Society, 55th meeting, (Abstract) 253.

Tullner, W. W., Gulyas, B. J. and Hodgen, G. D. (1975). Maternal estrogen and progesterone levels after hypophysectomy in early pregnancy and in term fetuses of newborn monkeys. Steroids 26, 625-633.

Tullner, W. W. and Hodgen, G. D. (1974). Effects of fetectomy on plasma estrogens and progesterone in monkeys (*Macaca mulatta*). Steroids 24, 887-897.

RECOMMENDED REVIEWS

Baker, T. G. (1966). A quantitative and cytological study of oogenesis in the rhesus monkey. J. Anat. 100, 761-776.

van Wagenen, G. and Simpson, M. E. (1965). Embryology of the ovary and testis *Homo sapiens* and *Macaca mulatta*. Yale University press, New Haven.