REPRODUCTION IN THE FERRET

I. THE EFFECT OF OVARIECTOMY ON THE COURSE OF GESTATION

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Summary. The role of the ovaries in the maintenance of pregnancy was studied in the ferret. Ovariectomy at the time of implantation showed that some embryos survived for 7 days after the operation but all were destroyed after 10 days, although the trophoblast continued to grow and at a much faster rate than normal. Ovariectomy performed after implantation showed that no fetal development occurred when the ovaries were removed at Day 21 *post coitum*, but that fetuses developed for an appreciable length of time in animals ovariectomized on Days 23 to 27 *post coitum*. Ovariectomy in late gestation resulted in speedy expulsion of the fetuses. An increase in the placenta:fetus ratio did not alter the response to ovariectomy in late gestation. The uteri in all ovariectomized animals showed progesterational endometria when examined shortly after expulsion of the fetuses.

INTRODUCTION

The presence of CL is a universal feature of reproduction in eutherian mammals (Asdell, 1946; Harrison, 1962). The effect of excision of CL or removal of both ovaries on the course of pregnancy has been reported in a variety of vertebrate species (Courrier, 1945; Amoroso & Finn, 1962), and the importance of the secretions of the CL during implantation has been stressed in these and other reviews on the endocrinology of early pregnancy (Deanesly, 1960a). In all viviparous mammals the ovaries are essential for the establishment of early pregnancy and, with the exception of the guinea-pig (Loeb & Hesselberg, 1917; Deanesly, 1960a, b), the armadillo (Buchanan *et al.*, 1956; Enders & Buchanan, 1959) and the quokka (Tyndale-Biscoe, 1963), for nidation and implantation of the fertilized ovum. In animals in which pregnancy continues in the absence of the ovaries an alternative source of progesterone has been postulated (Newton, 1949) and is probably the placenta (Neher & Zarrow, 1954; Pearlman, 1957; Short, 1957; Short & Moore, 1959; Zander, 1959).

Although the ferret has been used in studies of reproduction, information on the endocrine mechanisms responsible for the maintenance of pregnancy in this animal is meagre. McPhail (1935) performed ovariectomy on two 35-day pregnant ferrets, one of which aborted non-viable fetuses 3 days after the operation while the other gave birth to a premature litter of eight at Day 40 and

successfully reared some of them. More recently, Buchanan (1969) bilaterally ovariectomized ferrets at Days 4 to 10 *post coitum* (*p.c.*), well before implantation. The blastocysts did not implant but survived for about a week beyond the normal time of implantation. Ferrets ovariectomized on Day 10 *p.c.* and examined 3 to 5 days later did not show implanted blastocysts, but at Day 20 or later a uterine reaction was found that led Buchanan to infer that implantation might have occurred later than usual. He suggested that the ferret uterus, once stimulated by ovarian hormones, is capable of prolonged response without further stimulus. Hormonal requirements for implantation and embryonic development in the ferret were studied by Wu & Chang (1972, 1973). Their results indicate that only progesterone is required for implantation and subsequent embryonic development. In animals ovariectomized on Days 7, 8 or 9 of pregnancy, and treated with progesterone alone or in combination with oestrogen, implantation occurred at the normal time, on Day 13, except for those ovariectomized on Day 7 in which implantation was delayed for several days (Wu & Chang, 1973). In all animals, embryonic development to Day 26 was more retarded than that of ferrets ovariectomized on Day 13 and injected with the hormones.

The embryology and placentation of the ferret has been studied by Strahl & Ballman (1915), and the placental labyrinth (Lawn & Chiquoine, 1965), implantation (Enders & Schlafke, 1972) and the uterus in early pregnancy (Gulamhusein & Beck, 1973) have been investigated.

The current experiments were designed to study the effects of ovariectomy on the course of gestation in the ferret.

MATERIALS AND METHODS

Forty-one female ferrets, of unknown reproductive history, were purchased from a recognized dealer. The animals were kept in a room maintained at 70 to 75°F and were accommodated in modified guinea-pig cages fitted with wide-mesh bottom grids. They were fed a diet consisting of tinned meat and milk. Once a week the tinned meat was substituted with fresh meat. Water was always available. The mating of the animals was supervised and accurate dating of pregnancies was obtained. The day following copulation was considered as Day 1 of pregnancy. Eleven females were used as controls for endometrial reactions and for placental and fetal development at 13, 17, 20, 27, 30, 33, 34, 36 and 42 days of pregnancy.

Nembutal (Abbott Laboratories), given intraperitoneally in a dose of 30 mg/kg body weight, was used to induce anaesthesia. The ovaries were removed through a mid-ventral incision after ligation of the ovarian vessels. The conceptual swellings and interconceptual areas were measured with a mm vernier. The number of conceptuses was also recorded. The animals recovered consciousness shortly after the operation and by the following day appeared alert and in good condition.

At autopsy whole uterine tracts of early pregnancies were removed and treated according to the method of Orsini (1962) in which the uteri were eventually cleared and stored in benzyl benzoate. Uterine segments and placentae

of animals at other stages of pregnancy were prepared for histology in the normal way and stained with haematoxylin and eosin. Histological sections of the uteri cleared in benzyl benzoate were also prepared. Weights and crown-rump (C-R) measurements of fetuses were recorded. The term 'resting phase' (Dawson & Kusters, 1944), was used to describe the state of the endometrial reaction when the enlarged surface epithelial cells have been replaced by simple low columnar epithelium and where the deep uterine glands become restricted to areas close to the surface epithelium (Pl. 1, Fig. 1). The general term 'progestational phase' is used to describe the pregnancy endometrium as detailed by Hammond & Marshall (1930). The existence of enlarged surface epithelial cells together with a fibrous stroma is regarded as a measure of minimum progestational response.

RESULTS

Effects of ovariectomy on implantation

Three animals, Nos 40, 41 and 79, were ovariectomized on Day 13 *p.c.* and were killed 4, 7 and 10 days afterwards, respectively. In Nos 40 and 41, most of the embryos were dead although some survived as judged by their appearance and the presence of functional fetal capillaries in the allantochorionic mesoderm. In No. 79 all the embryos were dead 10 days after ovariectomy. Although the diameter of the gestational swellings at autopsy in the operated animals showed an increase, this was always less than in the control animals (Pl. 1, Figs 2 and 3). Sections through the conceptuses of dead embryos revealed gross infolding of the endometrium (see Pl. 1, Fig. 4, and Pl. 2, Figs 5, 6 and 7). The trophoblast continued to proliferate and invade the endometrium, giving rise to an incipient labyrinth a few days after ovariectomy (Pl. 2, Fig. 8), sooner than in the control (Pl. 3, Fig. 9). The trophoblastic invasion 7 days after ovariectomy was so advanced that the placenta was well developed and the trophoblastic villi abutted the deep glandular layer (Pl. 3, Fig. 10), although the trophoblast was only half-way through the endometrium in controls (Pl. 3, Fig. 11). Ten days after ovariectomy the placenta developed further and showed increase in size and complexity of the labyrinth (Pl. 3, Fig. 12). Both the trophoblast and the maternal capillaries appeared normal but allantoic vessels were absent from the labyrinth. The placenta contained many haemorrhagic foci in the junctional zone and a few grossly dilated maternal capillaries had ruptured on the luminal side (Pl. 3, Fig. 13). Progestational changes were still apparent in the uterine epithelium 10 days after ovariectomy.

Ovariectomy in early gestation

Bilateral ovariectomy was performed on Days 21, 23, 25 and 27 of gestation. At each stage two animals were used; one was killed 5 days after ovariectomy and the other at Day 39, except for those ovariectomized at Day 27 which were killed 1 day and 5 days after expulsion of the fetuses. The results are shown in Table 1.

The placentae of the surviving fetuses of Nos 29 and 31 appeared normal; when the embryos had died the only noticeable feature was absence of the allantoic vessels. The epithelium of the uterine surface and that of the glands showed

EXPLANATION OF PLATES 1 TO 3

Material from pregnant ferret uteri. cm, Chorionic mesoderm; dug, deep uterine gland; gs, glandular symplasma; ht, haematoma; mc, maternal capillary; mct, maternal connective tissue; myo, myometrium; pl, placental labyrinth; tr, trophoblast; ug, uterine glands; ugc, uterine glandular epithelium.

PLATE 1

FIG. 1. An endometrium in the 'resting phase'. The proliferative surface epithelium has been replaced by simple low columnar epithelium. $\times 111$.

FIG. 2. Benzyl-benzoate cleared uterine tract 7 days after ovariectomy at Day 13 *p.c.* Note the haemorrhage in gestational swellings and in interconceptual areas. Compare with Fig. 3 (not to the same scale).

FIG. 3. Benzyl-benzoate cleared uterine tract at Day 20 *p.c.* in an intact ferret. Dark opacities at antimesometrial apices represent haematomata.

FIG. 4. Blastocyst swelling 4 days after ovariectomy at Day 13 *p.c.*, showing marked infolding of the endometrium. The trophoblastic invasion is far ahead of that in the control (Fig. 6). $\times 10$.

PLATE 2

FIG. 5. Blastocyst swelling 7 days after ovariectomy at Day 13 *p.c.*, showing marked infolding of endometrium (compare with Fig. 7). The trophoblast forms a complex placental labyrinth (pl) eroding the entire glandular epithelium. Note progestational changes in the endometrium on the mesometrial side. A haematoma (ht) is formed on the antimesometrial side. $\times 9.5$.

FIG. 6. Blastocyst swelling at Day 17 *p.c.*, ovaries intact. $\times 9.5$.

FIG. 7. Blastocyst swelling at Day 20 *p.c.*, ovaries intact. Note the limited trophoblastic invasion compared to that in Fig. 5. A haematoma (ht) is formed at the antimesometrial side. $\times 7$.

FIG. 8. Part of the endometrium shown in Fig. 4, 4 days after ovariectomy at Day 13 *p.c.* Trophoblastic invasion of the uterine endometrium is far more extensive than in the control (Fig. 9) as judged by the degree of trophoblastic (tr) penetration and extent of symplasma (gs) formation. A primitive labyrinth is formed on the luminal side. $\times 116$.

PLATE 3

FIG. 9. Part of the endometrium in Fig. 6. The trophoblastic invasion (tr), still limited to a few columns of solid trophoblasts and a few trophoblastic villi, did not advance far beyond the surface epithelium. $\times 133.5$.

FIG. 10. Part of the placental labyrinth in Fig. 5 (7 days after ovariectomy at Day 13 *p.c.*). The trophoblast (tr) formed a well developed labyrinth except that the allantoic vessels were absent (the embryo in this gestational swelling was destroyed). Compare the extent of labyrinth formation with that in Fig. 11. $\times 116$.

FIG. 11. Part of the placental labyrinth and endometrium in Fig. 7 (Day 20 of normal pregnancy). The trophoblast (tr) formed a primitive labyrinth and traversed the inner half of the endometrium. The vascular allantoic mesoderm established contact with the chorion. $\times 133.5$.

FIG. 12. Part of the placental labyrinth in Fig. 13 (10 days after ovariectomy at Day 13 *p.c.*). Note the advanced development of the labyrinth. Allantoic vessels are absent. The X-cells resemble the giant decidual cells of the cat placenta. These cells are not present in the normal ferret placenta. $\times 116$.

FIG. 13. Gestational swelling 10 days after ovariectomy at Day 13 *p.c.* Note gross infolding of endometrium and development of a complex labyrinth. Also observe haemorrhages in the junctional zone and on the fetal surface of the placenta. The endometrium on the mesometrial side shows progestational changes. $\times 9.5$.

FIG. 14. The endometrium 5 days after ovariectomy on Day 29 *p.c.* and directly after abortion of fetuses shows a well preserved progestational surface epithelium. $\times 111$.

PLATE 1

(Facing p. 430)

Table 1. Effects of ovariectomy on early gestation in ferrets

Ovariectomy				Dimensions of conceptuses and interconceptual areas (mm)*						Comments
No. of ferret	Stage (days p.c.)	No. of healthy conceptuses	Autopsy (days p.c.)	No. of healthy fetuses	At ovariectomy			At autopsy		
					Breadth	Length	I.C.A.	Breadth	Length	
29	21	9	26	3	21.1±0.2	15.1±0.3	4.0±0.2	14.8±0.5	15.5±0.5	7.0±0.2
30	21	9	39	0	21.3±0.2	15.2±0.3	4.0±0.2	8.5±0.3	—	7.0±0.3
31	23	6	28	1	21.6±0.2	16.0±0.2	5.0±0.4	17.0±0.3	11.2±0.4	8.5±0.3
32	23	13	39	2	21.3±0.2	16.0±0.2	5.0±0.2	15.5	—	15.5
33	25	11	39	3	23.2±0.1	21.0±0.3	5.5±0.1	15.5±0.1	—	11.5±0.2
34	25	9	30	9	21.5±0.2	19.3±0.1	5.5±0.2	20.9±0.3	27.4±1.2	11.4±0.7
36	27	8	36	3	24.1±0.2	24.4±0.2	8.0±0.2	16.5±0.2	—	14.5±0.2
37	27	14	42	8	23.4±0.3	21.6±0.9	17.0±0.1	10.0±0.1	—	9.0±0.1
										Aborted Days 37 to 38
										Aborted Day 34
										Aborted Day 35
										One fetus aborted
										Day 35 and seven
										Day 37

Some of the fetuses were maimed by their mothers and it is likely that fetuses unaccounted for were eaten.

* Means ± S.E.M.

progestational changes. At autopsy of No. 32 the uterine horns were empty except for one placenta at the cranial end of the left horn. The endometrium was in the progestational state and one cotyledon of the retained placenta was necrotic while the other retained its normal appearance including evidence of fetal circulation. The uterus of No. 30 showed marked involution. The endometrium retained slight progestational changes in the form of a single layer of enlarged surface epithelial cells and necrotic placental tissue was found in some parts of the uterine lumen.

The development of the fetuses of No. 34 was normal (mean weight 1.2 g, length 27.7 mm) compared with fetuses of intact animals at Day 30 (mean weight 1.0 g, length 27.7 mm). The placentae were histologically normal and the labyrinth was well developed. The endometrium showed marked progestational changes. Two of the aborted fetuses of No. 33 were intact and the mean weight (2.6 g) and length (43.0 mm) were only slightly less than that of fetuses at Day 34 (mean weight 3.8 g, length 43.6 mm). The third fetus was maimed by the mother. At autopsy both uterine horns were empty and the endometrium revealed progestational changes.

The expelled fetuses of No. 36 reached a mean weight of 4.1 g and a length of 44.5 which compared well with normal weights and measurements for Day 36 of pregnancy (mean weight 4.6 g, length 52.7 mm); the uterine horn had a progestational endometrium and an oedematous stroma. The aborted fetuses of No. 37 weighed 4.0 g and were 51.5 mm in length; the uterine horns showed advanced involution and were in the 'resting phase'.

Ovariectomy in late gestation

Ovariectomy was conducted on each day from Day 29 to Day 37 of gestation. The number of animals used at each stage is indicated in Table 2. After ovariectomy the fetuses were retained in the uterus for periods extending from 3 to 5 days and the animals were killed shortly afterwards. The fetuses continued to develop but at the time of abortion they were generally not as heavy as fetuses from normal animals at the same stages of pregnancy. The number of viable fetuses increased the nearer ovariectomy was performed to term.

Most uteri showed progestational changes (Pl. 3, Fig. 14), but in one animal in which abortion was completed by Day 37 and autopsy was performed at Day 42, the endometrium was in a 'resting phase'.

Placenta:fetus ratio

From the results of the ovariectomies performed in late gestation, it was decided to test whether the creation of a high placenta:fetus ratio could modify the response to bilateral ovariectomy. Four animals were used in this experiment. Bilateral ovariectomy and removal of the 'extra' fetuses were performed in a single operation (Table 3). Abortion occurred within 3 to 5 days after operation but the fetuses had continued to grow (Table 3). The endometrium appeared histologically similar to that of animals ovariectomized in late gestation.

Table 2. Development of ferret fetuses following ovariectomy in late gestation (Mean $\pm$ S.E.M.)

No. of ferrets	At ovariectomy				At abortion			
	Day	No. of fetuses	Wt of fetuses* (g)	C-R length of fetuses* (mm)	No. of fetuses		Wt of fetuses (g)	C-R length of fetuses (mm)
					Alive	Dead		
2	29	11			33, 34	0	11	1.82 $\pm$ 0.18
1	30	4	0.99 $\pm$ 0.02	27.7 $\pm$ 0.2	35	0	4	3.05 $\pm$ 0.02
1	31	7			35	0	7	3.92 $\pm$ 0.15
1	32	5			35	0	5	3.42 $\pm$ 0.09
1	33	11	2.57 $\pm$ 0.06	41.0 $\pm$ 0.3	37	7	4	6.20 $\pm$ 0.26
4	34	26	3.79 $\pm$ 0.16	43.6 $\pm$ 0.7	37, 39(3)	10	16	54.8 $\pm$ 0.7
2	35	11			40, 41	8	3	57.1 $\pm$ 0.5
1	36	3	4.55 $\pm$ 0.15	52.8 $\pm$ 0.5	40	3	0	8.02 $\pm$ 0.39
1	37	8			40	6	2	8.66 $\pm$ 0.19
								8.11 $\pm$ 0.22
								59.4 $\pm$ 0.7
								59.2 $\pm$ 0.5
								58.2 $\pm$ 0.4

All the fetuses of two control ferrets were born alive at Day 42; mean weight 8.38 $\pm$ 0.21 g, length 64.03 $\pm$ 0.4 mm.

* Data from ferrets used in other experiments.

Table 3. The effect of alteration of placenta: fetus ratio in ovariectomized ferrets

No. of ferret	Ovariectomy (days p.c.)	Placenta:fetus ratio	Day of abortion	Weights and dimensions of fetuses*			
				At ovariectomy		At abortion	
				Weight (g)	C-R length (mm)	Weight (g)	C-R length (mm)
43	34	2:1	37	2.54	39.0	3.85	51.0
50	30	9:4	34	0.88 $\pm$ 0.0	26.5 $\pm$ 0.2	3.19 $\pm$ 0.10	42.3 $\pm$ 0.6
51	30	9:4	34	0.78 $\pm$ 0.02	26.5 $\pm$ 0.2	2.01†	44.0†
53	30	7:2	35	1.10 $\pm$ 0.04	28.5 $\pm$ 0.5	3.98	44.6

The mean weight of fetuses at 34 days of pregnancy is 3.8 g and the C-R length is 43.6 mm.

* Mean $\pm$ S.E.M.

† Mean weight and length of two developing and apparently healthy fetuses; the two other fetuses were degenerate and resorbing.

Physical transformation of the gravid uterus

From an early stage of pregnancy the embryonal sac distends the width of its locular compartment to near maximum. Thereafter only a slight increase in the width of the conceptus is observed. In contrast, extension of the conceptus along the horn increases progressively with advancing pregnancy, encroaching into the adjacent interconceptual areas. By Day 27 to 28 of pregnancy the length of the conceptus becomes equal to the breadth and by Day 34 to 36 the uterine horn is converted into a tube of uniform diameter.

DISCUSSION

The fact that some embryos continued to develop for at least a week in the absence of the ovaries does not necessarily mean that progesterone is not required for nidation and implantation of the ovum in the ferret. It may be that by the time the ovaries were removed in these experiments the endometrium was adequately sensitized to allow implantation and that subsequent development of the embryos may have been maintained by progestational hormones produced by the developing placenta. A similar finding of continued fetal development after ovariectomy in early pregnancy has been reported in the guinea-pig by Loeb & Hesselberg (1917) and Deanesly (1960a, b, 1963a, b), but fetal development continued for a longer period after ovariectomy than is reported here for the ferret.

It has been shown in the present experiments that reduction in the size of the conceptus and marked infolding of the endometrium occurs after ovariectomy and that this may contribute to the death of the embryos. The gross infolding of the endometrium that followed ovariectomy, however, did not affect the development of the haematoma. It may thus appear that the development of this organ, to which a role in the nutrition of the fetus has been suggested (Amoroso, 1952), is independent of the fetus in the same way that the trophoblast proved to be.

Ovariectomy after implantation showed that no advanced fetal development occurred when the ovaries were removed at Day 21, but that fetal development continued for an appreciable period when ovariectomy was performed from Day 23 to Day 27, although only a small number of fetuses in the litter continued to develop and expulsion of the fetuses did eventually occur. The rate of fetal development in the absence of the ovaries was not as great as in normal pregnancy and Deanesly (1963b) has shown that, when guinea-pigs were ovariectomized 3 to 5 days *post coitum*, reduction in the size of the conceptus commenced by Day 14 and preceded death of the embryos. Since the placenta of the ferret is not fully developed by Day 23, it would be natural to suppose that it is supporting fetal development through production of progestational hormones, and that this function improves with the development of the placenta. Ovariectomy at Day 29, however, resulted in speedy expulsion of the fetuses though they continued to develop during their short period of stay in the uterus. The striking discrepancy in the time during which the placenta can support fetal development after ovariectomy may be related to the physical change that occurs

between Days 27 and 29 in the gravid horn and which results in increasing distension of the interconceptual areas.

In all instances in which living placentae were detected in the uterine horns, however long after removal of the ovaries, progesterational changes in the endometrium were maintained. Although these changes were less extensive than in the intact animal at the corresponding stage, they were significant in that they show that an extra-ovarian source of progesterone exists and that this source may be the placenta. The results of the experiment in which the placenta:fetus ratio was changed show that, apart from the operative shock, the procedure did not modify the response of the ferret to bilateral ovariectomy in late gestation, and that the putative extra progesterone was not beneficial.

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