IS OVULATION IN DOLPHINS, *STENELLA LONGIROSTRIS* AND *STENELLA ATTENUATA*, ALWAYS COPULATION-INDUCED?

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ABSTRACT

This study of 58 nonpregnant uteri and ovaries of the spinner dolphin, *Stenella longirostris*, and the spotted dolphin, S. attenuata, was undertaken to ascertain whether ovulation is copulation-induced (reflex) or occurs spontaneously. Control specimens of immature, pregnant, and lactating females were examined also and were used to define the normal reproductive events in uteri and ovaries of these species. No differences were found between the two species. In about one half of the specimens we found active corpora lutea of recent origin and in another 15% of *S. attenuata* and 29% of *S. longirostris*, corpora had fibrous centers but were hormonally active. No embryos were found and the endometrial changes were such that early but unobserved pregnancies could be excluded. In 35% of the specimens from *S. attenuata* the macroscopic diagnosis of corpus luteum was erroneous, while this was true of 21% in *S. longirostris*. The corpora were degenerating and more resembled early corpora albicantia histologically. In two of these specimens, endometritis was found and the endometrial histology gave evidence of abortion in three. These findings are evidence that these *Stenella* species may sometimes ovulate spontaneously and that macroscopic classification of corpora lutea in the past may frequently have been erroneous.

The reproductive physiology of the spotted dolphin, *Stenella attenuata*, and spinner dolphin, *S. longirostris*, has not been fully elucidated. In particular, it is presently unknown whether these dolphins ovulate spontaneously or on reflex after copulation. Some reasons to believe the latter have been presented for *Tursiops truncatus* (Harrison 1977) and the same is implied for other Cetacea. The finding of corpora lutea almost exclusively in pregnant animals is the basis for this assumption, and the purpose of this study is to examine the genital tracts of 58 nonpregnant animals with corpora lutea in detail in an attempt to resolve this question. In a detailed study of spotted dolphin, Perrin et al. (1976) found that of 242 females with corpora lutea, 229 (95%) were pregnant. In a similar study of spinner dolphins, Perrin et al. (1977) found that 2.8% of 536 adult females contained corpora lutea whose presence could not be explained by pregnancy, lactation, or abortion.

The distribution of species with and without reflex ovulation has been reviewed by Jöchle (1973). A surprisingly large number of species is listed as having exclusively or predominantly reflex ovulation, including Cetacea. Only primates, mouse and rat are cited as exceptions; however, modern studies suggest this to be an incomplete list. In several papers on Camelidae, reflex ovulation is well supported by experimental studies and by observations from abatoirs. Although camels and their South American relatives must be accepted as being reflex ovulators, the carefully controlled study by England et al. (1969) showed that "occasional spontaneous ovulation occurred during the height of the breeding season" in the llama, *Lama glama*.

In an attempt to gain additional information on the reproductive physiology of *S. longirostris* and *S. attenuata*, we examined the reproductive tracts of animals recorded to possess corpora lutea while not pregnant. Special attention was paid to ascertaining any reasons for the existence of these corpora lutea, such as early undetected pregnancy and an attempt was made to correlate the ovarian status with endometrial changes.

MATERIALS AND METHODS

Reproductive tracts of female *S. longirostris* and *S. attenuata* were collected at sea by observers during commercial tuna fishing operations in the southeastern Pacific Ocean (Perrin et al. 1976,

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Manuscript accepted October 1979.
FISHERY BULLETIN: VOL. 78, NO. 2, 1980.

1977). The organs were preserved in 10% Formalin[4] (Mallinkrodt) solution and stored at the Southwest Fisheries Center (SWFC), National Marine Fisheries Service, NOAA, La Jolla, Calif. At the time of capture, a variety of observations were recorded, including identified pregnancy, size of the fetus, and lactation. These data, dates and location of capture as well as related pertinent information, are recorded in logs at SWFC. Here also the ovaries were sliced at 1 mm intervals, and observations such as the presence of corpora lutea, corpora albicantia, and ovarian size were recorded and correlated with capture information. Those apparently gravid uteri whose ovaries had a corpus luteum were dissected further and, occasionally, small fetuses were identified by SWFC staff. In addition, the present authors carefully dissected three intact uteri of the 1977 catches where ovaries contained corpora lutea but in which pregnancy had not been recorded.

For the purpose of the present study the reproductive tracts of 53 nonpregnant dolphins, captured in 1976, and whose ovaries were recorded to contain corpora lutea, were examined in detail. A few specimens of 1975 and 1977 were also studied and are included, bringing the figure to 58 genital tracts. The tabulation of the reproductive condition of these tracts is shown in Table 1.

These specimens represent the majority of reproductive tracts recorded to possess unexplained corpora lutea in 1976. There were 67 in all but not all of the specimens were in suitable condition for inclusion in this study. Thus, several were too severely autolyzed for proper evaluation; in others, either the complete ovaries or uteri could not be located in the specimen collection.

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1.—Reproductive tracts of *Stenella* spp. studied because corpora lutea were found in the absence of pregnancy or lactation.

Species	1975	1976	1977
Stenella longirostris	0	38	0
S. attenuata	2	15	3

In an effort to better understand the macroscopic and histologic features of dolphins with known reproductive events, it was desirable to select suitable control material from the same stored collection. Six groups of specimens were selected for this purpose and consisted of the following specimens: Group I, 3 tracts of immature *S. longirostris*; Group II, 6 tracts of mature female *S. longirostris* having no corpus luteum and, therefore, termed "resting"; Group III, 12 tracts of *S. longirostris* with early pregnancy, the embryos measuring from 1 to 53 mm long (1-5 g with placenta); Group IV, 6 tracts of later pregnant dolphins (4 *S. longirostris* and 2 *S. attenuata*) with fetal sizes ranging from 300 to 725 mm; Group V, 11 tracts of lactating, nonpregnant females (10 *S. longirostris* and 1 *S. attenuata*); Group VI, the experimental group, 58 tracts of nonpregnant females possessing a corpus luteum (38 *S. longirostris* and 20 *S. attenuata*; Table 1); for a total of 96 female genital tracts.

The gross examination of genital tracts by us ascertained the following standard information: Weight; length and width of uterine horns and cervices; thickness of uterine walls at standard locations. Histologic sections, stained with hematoxylin and eosin, were prepared from standard locations of tubes, uterine horns, lower uterine segments, and ovaries (Figure 1). Measurements of uterine mucosa and muscularis were made at low power microscopic examination with the aid of a calibrated ocular micrometer. At histologic examination the uterine findings (glands, secretion, mitoses, edema, hyalinization, inflammation) were compared with the ovarian activity. Relevant photomicrographs were made with a Zeiss Axiomat.

RESULTS

Controls

Group I, Immature Females

These three *S. longirostris* measured from 164 to 176 cm body length (Table 2) and possessed neither

TABLE 2.—Group I: Immature controls, *Stenella longirostris* captured on 20 February 1976.

Specimen no.	Dolphin length (cm)	Uterus weight (g)	Left horn of uterus: Length (cm)	Left: Endometrial fold (mm)	Left: Endometrial valley (mm)	Left: Myometrium (mm)	Right horn of uterus: Length (cm)	Right: Endometrial fold (mm)	Right: Endometrial valley (mm)	Right: Myometrium (mm)
1	176	47	10	1.49	0.60	0.54	9	2.09	0.75	0.60
2	168	44	9	1.34	0.39	0.60	9	1.07	0.36	0.62
3	164	22	9	1.10	0.60	0.42	7	1.07	0.36	0.54
			Average	1.31	0.53	0.52		1.41	0.59	0.58

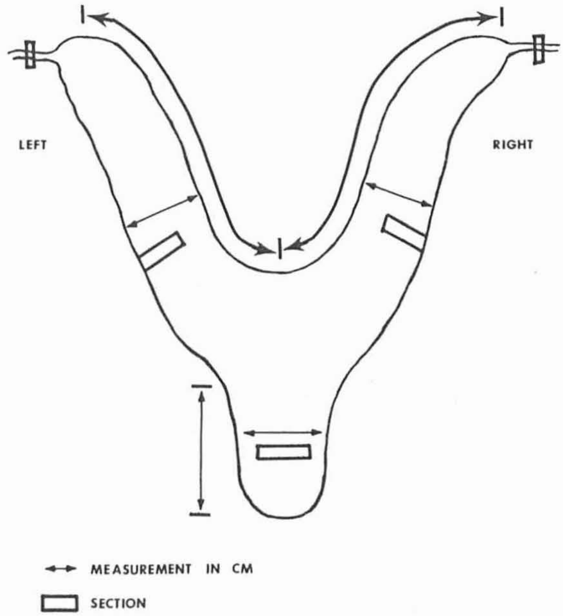

FIGURE 1.—Diagram of dolphin uterus. Arrows indicate measurements taken in *Stenella* spp. and squares denote location of histologic sections.

corpora lutea nor corpora albicantia. Because of their size it was assumed that they were at the verge of maturity. As shown in Figure 2, uterine horns were of equal size, there were no stretch marks and the endometrium was flat. Numerous Graafian follicles of varying stages of development were present in both ovaries, but there was no evidence of ovulation. The endometrium was thin and composed of tubular glands possessing neither secretion nor mitoses; the stroma was devoid of inflammation and edema or hyalinization (Figure 3). The fallopian tubes were small and empty. In all subsequent specimens, slides of fallopian tubes were examined. No relevant changes were observed and they are therefore not described further. Also, sections of the lower uterine segment were found to make no contribution in the assessment of reproductive state and are not included in this analysis.

Group II, Mature Females

These six *S. longirostris* measured from 173 to 186 cm in length and were adjudged to be mature

FIGURE 2.—Immature uterus of *Stenella longirostris* (no. 1, Table 2). Note the flat folds of endometrium, equal-sized horns, and lack of serosal stretch marks (center)

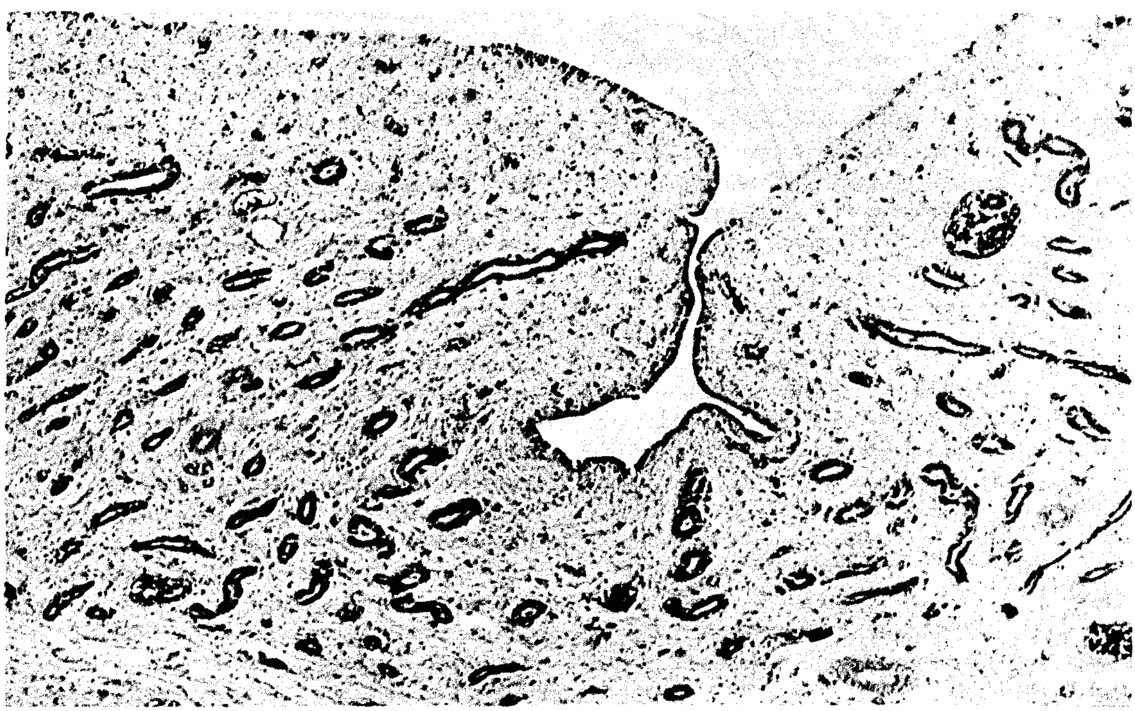

FIGURE 3.—Endometrium of immature *Stenella longirostris* (no. 3, Table 2). Tubular glands, little folding, thin endometrium, no secretion or mitoses (hematoxylin and eosin × 60).

because of their size and the presence of at least one corpus albicans. They were not lactating nor did they possess a corpus luteum. The appropriate measurements are set forth in Table 3. In general, the horns were asymmetrical but not so markedly as those of lactating females (Group V), with the exception of specimen no. 4 (Table 3). The wrinkled serosa and congestion of endometrium of this animal's left horn indicated recent pregnancy (Figure 4). All but one female of this group had "stretch marks" which we assumed to be an indication of previous pregnancy. Microscopic study of the corpora albicantia in this group allowed some correlation with the endometrial findings. Thus, the apparently most recently delivered uterus (Figure 4) had a still cellular corpus albicans (Figure 5) and, inflammatory cells, hemosiderin macrophages and stromal hyalinization were observed in the congested endometrium (Figure 6). In the others, judged to have had past pregnancies, the corpus albicans was less cellular, more hyalinized and shrunken. The endometrium did not appear to be stimulated, had thin epithelium, no mitoses but scattered macrophages were in the process of removing debris. Apparently later still, Graafian follicle development commences, uterine horns are of nearly equal size and endometrial glandular redevelopment occurs with mitoses, glandular convolutions, occasional epithelial vacuoles, and stromal edema (Figure 7).

TABLE 3.—Group II: Mature controls, *Stenella longirostris*, for 1976.

Specimen no.	Month of capture	Dolphin length (cm)	Uterus weight (g)	Left horn of uterus: Length (cm)	Left horn: Endometrial fold (mm)	Left horn: Endometrial valley (mm)	Left horn: Myometrium (mm)	Right horn of uterus: Length (cm)	Right horn: Endometrial fold (mm)	Right horn: Endometrial valley (mm)	Right horn: Myometrium (mm)
1	February	186	108	13	1.79	0.75	1.49	10	1.94	0.66	1.49
2	February	181	123	17	2.70	0.75	1.04	15	2.24	0.90	0.90
3	February	179	136	13	1.79	0.66	1.19	11	1.19	0.75	1.64
4	February	178	152	17	2.99	0.90	2.39	8	4.78	0.90	1.94
5	July	175	98	16	1.64	0.30	0.90	18	1.49	0.60	1.49
6	June	173	110	23	4.18	0.90	1.49	18	3.58	1.04	1.49
				Average	2.52	0.71	1.42		2.54	0.81	1.49

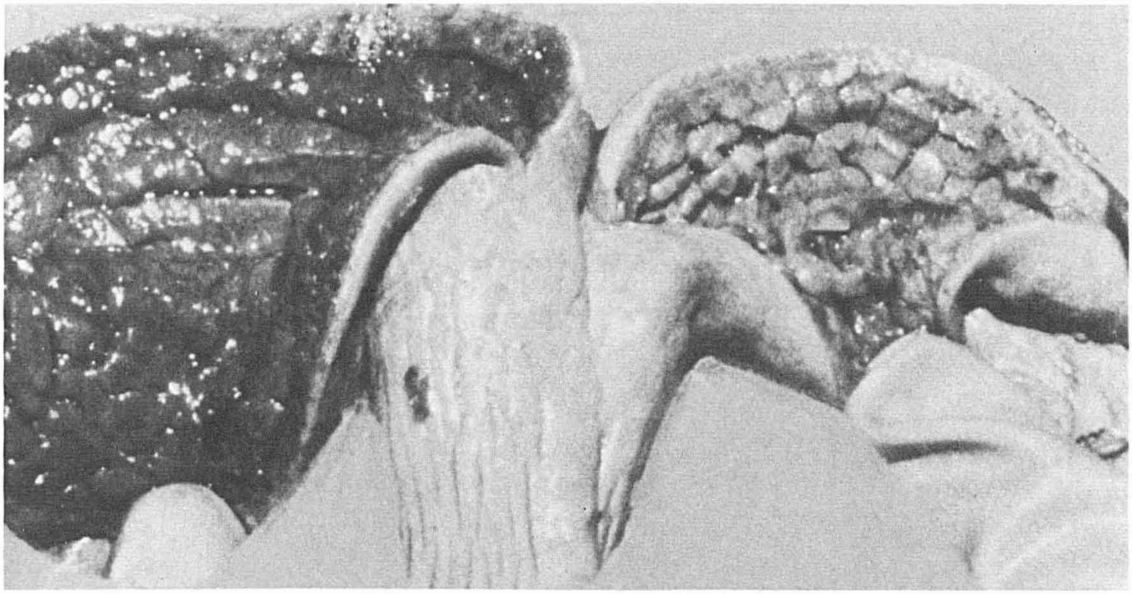

FIGURE 4.—Mature animal's uterus, presumably recently delivered (no. 4, Table 3). Note larger left horn with congestion and serosal stretch marks.

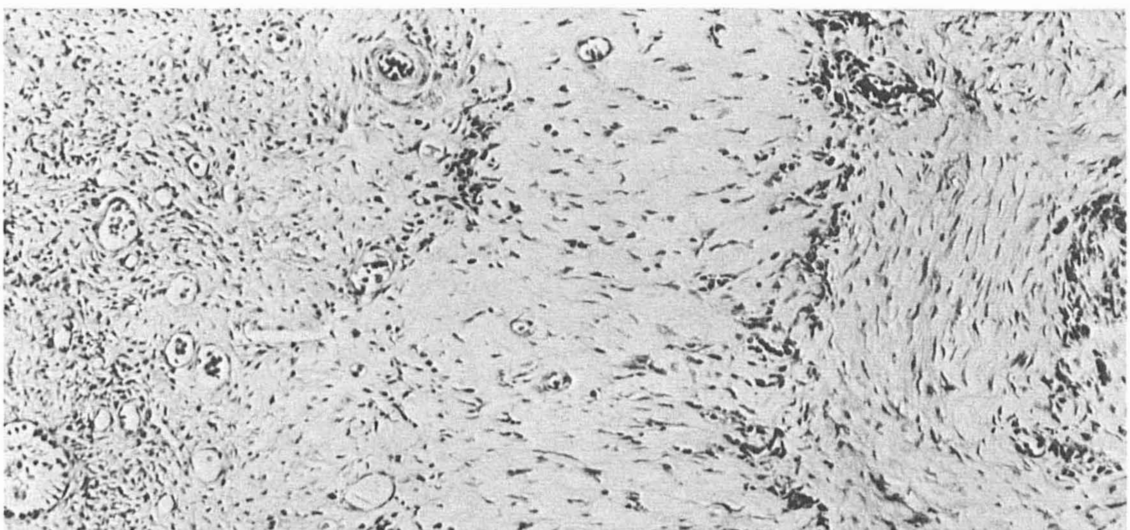

FIGURE 5.—Corpus albicans of animal in Figure 4. At right is former follicular cavity, replaced by young fibroblastic tissue. Dark cells in central (former luteal) band of corpus albicans suggest recent conversion of corpus luteum to corpus albicans (hematoxylin and eosin × 120).

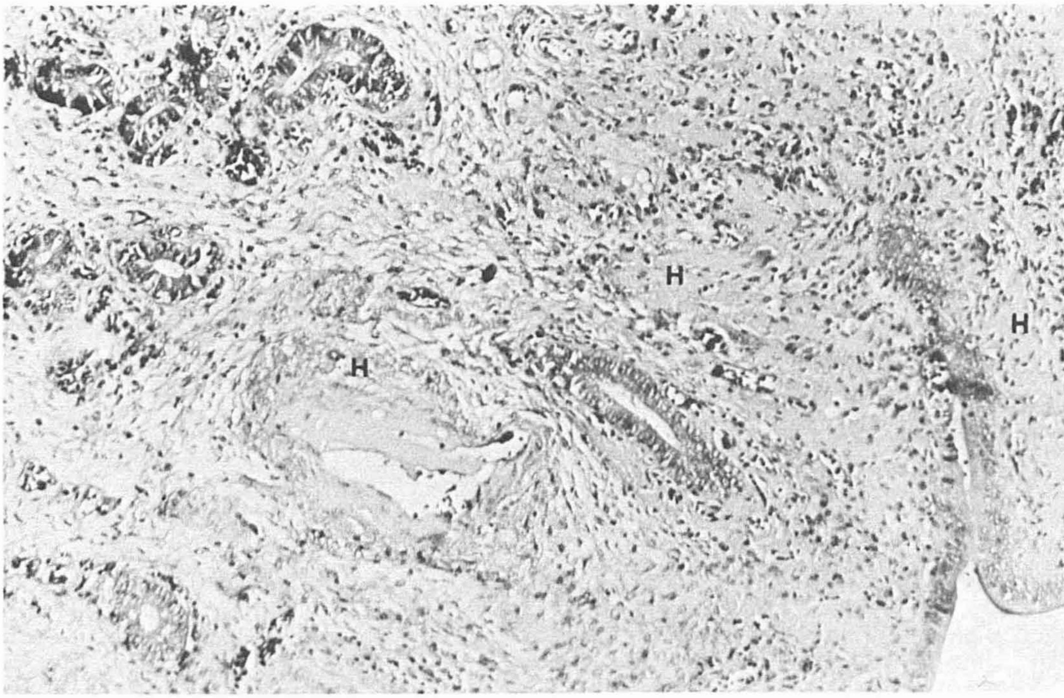

FIGURE 6.—Endometrium of animal in Figures 4 and 5, surface at right. Infiltration of stroma with macrophages; sparse glands; hyalinization of stroma and larger vessel walls at H (hematoxylin and eosin × 120).

Group III, Early Pregnancy

There were 12 *S. longirostris* in this group measuring from 166 to 188 cm in length (Table 4). With the exception of the first specimen listed, the left uterine horn was the larger. In this exceptional animal, the corpus luteum was also on the right and no corpus albicans was found in the left side. Uterine size and weight did not correlate well with embryonic size (1-53 mm) or weight (1-5 g). The embryos had been removed previously and their placentas had apparently not yet implanted. The larger (pregnant) horn had a congested endometrium and distended lumen (Figure 8).

This is an important control group for the experimental Group VI whose uteri were empty despite the presence of a corpus luteum. Thus, it is noteworthy that even in small embryos (Figure 9) the placental membranes are of appreciable size and they grow rapidly (Figure 10) so that they are not easily overlooked in dissections. Early limb bud development could be recognized in embryos sized 5 mm or more.

TABLE 4.—Group III: Early pregnant controls, *Stenella longirostris*. All from February 1976 except no. 11 captured in May 1976.

Specimen no.	Dolphin length (cm)	Uterus weight (g)	Left horn of uterus: Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Right horn of uterus: Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Fetus: Length (mm)	Weight (g)
1	176	140	10	6.87	1.49	1.49	15	5.37	1.49	1.49	1	1
2	172	171	20	—	—	—	12	5.67	1.19	1.49	2	1
3	178	145	15	5.07	0.90	1.49	12	4.03	1.04	1.94	2	1
4	181	208	28	2.69	1.19	0.96	20	2.69	0.90	0.78	5	1
5	188	163	20	2.83	0.75	0.04	15	2.99	0.75	1.04	5	2
6	172	192	19	5.97	1.19	1.04	12	6.87	1.79	1.49	5	2
7	166	204	17	6.87	0.75	0.90	11	7.46	1.34	0.75	5	2
8	176	212	25	5.37	1.49	1.04	15	3.88	0.60	1.19	5	2
9	167	227	19	6.87	1.19	1.94	13	—	2.39	1.49	5	2
10	184	229	28	2.09	0.36	1.04	15	3.58	0.90	1.19	5	2
11	180	144	17	4.78	1.19	1.04	13	2.99	1.19	1.19	8	2
12	180	187	23	1.04	0.35	0.90	16	2.09	0.30	0.90	53	5
			Average	4.68	0.99	1.17		4.33	1.16	1.25		

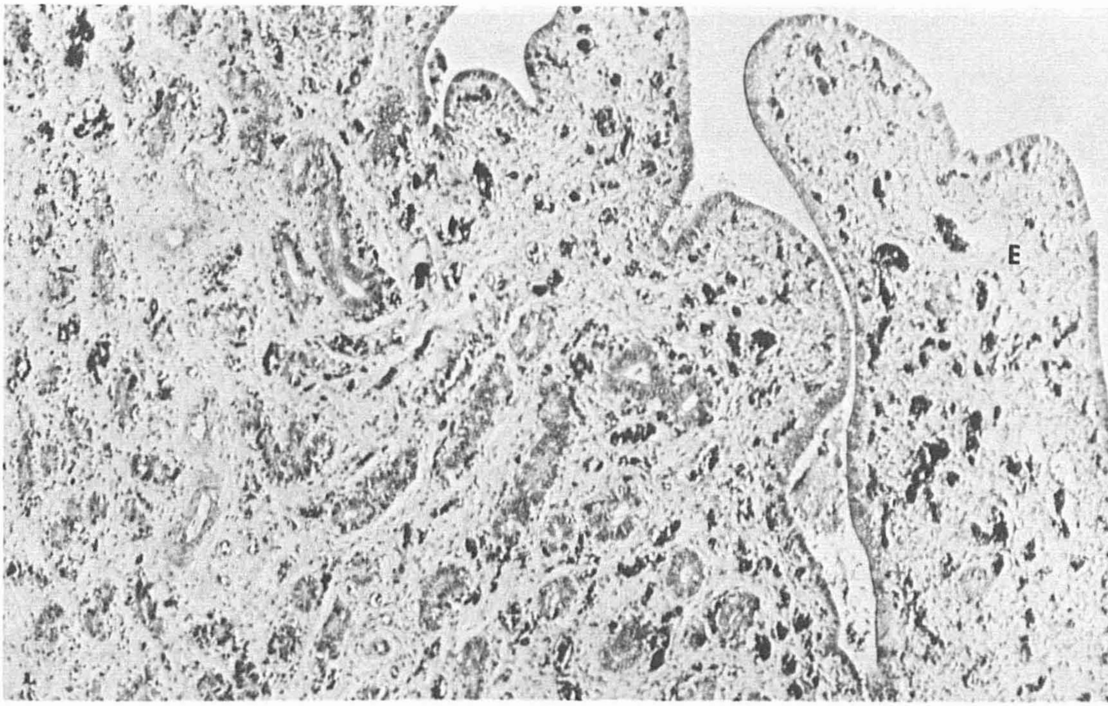

FIGURE 7.—Endometrium undergoing early stimulation, presumably after past pregnancy (no. 3, Table 3). Glands are more numerous and coiled. They contain occasional epithelial secretory vacuoles and mitoses. Focal edema (E) of stroma commences (hematoxylin and eosin × 60).

FIGURE 8.—Early pregnant uterus with distended left horn and hyperemia of endometrium (no. 4, Table 4).

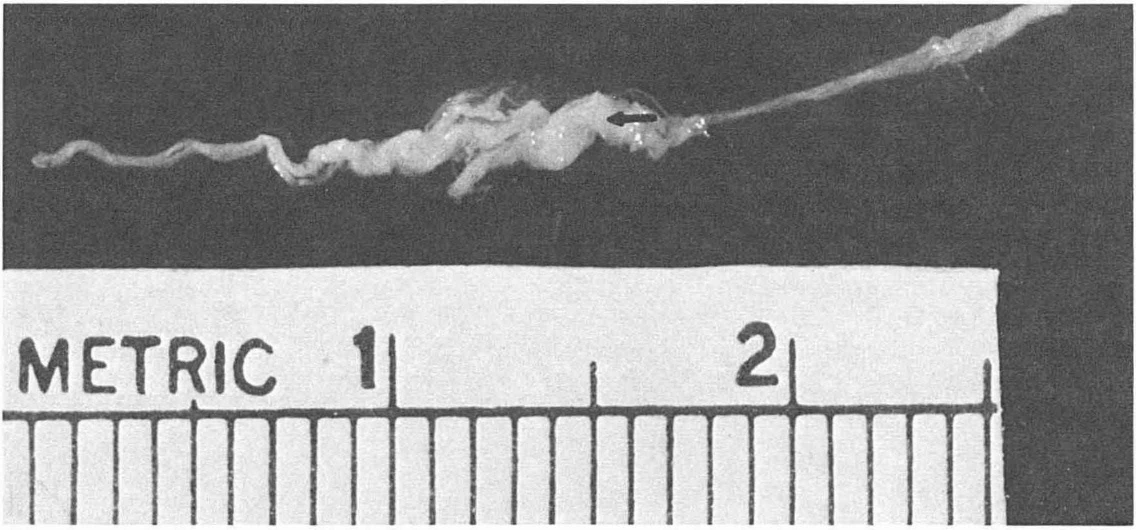

FIGURE 9.—Smallest (2 mm) normal embryo at arrow with long, filmy placental membranes (no. 3, Table 4).

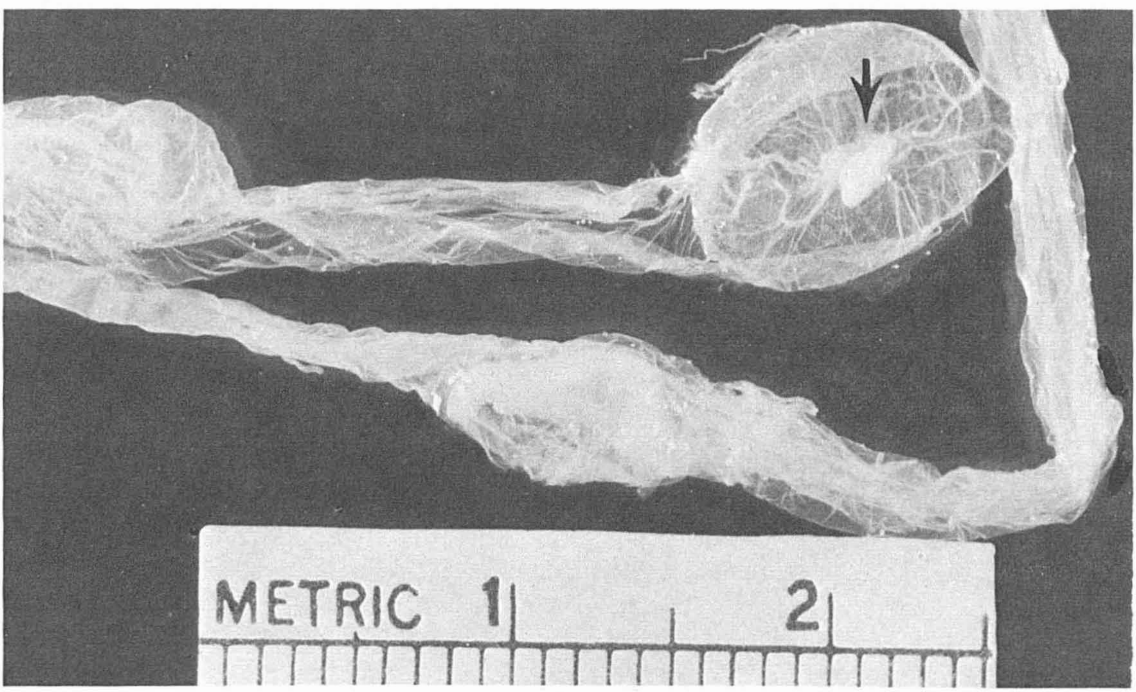

FIGURE 10.—Five mm embryo (arrow) with huge placental membranes (no. 9, Table 4).

The cells of the corpus luteum of all of these animals were well supported, and the central fibrin clot was invaded by vessels and fibroblasts in all but one animal (no. 3, Table 4; Figure 9). This was also the smallest embryo other than that of the aberrant right specimen (no. 1, Table 4). Its corpus luteum is shown in Figure 11 and is readily distinguishable from the small luteal mass found in the experimental group.

The endometrium had characteristic changes that should allow the histologic diagnosis of early pregnancy. First, the tips of endometrial folds be-

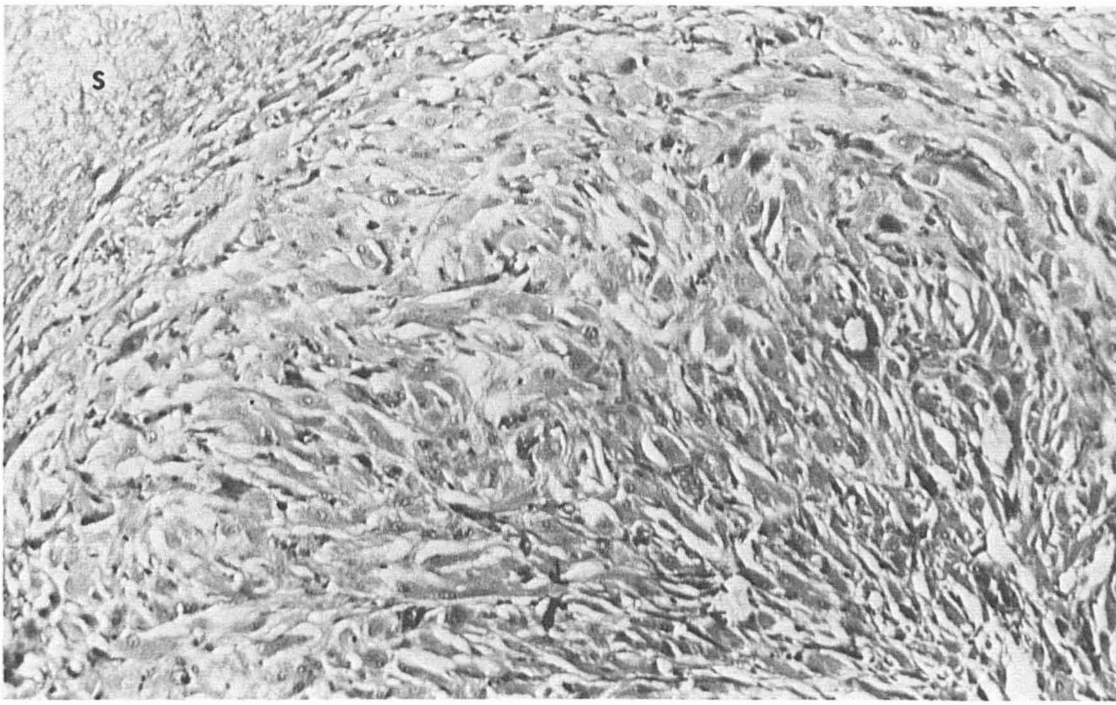

FIGURE 11.—Portion of active corpus luteum of earliest pregnancy with cavity at bottom right and ovarian stroma (S) at top left (no. 3, Table 4; hematoxylin and eosin × 100).

come edematous (Figure 12). At this stage the glands are empty and crowded in the basal portions. Their epithelium lacked mitoses. With advancing pregnancy the edema diminished while secretion accumulated in glands. Most characteristic, however, is the development of a rich capillary network with congestion, most notably in the superficial strata (Figure 13). Despite the presence of significant chronic endometritis in two cases, normally developing early embryos were found.

Group IV, Late Pregnancy

We selected four *S. longirostris* and two *S. attenuata* between 165 and 199 cm in length and embryos of 300-725 mm in length (the *S. attenuata* fetuses were lost at sea) for this comparison (Table 5). All implantations were in the larger left horn but a portion of the membranes traversed into the right horn. The large single corpus luteum of these six specimens was present in the left ovary.

The superficial endometrium was extremely arborized with distended glands into which the villi of the epitheliochorial placenta penetrated, forming an intimately interdigitating connection (Figure 14). The basal endometrium was edematous and glands contained secretion. With advancing gestation the villous arborization increased. The corpus luteum had characteristic appearance (Figure 15). Among the plump, eosinophilic luteal cells streamers of fibrocytes gave the first appear-

TABLE 5.—Group IV: Late pregnant controls. Specimen no. 1-4 *Stenella longirostris*, captured October 1978; no. 5-6 *S. attenuata*, captured July 1976.

Specimen no.	Dolphin length (cm)	Weight of placenta and uterus (g)	Left uterine horn				Right uterine horn				Fetus length (mm)
			Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	
1	197	617	—	—	—	0.60	—	—	—	0.75	300
2	182	703	42	—	—	—	21	—	—	—	429
3	165	700	60	2.69	0.60	0.75	26	5.97	1.49	1.19	557
4	168	982	64	1.79	0.60	0.60	20	—	—	—	725
5	—	730	—	—	—	1.79	12	—	—	0.75	—
6	—	965	—	—	—	2.08	21	—	—	0.60	—

FIGURE 12.—Endometrium of earliest pregnancy (Figures 9, 11; no. 3, Table 4). Uterine lumen (L) at right. Note the marked edema (E) in superficial stroma, congestion (C) and compact arrangement of secretory glands at left (hematoxylin and eosin × 50).

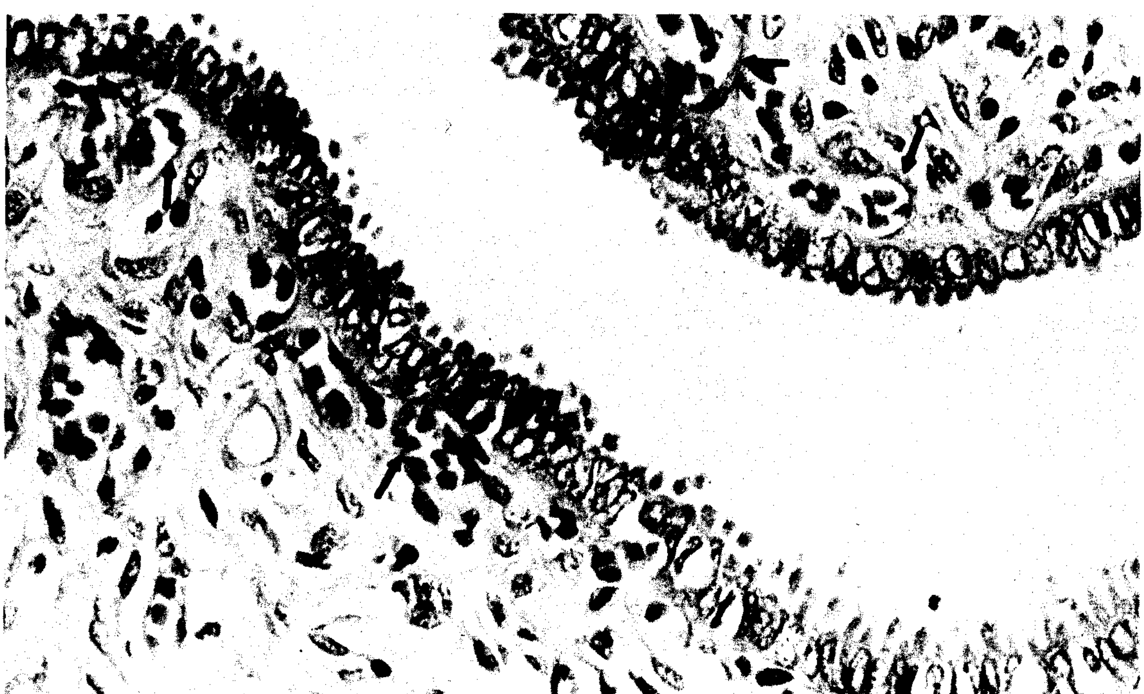

FIGURE 13.—Typical capillary distension (arrows) and neovascularization of endometrial stroma immediately beneath surface endometrial epithelium in early pregnancy (no. 9, Table 4; hematoxylin and eosin × 300).

FIGURE 14.—Placental attachment with basal endometrial glands (G) at left and interdigitating villi (V) within distended glandular lumina (no. 2, Table 5; hematoxylin and eosin × 80).

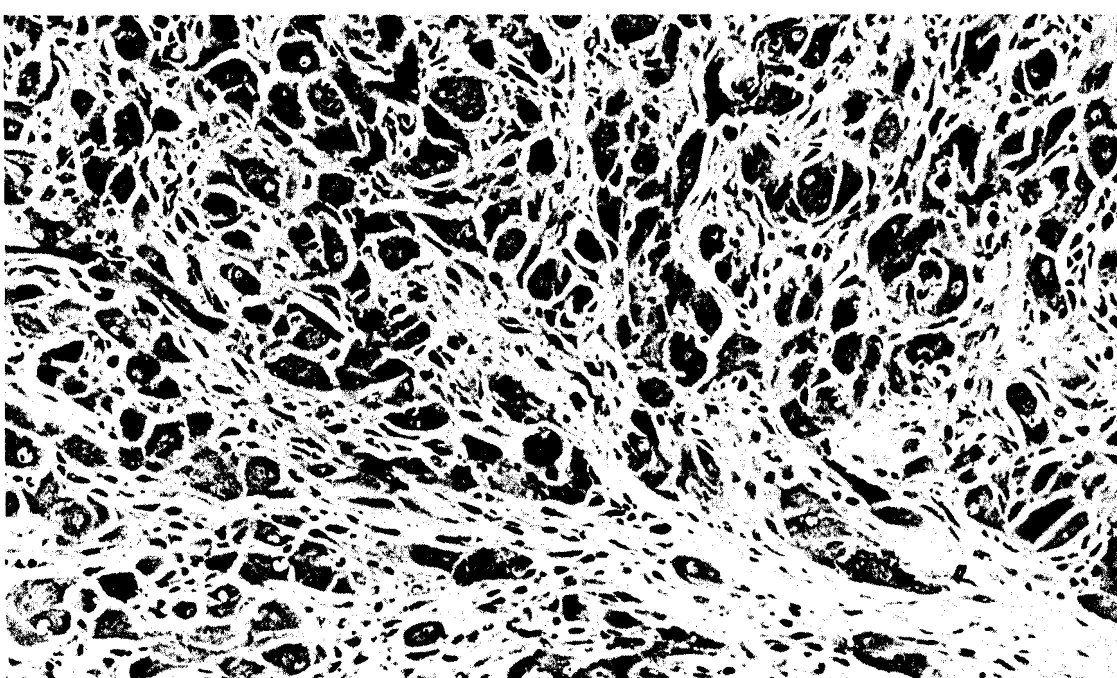

FIGURE 15.—Corpus luteum of uterus shown in Figure 14, the organized cavity is at bottom right. Note well-supported dark luteal cells with fibrocytes in between (hematoxylin and eosin × 150).

ance of degeneration. This connective tissue became less apparent with advancing pregnancy.

Group V, Lactating Females

Six specimens were selected measuring from 167 to 182 cm (*S. longirostris*) and one 202 cm (*S. attenuata*). They had been identified as lactating during dissection at sea. It is a heterogeneous group as judged by uterine size, endometrial histology, and the appearance of the corpora albicantia (Table 6). Some animals must have delivered very recently as evidenced by the marked disparity of uterine horn size, hyperemia, and the recent degenerative changes in the corpus luteum, while others had more equal horns and well-advanced corpora albicantia. No active corpora lutea were present while at least one corpus albicans was found in all specimens. Some corpora albicantia had chronic inflammatory cell infiltration and four ovaries had Graafian follicles maturing at various stages. Stretch marks were present on the uterine serosa and the endometrium had typical involutional changes histologically. Beneath the surface epithelium there was a thin zone of hyalinization in most specimens, while recently delivered animals had prominent, congested vessels in myometrium and endometrium. Some of these had hyalinized walls also. In a majority of endometria a mild chronic inflammatory infiltrate was present; one had acute pyometritis. No mitoses or edema was present, the endometria appeared "resting." Hemosiderin-laden macrophages in endometrium or lower uterine segment were present in three, presumably due to interstitial hemorrhage at recent delivery. Such deposits were found in only one other female (Control Group II) whom we suspect to have delivered recently but who was not recorded as lactating.

Experimental Groups

Group VI, Nonpregnant Animals With Corpus Luteum

The 58 animals of this group were not detected to be pregnant at sea but had a corpus luteum present in one of their ovaries. The group is divided into three subgroups made up of specimens with corpora lutea at different stages of development or involution.

GROUP VIa.—These 28 animals (19 *S. longirostris*; 9 *S. attenuata*) measured 163-191 cm in length, and the group was of special importance because the corpora lutea were judged to be the youngest. For this reason, an early pregnancy could have been overlooked. Two intact uteri were dissected meticulously, including the fallopian tubes. Neither embryos nor placentas were identified in any of the 28 specimens. From the dates of capture (Table 7) it will be noted that most (17) were captured in February, as is also true of early pregnant animals (Table 4). These figures must be interpreted with caution, however, because of different catch sizes on various cruises. The macroscopic findings were not uniform in that uterine weight varied between 90 and 467 g, the horn sizes varied considerably and endometrium was as often congested or mucus-covered as not. Only once was the corpus luteum found in the right ovary.

The histologic appearance of the endometrium correlated neatly with the corpus luteum development and grouping was undertaken accordingly. Those specimens whose corpus luteum had a central fibrin-filled cavity were placed into Group VIa; those whose cavity was replaced by fibrous tissue were adjudged to have ovulated earlier and

TABLE 6.—Group V: Lactating controls. Specimen no. 1-10 *Stenella longirostris*; no. 11 *S. attenuata*.

				Left uterine horn				Right uterine horn					
Specimen no.	Month and year of capture	Dolphin length (cm)	Uterus weight (g)	Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Graafian follicle	Corpus albicans
1	Feb. 1976	171	113	14	2.24	0.21	1.64	8	—	0.60	0.90	no	+
2	Feb. 1976	182	112	13	2.29	0.57	2.54	9	2.99	0.66	1.04	+	+
3	Feb. 1976	171	73	11	1.73	0.24	1.64	9	1.19	0.39	1.94	+	+
4	June 1976	171	65	12	1.34	0.30	2.99	11	1.79	0.30	2.09	no	+
5	June 1976	167	126	20	3.88	0.60	1.04	13	3.58	1.64	1.34	+	+
6	Feb. 1976	174	74	15	1.79	0.60	1.49	8	2.24	0.75	0.90	no	+
7	Feb. 1976	175	109	17	2.99	0.24	1.79	8	2.99	0.66	0.90	no	+
8	Feb. 1976	174	198	21	7.91	0.90	2.09	13	6.87	1.13	1.19	no	+
9	Feb. 1976	173	112	12	4.78	1.04	1.04	10	2.09	0.45	1.06	no	+
10	June 1976	173	60	13	1.49	0.75	2.09	12	1.19	0.60	0.75	+	+
11	Sept. 1977	202	550	—	1.64	0.75	2.09	—	1.64	0.60	1.34	no	+
		S. longirostris — Average			3.04	0.56	1.84		2.77	0.72	1.21		

TABLE 7.—Group VIa: Experimental group with early corpora lutea. Specimen no. 1-19 *Stenella longirostris*; no. 20-28 *S. attenuata*.

Specimen no.	Month and year of capture	Dolphin length (cm)	Uterus weight (g)	Left horn of uterus				Right horn of uterus			
				Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)
1	Feb. 1976	184	120	15	5.37	1.72	1.04	19	2.69	1.04	0.75
2	Apr. 1976	182	132	14	4.78	1.49	0.60	14	5.67	1.19	0.75
3	Feb. 1976	171	—	26	4.18	1.49	1.34	16	4.48	1.34	0.60
4	Feb. 1976	176	—	16	4.48	1.94	1.49	14	4.02	1.49	1.34
5	Feb. 1976	173	—	16	2.84	1.19	0.45	18	—	—	0.75
6	Feb. 1976	149	90	15	3.13	0.90	0.96	15	4.18	1.19	1.04
7	Feb. 1976	180	119	25	6.56	0.75	1.34	13	4.18	1.04	1.04
8	Feb. 1976	180	166	20	2.53	0.75	1.04	14	4.18	1.79	0.90
9	Feb. 1976	184	217	25	4.48	1.49	1.19	20	3.28	1.40	0.90
10	Feb. 1976	183	224	18	8.66	1.19	1.34	15	5.97	0.75	1.04
11	Feb. 1976	181	167	16	4.48	1.19	1.94	14	5.97	1.04	0.90
12	Feb. 1976	188	180	18	8.35	0.60	1.79	12	4.78	1.19	0.90
13	Aug. 1976	163	127	16	8.96	0.90	0.90	12	4.48	1.49	1.19
14	Feb. 1976	168	158	21	3.58	1.19	2.69	13	5.07	1.79	1.64
15	Feb. 1976	177	118	20	3.13	0.90	1.49	14	4.18	0.60	0.96
16	Feb. 1976	167	111	17	3.28	2.09	1.04	15	3.58	1.79	0.75
17	Feb. 1976	176	—	22	4.78	0.96	1.34	14	3.58	0.75	1.04
18	Feb. 1976	171	—	14	4.18	1.64	1.19	11	3.28	1.49	1.04
19	Aug. 1976	167	165	22	5.97	2.99	1.49	21	3.88	1.64	0.90
		S. longirostris — Average			4.93	1.33	1.29		4.30	1.28	0.77
20	Oct. 1976	174	190	29	3.88	0.60	1.49	18	3.28	0.84	2.09
21	Feb. 1976	189	192	23	2.98	0.60	1.49	19	2.38	1.19	1.49
22	Aug. 1976	176	390	35	7.46	1.49	1.64	15	—	1.19	1.49
23	Oct. 1975	191	156	25	4.93	1.04	1.34	14	3.58	1.04	1.34
24	Aug. 1976	174	333	26	7.16	1.79	1.94	16	2.99	0.75	1.19
25	May 1976	185	467	27	6.57	1.19	2.39	17	4.48	0.90	1.64
26	Apr. 1976	180	154	18	4.18	1.19	0.90	20	3.28	1.79	1.04
27	June 1977	185	190	12	2.69	0.90	1.19	17	1.79	0.30	0.75
28	June 1977	186	220	13	2.84	0.90	0.90	12	2.39	0.45	1.49
		S. attenuata — Average			4.74	1.01	1.48		3.02	0.94	1.39

placed into Group VIb. Because of the potential insight these specimens give into the dynamics of ovarian/endometrial relationships, these specimens are described in more detail.

When the Graafian follicle has recently ruptured, stromal vessels infiltrate the granulosa layer (Figure 16). The follicular lumen is filled with serous fluid and fibrin but rarely contains blood as in many other species. The corresponding endometrial stroma is edematous, the glands are tubular, lack secretory vacuoles and contain mitoses (Figure 17). When the corpus luteum is better established, the central cavity is more pronounced, capillaries have penetrated the granulosa layer, and, in contrast to its cells in specimens from early pregnancy (Figure 11), they possess less cytoplasm, being less plump (Figure 18). It should again be noted that the cavities rarely contain red blood cells. The endometrial glands at this stage are more crowded, have more coiling, and still possess mitoses, but the earliest appearance of epithelial cytoplasmic vacuoles occurs (Figure 19). The vacuolization appears to commence under the endometrial surface and penetrates slowly throughout the entire thickness of the endometrium (Figure 20). At the same time, fibroblastic infiltration of the corpus luteum cavity has taken place and the luteinized cell wall has folded remarkably. Very few mitoses were found in superficial endometrial cells at this stage and in the final stages before the central corpus luteum cavity has been completely filled in by fibrous tissue, the endometrial stromal edema disappears, to be replaced by coiled secretory glands (Figure 21). No mitoses exist, nor is secretion exuded into the glandular lumina. Such corpora lutea are expected to be associated with early pregnancy particularly because of the conspicuous size of granulosa luteal cells. Since no embryonic sacs were found, it is then also not surprising that the endometrium possesses different histologic features from those whose comparable corpora lutea were associated with early pregnancy (Figures 11-13). Perhaps this climaxes endometrial development before regression of corpus luteum occurs.

One of these specimens from an October catch (no. 20, Table 6) had a fresh corpus luteum with fibrin-filled cavity and unusual endometrium. The stroma beneath the surface epithelium had the typical hyalinization of the post partum state, yet, early secretory endometrium was found in deeper layers (Figure 22). Moreover, hemosiderin pigment was found. It would appear that this was the first and infertile ovulation after a recently past pregnancy which is further supported by the

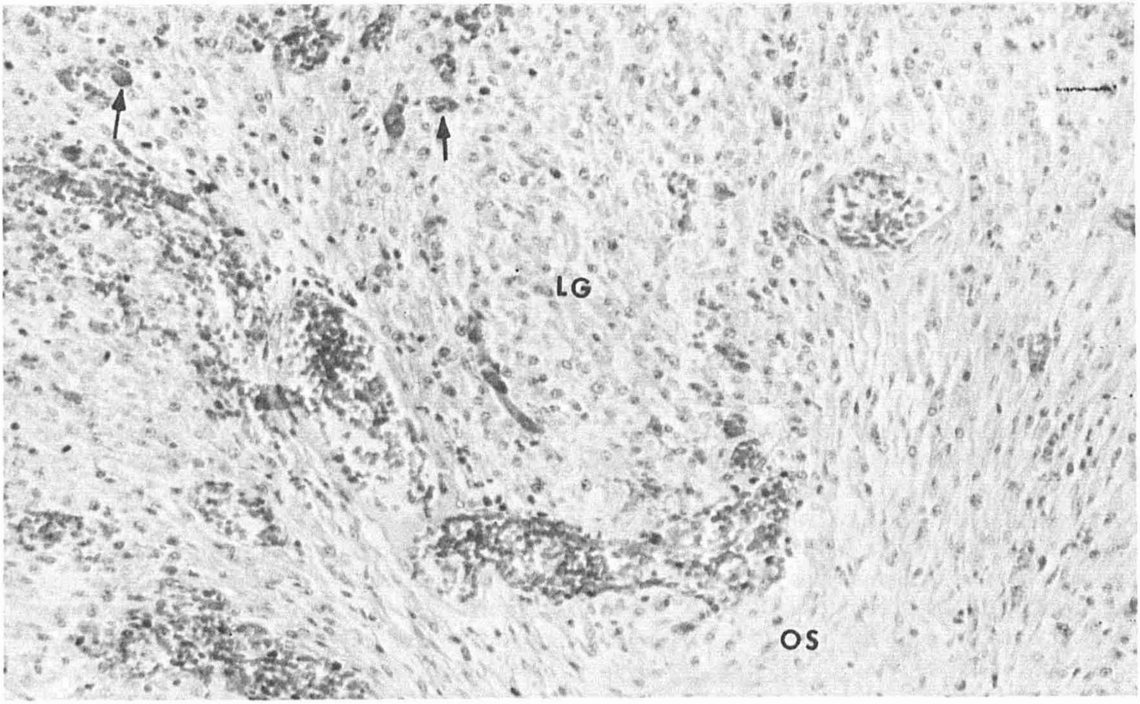

FIGURE 16.—Very fresh corpus luteum with focal hemorrhages (left), luteinized granulosa cells (LG) infiltrated by capillaries (arrows) sprouting from the ovarian stroma (OS) (no. 18, Table 7; hematoxylin and eosin × 400).

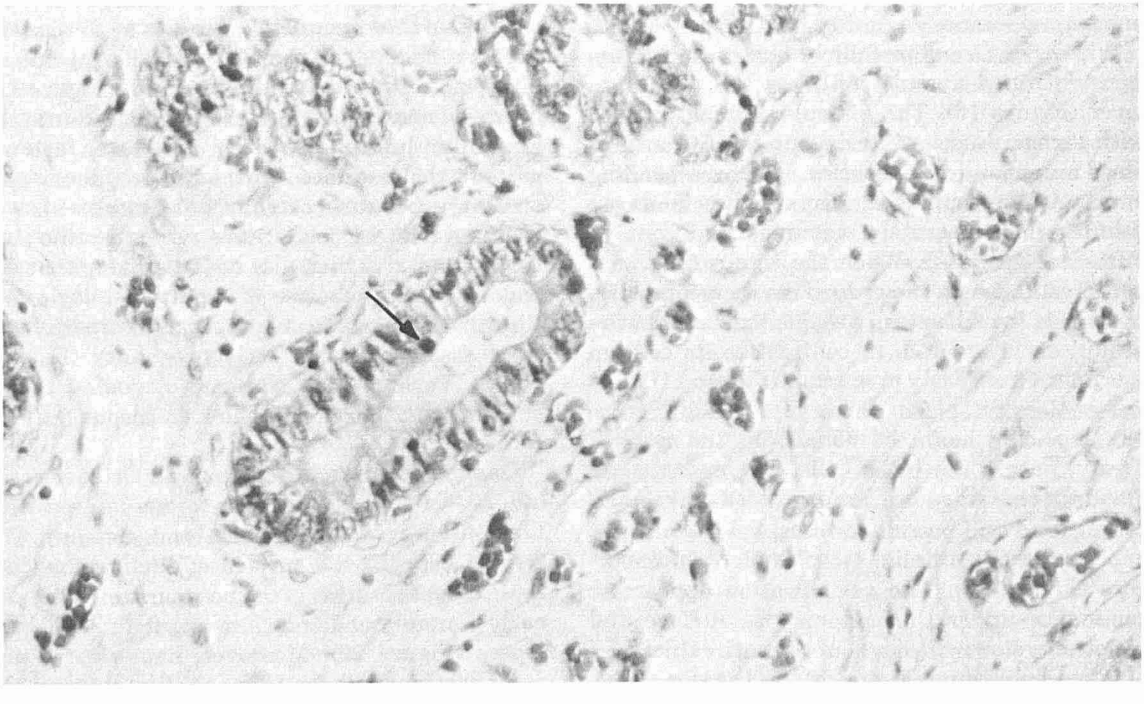

FIGURE 17.—Endometrium of specimen in Figure 16 with glandular mitosis at arrow. Note stromal edema (hematoxylin and eosin × 400).

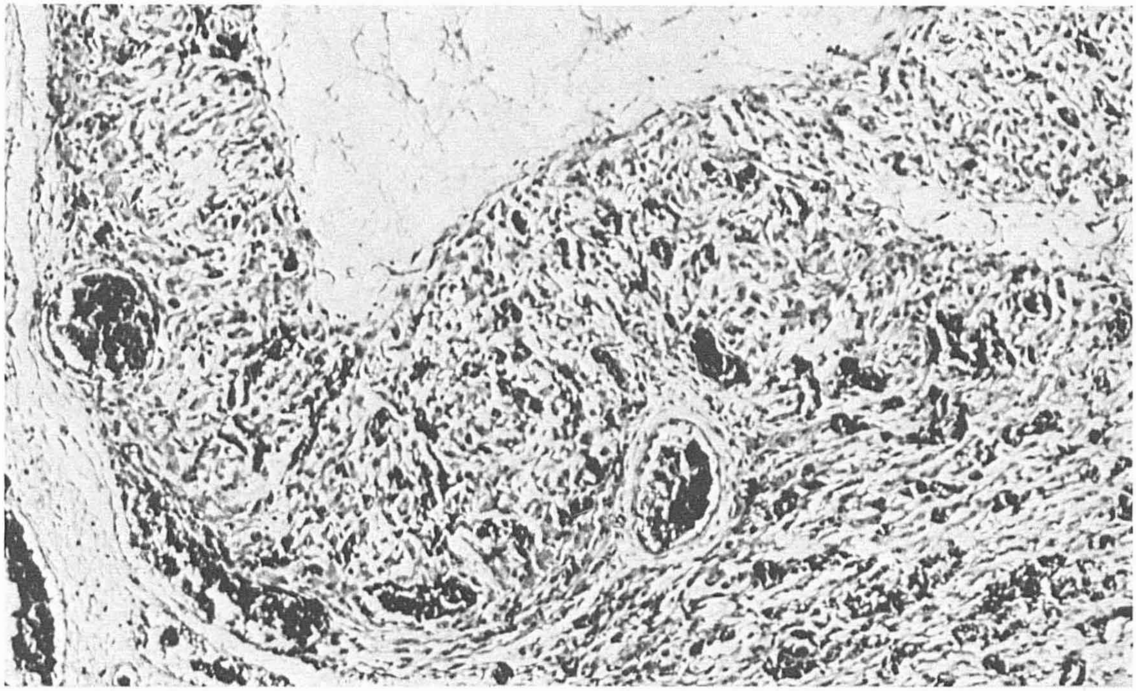

FIGURE 18.—Young corpus luteum in nonpregnant animal. Note central cavity (top) is filled with fibrin and granulosa layer is penetrated by capillaries (black). Size of granulosa lutein cells is much smaller than in Figure 11, an early pregnant specimen (no. 3, Table 7; hematoxylin and eosin × 100).

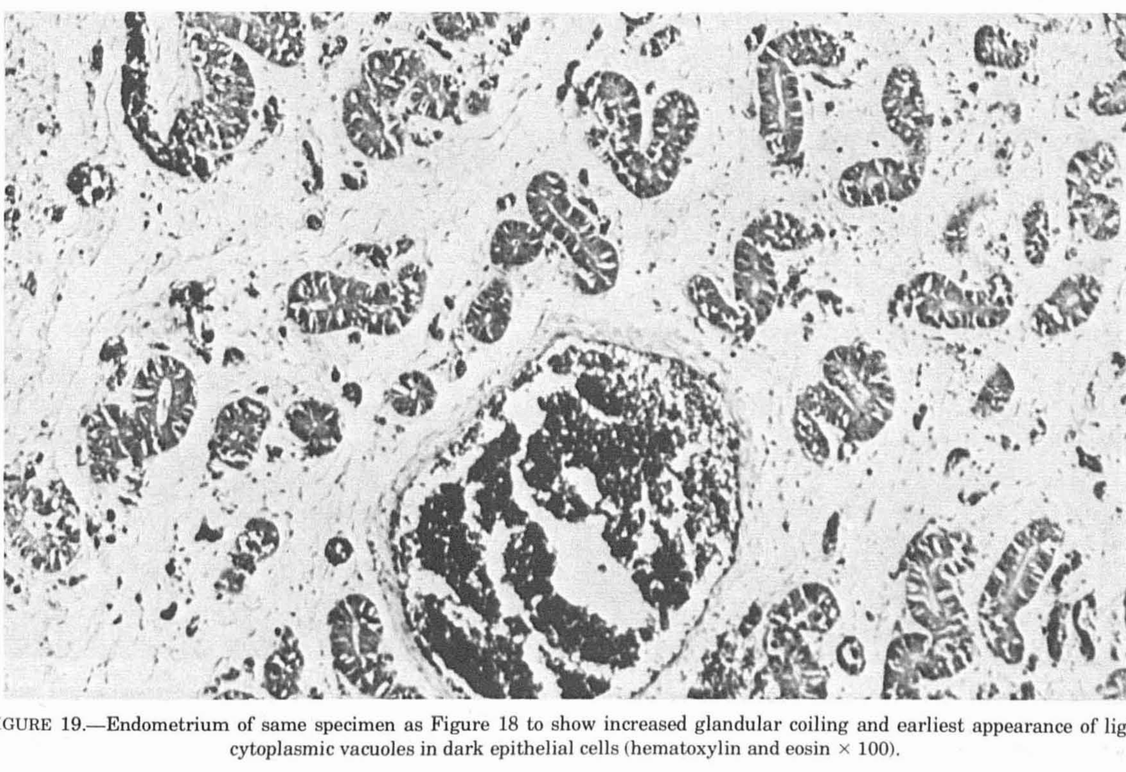

FIGURE 19.—Endometrium of same specimen as Figure 18 to show increased glandular coiling and earliest appearance of light cytoplasmic vacuoles in dark epithelial cells (hematoxylin and eosin × 100).

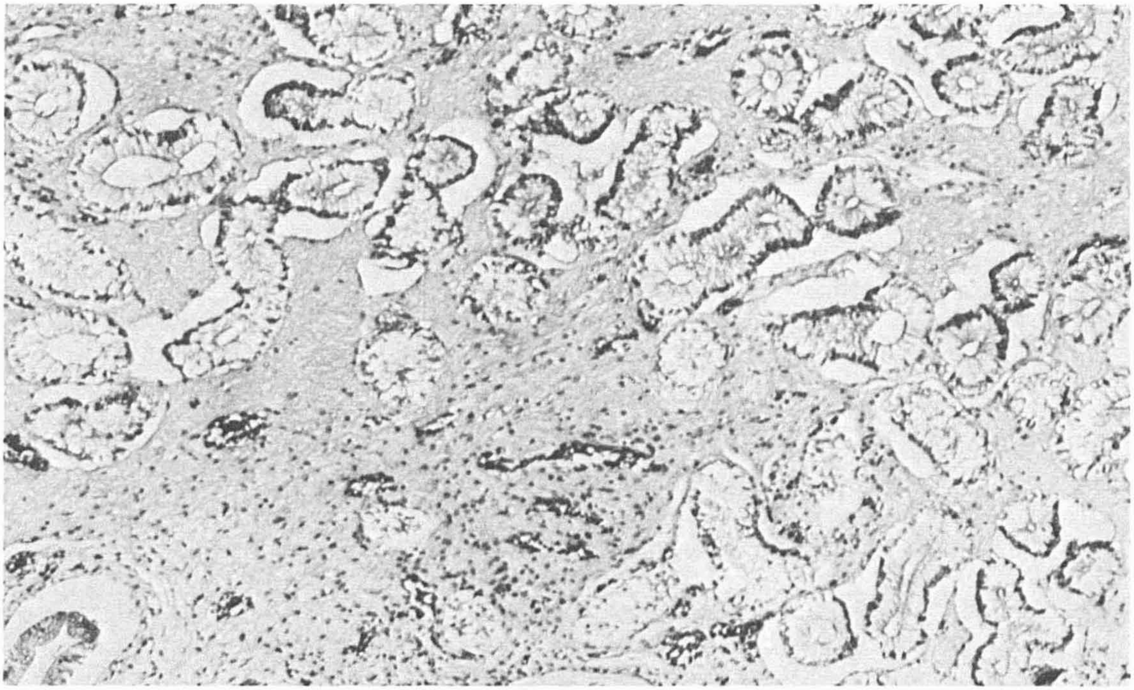

FIGURE 20.—Endometrium of nonpregnant *Stenella longirostris* with well-established corpus luteum, to show secretory vacuoles in endometrial glands as well as stromal edema (no. 10, Table 7; hematoxylin and eosin × 100).

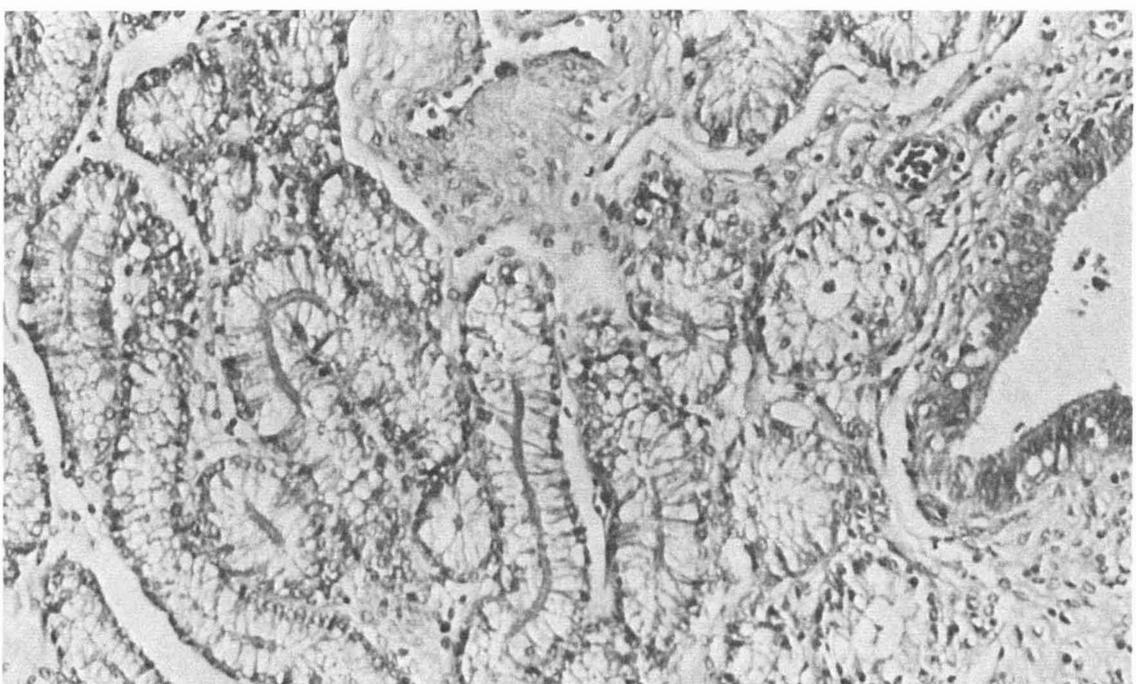

FIGURE 21.—Final stages of secretory change in endometrium of nonpregnant *Stenella longirostris* with advanced corpus luteum. Crowding of secretory glands occurs in stroma that has now lost its edema. No secretion in lumen. Endometrial cavity at right (no. 16, Table 7; hematoxylin and eosin × 100).

FIGURE 22.—Endometrium of presumably delivered *Stenella attenuata* whose ovary had a fresh corpus luteum. Endometrial cavity top left, hyalinized stroma (HyS) beneath surface epithelium and secretory glands (SG) beneath this layer (no. 20, Table 7; hematoxylin and eosin × 100).

marked discrepancy of the uterine horns (29 cm left; 18 cm right).

GROUP VIb.—These 15 animals (11 *S. longirostris*; 4 *S. attenuata*) were found to be nonpregnant but had well-established corpora lutea with completely organized cavities, the luteal cells appeared well supported (Table 8). Their lengths varied from 169 to 198 cm and uterine weights were between 110 and 580 g. One of these specimens had not been opened, was carefully dissected by us, and found to be nonpregnant. Again, most (12) were from February catches, many uteri had stretch marks from former pregnancy, and in two ovaries the corpus luteum was found on the right.

The secretory endometrium of almost all these

TABLE 8.—Group VIb: Experimental group with well-supported corpora lutea. Specimen no. 1-11 *Stenella longirostris*; no. 12-15 *S. attenuata*.

Specimen no.	Month and year of capture	Dolphin length (cm)	Uterus weight (g)	Left uterine horn				Right uterine horn			
				Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)	Length (cm)	Endometrial fold (mm)	Endometrial valley (mm)	Myometrium (mm)
1	Feb. 1976	191	281	21	5.97	1.19	0.66	18	5.07	1.64	0.60
2	Feb. 1976	169	—	17	2.23	1.64	0.90	14	1.94	1.19	0.84
3	Feb. 1976	180	—	19	4.18	1.04	0.75	13	3.28	1.04	0.75
4	Feb. 1976	184	142	18	7.16	0.84	1.49	16	6.57	1.49	1.79
5	Feb. 1976	180	149	22	5.67	0.90	1.19	16	4.33	1.04	1.19
6	May 1976	178	268	24	4.48	1.04	1.94	19	2.84	0.75	1.34
7	Feb. 1976	180	194	20	3.58	1.34	1.34	16	3.73	1.94	0.90
8	Feb. 1976	178	227	21	4.18	0.75	1.19	13	3.28	1.19	1.04
9	Feb. 1976	181	148	16	3.58	1.19	1.34	11	3.28	1.19	1.19
10	Feb. 1976	170	117	18	4.48	1.04	1.04	15	3.88	1.04	1.49
11	Feb. 1976	181	166	21	1.79	0.90	1.19	12	2.99	1.04	1.04
			S. longirostris — Average		4.30	1.08	1.18		3.74	1.23	1.11
12	Feb. 1976	193	250	24	5.67	2.09	1.34	23	2.84	1.19	0.90
13	Feb. 1976	184	110	11	3.28	0.90	1.04	10	4.18	0.90	0.84
14	June 1977	185	240	22	2.39	1.19	1.79	17	1.79	0.60	1.88
15	Nov. 1975	198	580	37	5.67	1.49	2.09	21	6.87	1.49	2.39
			S. attenuata — Average		4.25	1.42	1.57		3.92	1.05	1.50

specimens showed great similarity with those shown in Figures 20 and 21. The amount of edema varied slightly and so did the glandular vacuolization. No secretion was found within the glands although frequently there was thick mucus covering the endometrial surface on gross examination. The luteal cells were supported, not degenerated but less plump than in early pregnancy. The only difference was the complete fibrosis of the central cavity, probably insufficient findings for a meaningful separation of some specimens from the previous group VIa. Of course, no capillary proliferation of the superficial endometrium, so typical of pregnancy was evident.

One specimen is probably misclassified (no. 15, Table 8). Not only is this the largest uterus (580 g) with pronounced stretch marks (Figure 23) but also it exhibits a differing histologic appearance of the endometrium (Figure 24). This shows acute endometritis in a mucosa which otherwise had all of the characteristics of pregnancy. The corpus luteum was well supported with large plump cells. In all likelihood this *S. attenuata* had very recently delivered. This is from a November catch and it is impossible to determine whether this represented a full-term pregnancy since Perrin et al. (1976) showed that calving does occur in this species in the winter, although less commonly. It may well be that this inflammatory exudate in the superficial endometrial regions represents the normal immediately post partum event. This would explain the persistence of an active corpus

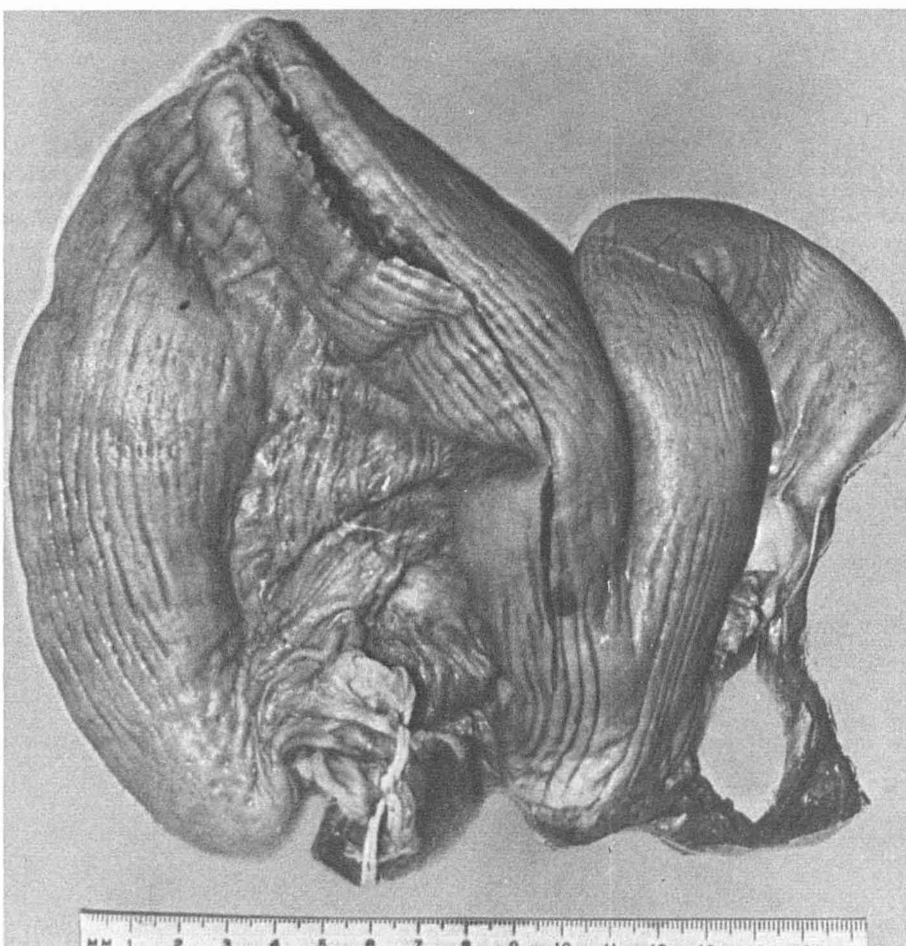

FIGURE 23.—Uterus of recently delivered *Stenella attenuata*, no. 15, Table 8. Note stretch marks of left horn, congestion and disparate size of horns.

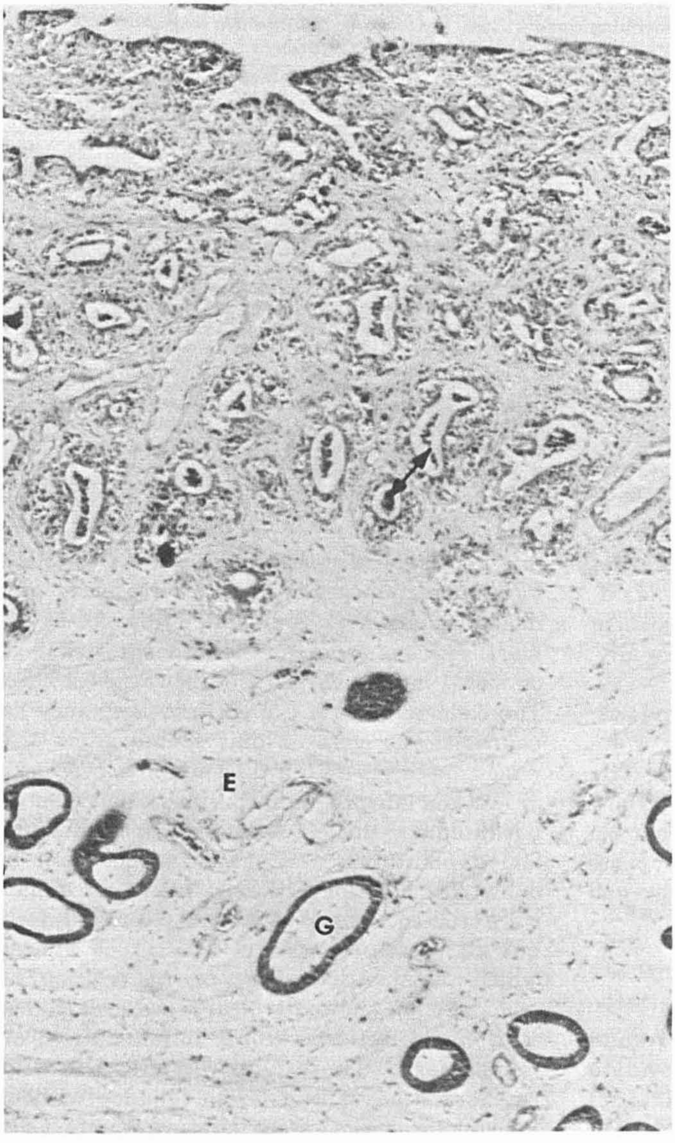

FIGURE 24.—Endometrium of uterus in Figure 23. The marked stromal edema (E) and distended glands (G) of basal endometrium are typical of late pregnancy. The superficial endometrium is infiltrated by polymorphonuclear leukocytes that exude into the glandular lumina (arrow). No surface stromal hyalinization has yet taken place. The former interdigitation of placental villi with surface endometrium can be imagined from the irregularities the surface presents (hematoxylin and eosin × 100).

luteum, presumably destined to involute shortly.

GROUP VIc.—This composes 15 animals (8 *S. longirostris*; 7 *S. attenuata*) measuring 162-193 cm whose features are listed in Table 9. These nonpregnant dolphins possessed histologically regressing corpora lutea. Eight animals in fact had corpora albicantia and were probably misclassified by the gross observations because the corpus luteum in each case was recently involuted, had still some vascularization and microscopic inflammation. Similarly, the endometria in these specimens usually had inflammation and hyalinization and were most likely recently delivered. This is further supported by the disparity in uterine horns. These animals were not listed as lactating either because this feature had been overlooked or perhaps the calves had died or were aborted. This material does not allow this differentiation.

Six of the remaining seven animals had corpora lutea with histologic signs of early degeneration. Two had late secretory endometrium and this would likely to have involuted upon further regression of the corpus luteum since pregnancy did not occur. Two animals had marked chronic en-

TABLE 9.—Group VIc: Experimental group with degenerating corpora lutea. Specimen no. 1-8 *Stenella longirostris*; no. 9-15 *S. attenuata*.

Specimen no.	Month and year of capture	Dolphin length (cm)	Uterus weight (g)	Left horn of uterus: Length (cm)	Left: Endometrial fold (mm)	Left: Endometrial valley (mm)	Left: Myometrium (mm)	Right horn of uterus: Length (cm)	Right: Endometrial fold (mm)	Right: Endometrial valley (mm)	Right: Myometrium (mm)
1	Sept. 1976	162	250	32	5.22	1.19	2.24	15	3.88	1.19	1.64
2	Feb. 1976	175	252	25	10.14	2.09	1.19	12	—	—	1.04
3	May 1976	183	273	25	4.93	1.04	1.19	15	3.88	1.19	1.04
4	Feb. 1976	179	—	23	4.78	0.90	1.19	14	4.48	1.94	1.34
5	Feb. 1976	185	—	21	2.09	0.90	0.90	16	1.19	1.04	0.75
6	Feb. 1976	186	—	22	2.24	0.75	0.90	16	1.04	0.45	0.90
7	Feb. 1976	186	291	25	5.37	1.19	1.34	14	3.58	0.90	1.04
8	Mar. 1976	189	197	17	5.37	1.19	1.64	13	3.28	1.04	1.04
			S. longirostris — Average		5.02	1.16	1.32		3.05	1.11	1.10
9	Aug. 1976	172	145	—	1.64	0.45	0.90	—	2.09	0.60	0.60
10	Feb. 1976	180	122	18	5.79	1.34	1.64	15	6.57	1.19	1.04
11	Feb. 1976	185	—	23	3.13	0.90	1.34	18	2.39	0.60	1.04
12	May 1976	191	205	18	4.18	0.75	1.79	18	2.39	0.60	1.79
13	June 1976	193	375	22	8.80	1.94	2.09	15	6.42	1.79	1.79
14	Aug. 1976	179	356	30	6.57	1.04	1.79	16	5.07	0.75	0.90
15	Apr. 1976	185	305	25	4.18	0.90	1.49	13	3.28	0.90	1.64
			S. attenuata — Average		4.90	1.05	1.60		4.03	0.92	1.26

dometritis. Two others had marked endometrial stromal edema in addition to having horns of different lengths. The possibility of abortion exists in these animals but this cannot be proven. Finally, one animal of this heterogeneous group (no. 12, Table 9) had equal uterine horns (18 cm) and possessed corpora albicantia in addition to a degenerating right-sided corpus luteum. The endometrium with hyalinization and capillary development suggested recent pregnancy but because of the size of the uterus (205 g) it appears likely that a recent right-sided pregnancy had ended in abortion.

DISCUSSION

Although conclusive proof is lacking, the results support the notion that dolphins may ovulate spontaneously, at least at times. Control material of different reproductive phases (96 specimens) of the two species of dolphin investigated, the eastern spinner dolphin, *S. longirostris*, and the spotted dolphin, *S. attenuata*, provided a life history of the macroscopic and microscopic events in the uteri and ovaries of these species for the first time. The principal findings were the following: In three immature animals neither corpora lutea nor corpora albicantia were present in the ovaries but Graafian follicles were forming. The endometrium had tubular glands. Six mature animals without corpus luteum all were found to have one or more corpus albicans, the horns were disparate in size, five had stretch marks as an excellent sign of former pregnancy, in most the inactive endometrium possessed inflammatory cells, hyalinization, had hemosiderin macrophages as indications of former pregnancy. Eleven of twelve early pregnant animals with embryonic sizes of 1-53 mm had the pregnancy in the left horn; one in the right on which side the corpus luteum was also located. The endometrium of the earliest pregnancy had characteristic changes that should allow pregnancy diagnosis even if the embryo is overlooked or lost. Six late pregnant specimens had their corpus luteum in the left ovary, typical epitheliochorial implantation and again characteristic endometrial histology. Eleven lactating females were studied, none of which had a corpus luteum, but all possessed at least one corpus albicans which was in various stages of degeneration. The endometrium showed typical regressive changes with hyalinization, chronic inflammation, and hemosiderin. These features will also allow categorization of uteri from females with unknown reproductive state in future studies. Of the 58 animals with macroscopically diagnosed corpora lutea and no detectable pregnancy, only 43 in fact had an active corpus luteum, the remaining 15 specimens had corpora albicantia in various stages of regression. Three corpora lutea were on the right, the remaining 40 were on the left, confirming the usual finding of Cetacea that the left side predominates in reproduction. When a correlation was sought between the development of the corpus luteum and endometrial changes a well-delineated cycle of proliferation and edema to secretory stages emerged. No changes indicative of early pregnancy were seen and three intact uteri contained no embryos. Thus, clearly, ovulation does occur without pregnancy ensuing. In three specimens the findings are consistent with abortion, two had marked endometritis. These latter findings are summarized in Table 10.

TABLE 10.—Group VI: Summary of experimental, nonpregnant animals; 38 *Stenella longirostris*, 20 *S. attenuata*.

S. longirostris:		
19	(50%)	Fresh corpus luteum
11	(29%)	Well-established corpus luteum
8	(21%)	Degenerating corpus luteum—wrong gross diagnosis (2 endometritis; 2 unimplanted abortion?)
S. attenuata:		
9	(45%)	Fresh corpus luteum
3	(15%)	Well-established corpus luteum
1	(5%)	Misclassified, recently delivered
7	(35%)	Degenerating corpus luteum—wrong gross diagnosis (1 recent abortion?)

The findings of Perrin et al. (1976, 1977) indicate that over 90% of female genital tracts of *S. longirostris* and *S. attenuata* contain a corpus luteum because of their pregnancy. The possibility that abortions of implanted pregnancies occur frequently can be ruled out from the histologic appearance of the endometria in the majority of the specimens from the experimental group. Early embryonic death remains a possibility although no evidence can be adduced for this. The present study provides strong evidence that spontaneous ovulation may occur in these species, at least that the presence of a corpus luteum is not indicative of pregnancy. Moreover, recent and as yet unpublished results from our laboratory (J. Sawyer) have identified hormonally the occurrence of spontaneous ovulation in a *Delphinus delphis* female that was kept in isolation. Evidence for regression of corpora lutea in nonfertile cycles was also found in our experimental group. It is possible then that either ovulation is induced in a majority of cycles or that a majority of dolphins become pregnant when ovulating. In either case, it is not likely that a count of corpora albicantia in dolphin ovaries accurately reflects the number of past pregnancies. We were unable to differentiate with certainty between the histologic appearance of a corpus luteum of early pregnancy and one from nonpregnant animals and cannot accept the alleged feasibility of the diagnosis of pregnancy from the histology of only a corpus luteum. The endometrial histology is a better guide. Whereas accessory corpora lutea have been found in other odontocetes (Brodie 1972), no such structures were encountered in the genital tracts of these two *Stenella* species.

It would be useful to know from aquaria with accurate historical information and pathologic study at death whether older females that have been kept in isolation since youth possess corpora albicantia. Inasmuch as the question of artificial insemination of exhibited animals has been raised in the past (Hill and Gilmartin 1977) it can be concluded that it would appear feasible without the need of superovulation.

From a practical standpoint it is suggested that the following points be considered in future studies. First, it would have been helpful to have had a histologic sample of mammary gland and vagina in all specimens to enhance histologic correlations. Moreover, inasmuch as the hormonal cycle of *D. delphis* is now being defined by modern endocrine techniques undertaken by sequential sampling of captive specimens it may be useful for future studies to save, for endocrine analysis, an aliquot of frozen serum. Potentially, such a study would clarify the status of equivocal corpora lutea. At the same time storage of fetal serum would allow insight into the fetal endocrine behavior which is known to differ so markedly between species. Perhaps such comparative studies will ultimately give additional insight into the phylogenetic descendency of Cetacea.

This study extends the sparse literature that exists on the morphology of the female reproductive organs. In general, the endometrial cycle is similar to that illustrated schematically for several whales (Slijper 1966) and for *Globiocephala melaena* (Harrison 1949). In this latter species Harrison (1949) also described the small superficial anastomosing vessels beneath the uterine epithelium and, in three recently ovulated specimens, he was unable to identify products of conception. In a later contribution, Harrison et al. (1969) suggested that while ovulation in *Tursiops truncatus* appears to be of a reflex nature, that of *S. graffmani* and *Lagenorhynchus obliquidens* may be spontaneous as in *G. melaena*. Our own findings cited above suggest, however, that in *D. delphis* spontaneous ovulation occurs and one wonders whether this then may not be the rule in Cetacea. A resolution can come only from longitudinal endocrine studies of isolated females from different species. Finally, as was the case in most other studies of the morphology of Cetacean reproductive organs, we observed no significant pathologic features in these specimens other than the endometritis described and occasional parametrial parasitic granulomata.

ACKNOWLEDGMENTS

The encouragement and constructive criticism of W. F. Perrin is warmly acknowledged. The study was performed under contract NAS07-35234. The original slides, additional data, photographs, negatives, and an expanded manuscript are on file at the Southwest Fisheries Center, La Jolla Laboratory, National Marine Fisheries Service, NOAA, La Jolla, Calif.

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