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TECHNIQUES FOR THE PREPARATION AND EXAMINATION OF REPRODUCTIVE SAMPLES COLLECTED FROM DOLPHINS IN THE EASTERN TROPICAL PACIFIC

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U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southwest Fisheries Science Center

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ABSTRACT

This report describes the standardized methods that are used for the collection and processing of reproductive samples collected from eastern tropical Pacific dolphins. For 20 years, consistent sampling and processing procedures have been used to ensure the continuity and integrity of the data. Descriptions of laboratory rough-sorting and fine processing of uteri, fetuses and testes have been included along with a brief description of both ovary and testis histology. In addition, criteria for the identification and classification of the stages of regression of ovarian scars and the determination of sexual maturity from the testis are described and illustrated.

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INTRODUCTION

During the past twenty years biological data have been collected from dolphins incidentally killed in the yellowfin tuna purse-seine fishery in the eastern tropical Pacific (ETP) (Oliver 1991). The species most commonly killed in the fishery are the spotted dolphin, Stenella attenuata; the spinner dolphin, Stenella longirostris; the common dolphin, Delphinus delphis; and the striped dolphin, Stenella coeruleoalba. Purse-seining for yellowfin tuna in the ETP began in the late 1950s. The collection of data to document the kill and estimate population parameters began in 1966, although the collection procedures were not standardized until 1974. The data are used to estimate the reproductive potential of each dolphin population as well as to evaluate the long-term effects of fisheries exploitation on the dolphin species involved. This is accomplished by examining trends in reproductive parameters such as pregnancy rates and age at attainment of sexual maturity (ASM) (Perrin & Reilly 1984). The collected data are used to determine the fraction of sexually immature and mature animals incidentally killed, along with the fraction of mature females which are pregnant, lactating or resting. Ovulation rate, annual pregnancy rate and length of the calving interval are also estimated (Perrin and Reilly, 1984; Myrick et al. 1985). ASM is calculated from a sample of teeth that have been aged (Myrick et al. 1983) and the percent of reproductively mature animals in each age class.

In the field, biologists (i.e., observers) collect life history information and record data on species, stock, total body length, sex, and reproductive condition of females (for instance, whether they are pregnant or lactating). Specimen sample materials are collected and recorded by the observer on the "Cetacean Life History Form" (Fig.1). Tissue samples, such as gonads, jaw sections and small fetuses (<25 cm.) collected are preserved in formalin and returned to the laboratory for further processing (Myrick, 1986).

In this manual we describe the laboratory processing of gonads and fetuses collected by observers in the field. Although preparation techniques described here are specifically for ETP species, they may be readily applicable to other cetacean species as well. The collection and processing of other specimen materials has been described by Perrin et al. (1976; fetuses), Myrick et al. (1983; collection and preparation of teeth), Bernard and Hohn (1989) and Schwartz et al. (1992; stomach contents) and Hohn et al. (1986; reproductive tissues and skin and liver tissues collected for mtDNA analyses and chemical pollutant studies).

METHODS

Rough-sorting

"Rough-sorting" is the term used for the overall preparation, external examination and cataloging of biological specimens after they are returned to the laboratory. During rough-sorting of

gonads for females, the ovaries and uteri are examined. A pregnancy may be detected that was not noted in the field by the observer. A step-by-step procedure for processing the ovaries and uteri is detailed in Table 1. Fetuses are only returned to the laboratory for processing if they are less than 25 cm in length (Table 2); otherwise they are measured, sexed and discarded in the field by the observer as they are too large to collect. During rough-sorting of gonads for males, testes and epididymides are weighed and a small tissue sample of each is retained for further histological preparation (Table 3).

Determination of sexual maturity

Females: the ovarian cycle begins with the development of a follicle in the ovary and ends with the release of an oocyte or the degeneration (atresia) of the follicle. The follicle contains a small oocyte, a layer of granulosa cells and a basement membrane. Maturation of the follicle includes growth of the oocyte, an increase in the number of layers of granulosa cells and the division of the cell wall membranes into two layers. Through gradual transition (Fig. 2), the final antral stage, the Graafian follicle, is produced. At this point in the cycle, the cavity is fluid filled (Fig. 3). The oocyte undergoes meiosis and one set of chromosomes remains in the cell, now called the secondary oocyte. The secondary oocyte is released at ovulation.

After ovulation, the follicle walls may be transformed into an endocrine gland, the corpus luteum (Fig. 7), (Perrin and Donovan 1984). If a pregnancy ensues, the corpus luteum is referred to as the corpus luteum of pregnancy (CLP) and persists as a secretory organ, producing the hormones necessary to maintain pregnancy. If no pregnancy occurs, the corpus luteum is referred to as the corpus luteum of ovulation (CLO) and regresses, becoming a corpus albicans or scar on the ovary.

In appearance, the corpus luteum is a globular 'bulge' on the surface of the ovary. Its interior is composed primarily of yellow-colored granulosa cells arranged in convoluted layers which are interspersed with connective tissue and blood vessels (Fig. 7); the outer surface is covered with a network of blood vessels. Some corpora lutea have a stellate, gelatinous central cavity surrounded by the glandular element arranged in distinct folds; these septa, which carry blood vessels, radiate inward from the periphery. In others, the central cavity is filled with connective tissue. (Harrison and Weir 1977; Chittleborough 1954).

After ovulation or at the termination of a pregnancy, a continuous process of regression of the luteum occurs. The amount of connective tissue between lutein cells increases and becomes hyalinized (hyaline is a transparent-to-translucent crystalline tissue containing little fibrous tissue). The remaining tissue of the luteal degeneration is called the corpus albicans (CA). This tissue is divided into stages of regression for easy classification and study. Assuming that the regression rate is constant, the

amount of luteal tissue remaining is inversely proportional to the amount of time that has elapsed since the ovulation which preceded it. The remaining corpora albicantia are records of past ovulations, not of pregnancies.

Corpora albicantia stages have been described for Pontoporia (Harrison et al., 1981), Stenella (Perrin et al. 1976) and Globicephala (Marsh & Kasuya 1984) based on color and composition and the amount of hyaline material present. The classification system defined by Perrin et al. 1976 and used at the Southwest Fisheries Science Center is described in detail under Equipment and Procedures for Corpora Reading (counting) and Classification of Corpora Albicantia (Tables 4 and 5) and is illustrated in Figures 4-8.

Other stages of degeneration, according to categories recommended by Marsh and Kasuya (1984), are identified as: (1) early, fibrous tissue replaces primary luteal cells, (2) medium, connective tissue is shrunken, hyalinized, (3) old, a continuation of stage 2, with blood vessels making up a greater part of the structure. These stages would correspond to Figures 4-6.

Sexual maturity in female dolphins is determined by the presence of one or more corpus lutea or a corpora albicantia. Most attempts to distinguish the CAs of ovulation from those of pregnancy have not been successful (Perrin and Donovan 1984). The distinguishing feature between the two types of CAs is thought to be the amount of amorphous, hyaline material present (Collet & Harrison 1981). Some workers think that the CAs of pregnancy persist for life (Perrin et al. 1976; Collet and Harrison 1981). Marsh & Kasuya (1984) state that CAs persist for life in Globicephala macrorhynchus and that in young animals, CAs regressed to the oldest CA stage within two years. In pregnant and older animals, the rate of CA regression is greatly reduced. In Pontoporia, the CAs may persist for a maximum of four years before being reabsorbed completely (Harrison et al. 1981).

Not all ovarian follicles produce an oocyte. In some, the normal follicular development cycle undergoes follicular atresia, ovulation does not occur (Perrin and Donovan 1984) and atresia gives rise either to secondary interstitial tissue or to accessory corpora lutea, both of which may be hormonally active. Accessory corpora lutea are termed atretic follicles without luteinization and form fibrous bodies (corpora atretica b) that are probably reabsorbed slowly (Marsh and Kasuya 1984). Atretic follicles that do become lutealized eventually regress and may be the corpora albicantia (seen when counting ovarian scars of delphinids), labeled "Type 5" in Table 5 and Figure 8.

In Pontoporia (Harrison et al. 1981), the size of the corpus luteum in its earliest stages of development is variable and seems to be dependent on the way the follicle ruptures. If it ruptures completely, the corpus luteum begins development and is quite small. If it does not rupture completely, the small opening or pore is plugged and the follicle is lined with luteal cells but is filled with blood. In this case, the corpus luteum diameter is quite large. These differences indicate that, until the luteum is

fully developed, the degree of development cannot be estimated by measurement alone. In addition, the size of the corpus luteum during the course of the pregnancy varies by species. In spotted dolphins, the corpus luteum decreases in size during gestation (Perrin et al. 1976) while in Pontoporia (Harrison et al. 1981), the size of the luteum increases slightly.

Males: Spermatogenesis is a complex process of continual cell differentiation. Stages of spermatogenesis can best be described by following the sequence of development of spermatogonia. Through mitosis, one spermatogonium produces two cells: one is called the "A" cell and the other the stem cell. The "A" cell divides into intermediate spermatogonial cell types, which divide into "B" type spermatogonia. The "B" type spermatogonia divide into diploid primary spermatocytes, which are in the early stages of meiosis. At the end of the first phase of meiosis, these become haploid secondary spermatocytes which almost immediately enter the second meiotic division to become spermatids. Spermatids are the most numerous and most developed layer in the tubule epithelium. Through several stages of spermiogenesis, the spermatids are transformed into spermatozoa. Structurally, four classes of germinal cells, the spermatogonia, the spermatocytes, the spermatids and the spermatozoa are arranged in concentric layers in the seminiferous tubules. The most primitive cells (spermatogonia) are located near the periphery of the tubule, while the most developmentally advanced cells (spermatozoa) are located on the border of the lumen (Banks 1986; Guraya 1987). Histological preparations necessary for determination of stage of sexual maturity are made by thin-sectioning (5-10 μm) testes or epididymal tissue and staining these with hematoxylin and eosin. Sexual maturity in male dolphins is determined by examination of stained histologic sections and noting the presence of spermatozoa and the size of the seminiferous tubules (Hohn et al. 1985), by testis and epididymis weight (Perrin et al. 1977; Perrin and Henderson 1984), by seminiferous tubule diameter (Perrin and Henderson 1984) and by percentage of mature tubules present (Kasuya and Marsh 1984).

Collet and St. Girons (1984) defined three stages of sexual maturity: immature, prepubescent and mature. In their study of male spotted dolphins, Hohn et al. (1985), called these stages immature, pubertal and mature (Figures 9,10,11). The immature stage is characterized by relatively small, circular tubules with no lumen. These tubules contain only spermatogonia and abundant interstitial tissue. The pubertal or prepubescent stage has much larger, slightly elongated tubules and the interstitial tissue occupies little space between the tubules. Generally, spermatogonia and spermatocytes are present but there are no spermatozoa, although a few of the tubules may indicate sexual maturity by having spermatozoa present. In the mature stage, tubules are large in diameter and are elongated, with little interstitial tissue present. All stages of spermatogenesis are present in the tubules and the lumen is large. Virtually every tubule has spermatozoa although this may vary seasonally.

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Table 1. Rough-sorting of Female Reproductive Tissues

1. First, the left ovary is processed. A check is made to make sure the specimen tag with the specimen number[1] is on the left uterine horn. If necessary, lay out the reproductive tract with the dorsal surface uppermost (the dorsal surface has deep longitudinal wrinkles on the outer surface of the vagina).

2. The left ovary is removed from the left horn with dissecting scissors and the number of scars (corpora albicantia) visible on the surface are counted. This count is recorded on the Cetacean Life History Form in pencil (see Coding Format, Appendix 1).

3. The ovary is weighed to the nearest tenth of a gram and the weight recorded on the Cetacean Life History Form.

4. The ovary is checked for presence of a corpus luteum. If a CL is present, it should be noted on the Form according to the appropriate code (Appendix 1).

5. The corpus luteum is measured with dial calipers and the three greatest diameters are recorded in ink on the Cetacean Life History Form. Measurements are comparable to length, width and depth, even if the luteum is spherical.

6. Steps 2-5 are repeated for right ovary.

7. Left and right ovaries are stored in separate vials in 70% ethanol (ETOH) with a label stating the specimen number, and an "L" or "R," indicating left or right ovary. For example: ABC0123 L and ABC0123 R. Vials are placed in a box in specimen number order. The outside of the box is marked with a label indicating "OVARIES", along with the cruise number from which the samples came, observer initials used in specimen numbering, date the samples were taken, and the species of dolphins.

8. Further examination of the ovaries to classify and measure corpora types is completed later in the laboratory (See Corpora Reading).

[1]The specimen number is the observer's initials and a number indicating the number of specimens worked on by that observer. Example "ABC0123" indicates that it is the one hundred twenty-third animal collected by observer ABC.

Table 2. Rough-sorting of Fetuses

1. If a corpus luteum is present, the reproductive tract is checked carefully for the presence of a fetus. Starting with the horn that had the corpus luteum on the ovary, a slit is made in the wall of the uterine horn using a knife or scissors, taking care not to cut too deeply. The opening is then enlarged using the fingers, and the uterus is torn open longitudinally from the base of the vagina to the end of the horn. The procedure is repeated on the other horn. If a fetus is found, it is indicated on the Cetacean Life History Form as "1"; if no fetus is found, a "2" is recorded.

2. A very small fetus may look like a slender, white thread. If such a thread is found, it is removed from the uterus with forceps and stored in a vial of 70% ethanol. No measurement is taken as the fetus is too small. Instead, the measurement is recorded as 0.1 cm.

3. Larger fetuses are removed from the uterus, leaving some of the umbilical cord attached to the fetus. The length is measured in mm with calipers, and the weight is taken. Fetal length and weight are entered on the Cetacean Life History Form.

4. The sex of the fetus is determined visually and recorded. If the fetus is too small to determine the sex, then a dissecting scope may be used.

5. Data collected on all fetuses are recorded in the "Fetus Log"; these include the specimen number of the pregnant female, the species, sex, length and weight of the fetus, and whether the fetus is in a separate container or is stored with the uterus. If it is stored in a separate container, the container is numbered sequentially according to a continuing list in the "Fetus Log." The container number is marked on the outside of the jar with a self-adhesive label.

6. A small label is placed in the container or a tag may be attached to the tail of the specimen. This tag is labeled in pencil with the specimen number of the pregnant female, cruise number, date of capture, latitude and longitude of capture, length of animal and species.

7. Fetuses in containers are placed in the fetus collection in container-number order.

Table 3. Rough-sorting of Male Reproductive Tissue

1. The specimen label on the testis is removed. The right testis is usually collected in order to keep the data consistent.

2. The epididymis is separated from the testis by cutting through the membranous connection located between the two tissues; any cysts are removed from the epididymis before it is weighed. The length of the testis is measured with the anthropometer and recorded on the Cetacean Life History Form.

3. The testis and epididymis are weighed together on the balance, then the testis is weighed alone. Both weights are recorded. If both left and right testes were collected, the weights are recorded separately.

4. A boning knife is used to slice transversely through the center of the testis at approximately mid-length. Another slice is made parallel to the first to obtain a cross-section about 1 cm thick. A cubic centimeter piece is then cut from the center of the section. (Marsh and Kasuya 1984, found no differences in the mean diameter and maturational status of the seminiferous tubules in different portions of the testis). A central portion of the epididymis (also about 1 cubic centimeter) is removed.

5. Both the testis and the epididymis samples are stored in vials of 70% ETOH. Labels are placed in the vials listing specimen number and the letter "R" for right testis and "EPI" for the epididymis. For example: ABC0123 EPI and ABC0123 R.

6. All testis and epididymis vials from one cruise are stored together in a small cardboard box (7 1/8" x 4 1/4" x 2 3/4"). This is labeled (in marking pen) on one end, with observer initials, species, the word "TESTES," cruise number, and year.

7. Testis boxes are stored in cruise number order.

Table 4. Equipment and Procedures for Corpora Reading

Equipment:

Microscope- a binocular dissecting scope set at 10x, used with a 10 mm micrometer disk

Dial calipers

Scalpel- a number 8 handle with a number 60 autopsy blade

Beaker - about 100 ml.

Forceps - generally two pair are used: a blunt-tipped 150 mm forceps to handle whole ovaries and a fine-tipped 120 mm forceps to manipulate slices of the ovary.

Plastic Slide - an opaque, white plastic slide of at least 13 x 7 cm is used as a platform for slicing ovaries.

Procedures:

1. A pair of ovaries (left and right ovaries from the same specimen) are selected to slice. The left ovary is removed from its vial, rinsed in a beaker of water, dried on a paper towel, placed on a plastic slide and sliced with a scalpel into 1 mm serial sections. The sections are arranged on the slide in the order that they were sliced, each section falling to the right in the row from right to left. Each succeeding row is also arranged right to left until the ovary is completely sliced.

2. To classify the corpora albicantia (Table 5), each corpus is measured with dial calipers from the surface scar to the maximum internal depth. Diameter measurements are made to the nearest 0.1 mm on the section that has the most definitive characteristics of the stage represented by the corpus. The beginning and ending of each corpus should be carefully included in the measurements as the corpus usually extends through a number of sections. When all the corpora on the ovary have been classified and measured, the micrometer disk in the eyepiece is used to measure the diameter of the largest Graafian follicle. All information is coded and recorded in ink on the Cetacean Life History Form.

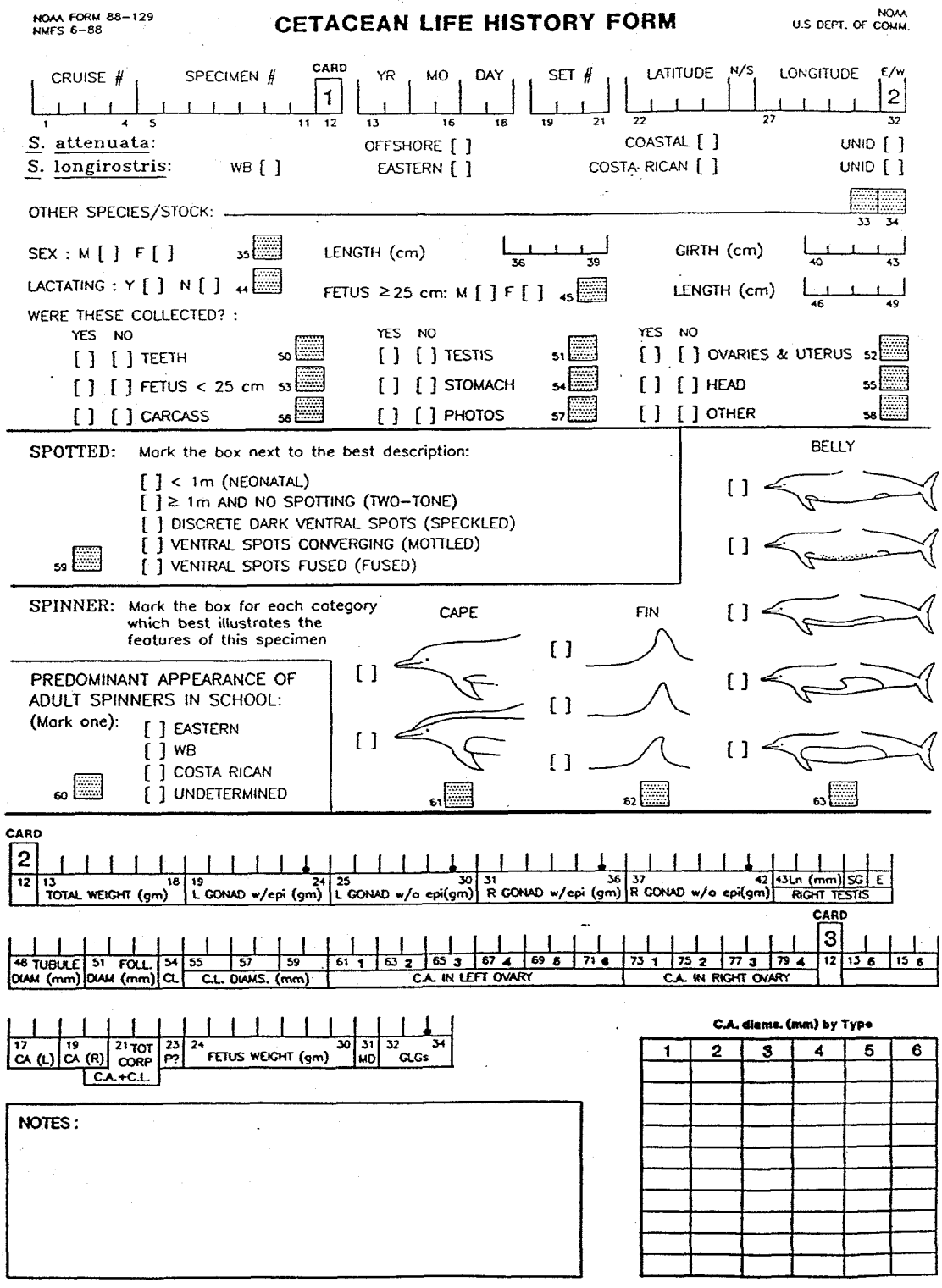
Table 5. Classification of Corpora Albicantia

	Type 1 (Fig. 4)
External structure	Surface raised, smooth or slightly wrinkled; appears as small corpus luteum.
Internal structure	Cortex solid luteal tissue white or yellow interspersed with white connective tissue; some remnants of vascularization.
Size	Diameter 3.5 to 15.5 mm.; average 7.0 mm.
Comments	This type is first stage of degeneration of the corpus luteum. Females with two or more corpora usually have one type 1 corpus although as many as five may be present.
	Type 2 (Fig. 5)
External structure	Surface raised and wrinkled.
Internal structure	Interior white to yellow with traces of luteal cortex and vascularization. Center constructed mainly of white connective tissue either loosely constructed or solid. Some vascularization.
Size	Diameter 3.0 to 12.0 mm, average 6.0 mm.
Comments	Less integrated in structure than type 1; evidence on accumulation rate suggests that this type is a mixture of regressed corpora lutea and corpora of ovulation.
	Type 3 (Fig. 6)
External structure	Surface not raised; scar usually smaller than type 2 and heavily wrinkled. May be flattened against the surface or pedunculate, surface flattened and extending deep into the ovary.
Internal structure	Primarily white connective tissue; may have deep yellow stains around the white center.

Table 5. Continued.

Size	Diameter 2.0 to 8.5 mm, average 3.5 mm.
Comments	Catch-all category for all small compact corpora with surface scars (usually) and internal structure. Includes both regressed corpora lutea and corpora representing ovulation and other events. When many corpora are present, some of this type may be present but not visible at the surface.
	Type 4 (Fig. 7)
External structure	Corpus flattened against the edge of a new corpus luteum. Slight surface wrinkles.
Internal structure	Interior structure of type 2 or type 3 but distorted by the new corpus luteum.
Size	Diameter 2.0-15.0 mm.
Comments	Distortion from c. luteum prevents categorizing as type 2 or 3.
	Type 5 (Fig. 8)
External structure	Surface trace very slight or absent.
Internal structure	No concentrated connective tissue, deep yellow- or orange-stained area.
Size	Diameter 0.5 to 5.5 mm, average 2 mm.
Comments	This type is most likely the end result of regression of an atretic lutealized follicle.
	Type 6
External structure	A surface scar is found in this stage but no discernable internal structure is present. Possibly the final result of a corpus luteum after a long period of degeneration. (No figure is available as these scars are rare).

Figure 1. Cetacean Life History Form.



NOAA FORM 88-129
NMFS 6-88

CETACEAN LIFE HISTORY FORM

NOAA
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CRUISE # | SPECIMEN # | CARD 1 | YR | MO | DAY | SET # | LATITUDE | N/S | LONGITUDE | E/W | 2

1 4 5 11 12 13 16 18 19 21 22 27 32

S. attenuata: OFFSHORE [ ] COASTAL [ ] UNID [ ]

S. longirostris: WB [ ] EASTERN [ ] COSTA RICAN [ ] UNID [ ]

OTHER SPECIES/STOCK: 33 34

SEX : M [ ] F [ ] 35 LENGTH (cm) 36 39 GIRTH (cm) 40 43

LACTATING : Y [ ] N [ ] 44 FETUS ≥25 cm: M [ ] F [ ] 45 LENGTH (cm) 46 49

WERE THESE COLLECTED? :

YES	NO		YES	NO		YES	NO	
[ ]	[ ]	TEETH 50	[ ]	[ ]	TESTIS 51	[ ]	[ ]	OVARIES & UTERUS 52
[ ]	[ ]	FETUS < 25 cm 53	[ ]	[ ]	STOMACH 54	[ ]	[ ]	HEAD 55
[ ]	[ ]	CARCASS 56	[ ]	[ ]	PHOTOS 57	[ ]	[ ]	OTHER 58

SPOTTED: Mark the box next to the best description: 59

[ ] < 1m (NEONATAL)
[ ] ≥ 1m AND NO SPOTTING (TWO-TONE)
[ ] DISCRETE DARK VENTRAL SPOTS (SPECKLED)
[ ] VENTRAL SPOTS CONVERGING (MOTTLED)
[ ] VENTRAL SPOTS FUSED (FUSED)

BELLY

[ ]
[ ]

SPINNER: Mark the box for each category which best illustrates the features of this specimen

CAPE FIN

[ ] [ ] [ ] 61 [ ] [ ] [ ] 62 [ ] [ ] [ ] 63

PREDOMINANT APPEARANCE OF ADULT SPINNERS IN SCHOOL: (Mark one): 60

[ ] EASTERN
[ ] WB
[ ] COSTA RICAN
[ ] UNDETERMINED

CARD 2

12 | 13 TOTAL WEIGHT (gm) 18 | 19 L GONAD w/epi (gm) 24 | 25 L GONAD w/o epi(gm) 30 | 31 R GONAD w/epi (gm) 36 | 37 R GONAD w/o epi(gm) 42 | 43Ln (mm) | SG | E | RIGHT TESTIS

CARD 3

48 TUBULE DIAM (mm) | 51 FOLL. DIAM (mm) | 54 CL | 55 57 59 C.L. DIAMS. (mm) | 61 1 63 2 65 3 67 4 69 5 71 6 C.A. IN LEFT OVARY | 73 1 75 2 77 3 79 4 C.A. IN RIGHT OVARY | 12 | 13 6 | 15 6

17 CA (L) | 19 CA (R) | 21 TOT CORP C.A.+C.L. | 23 P? | 24 FETUS WEIGHT (gm) 30 | 31 MD | 32 GLGs 34

C.A. diams. (mm) by Type

1	2	3	4	5	6

NOTES :

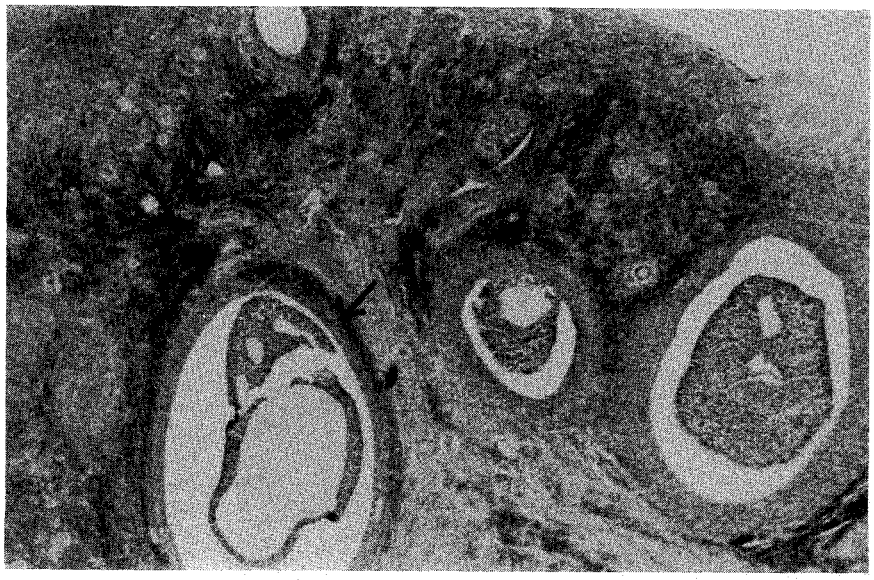

Figure 2. Graafian follicle.

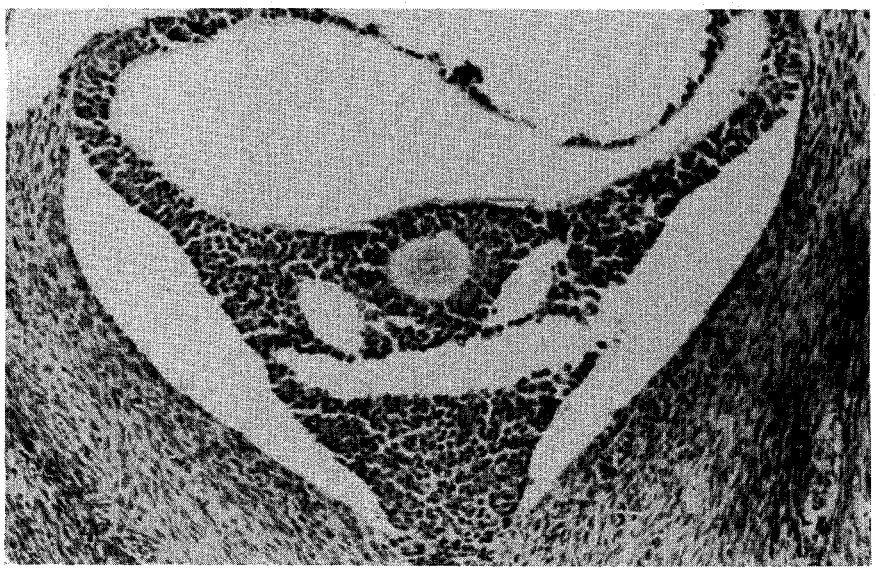

Figure 3. Egg in fluid-filled antrum.

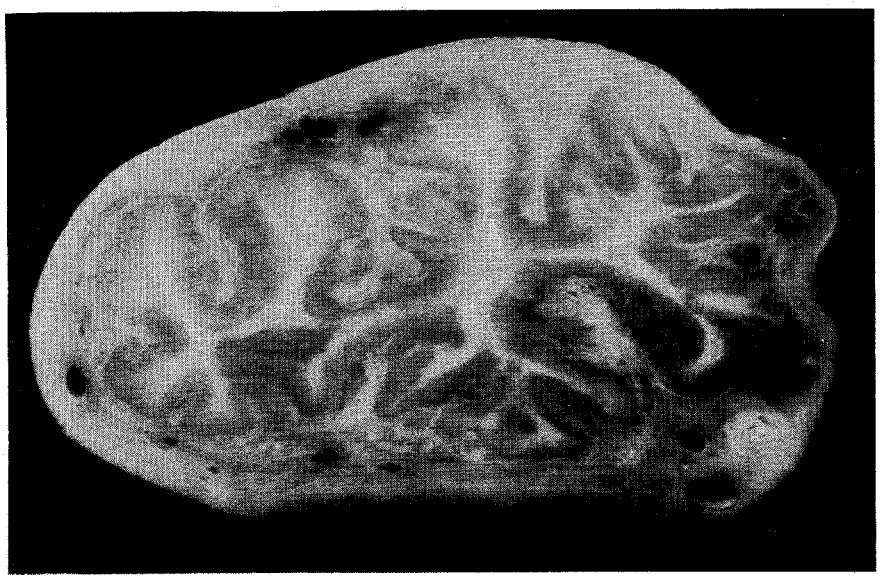

Figure 4. Type 1 corpus albicans.

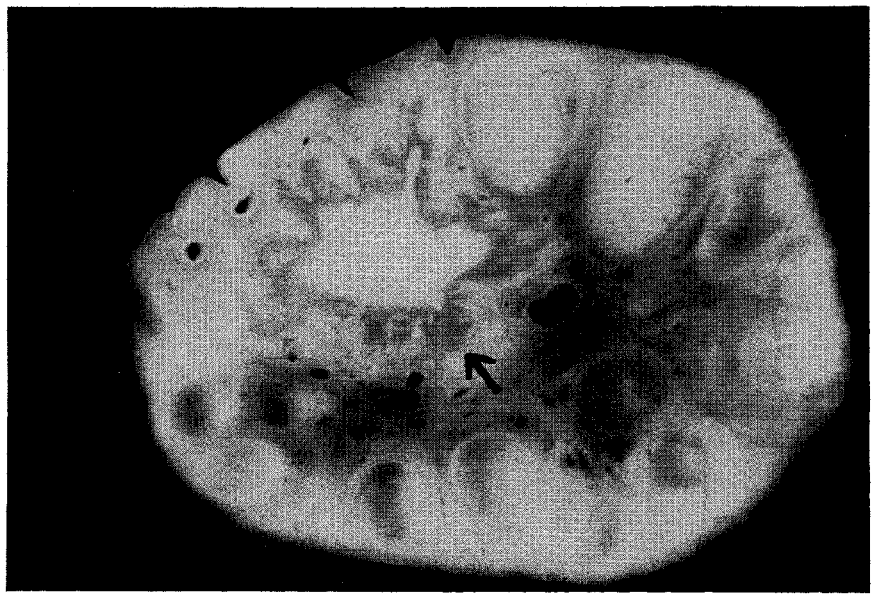

Figure 5. Type 2 corpus albicans.

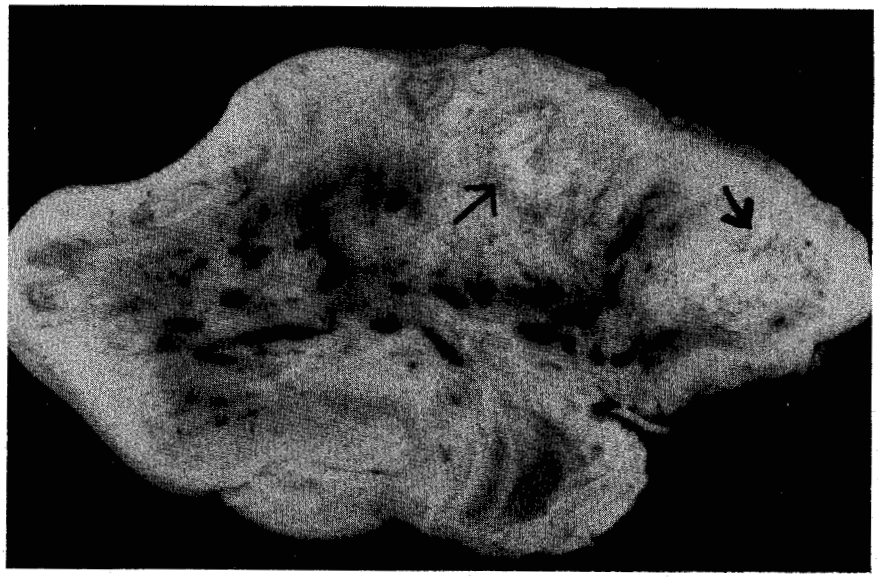

Figure 6. Type 3 corpus albicans.

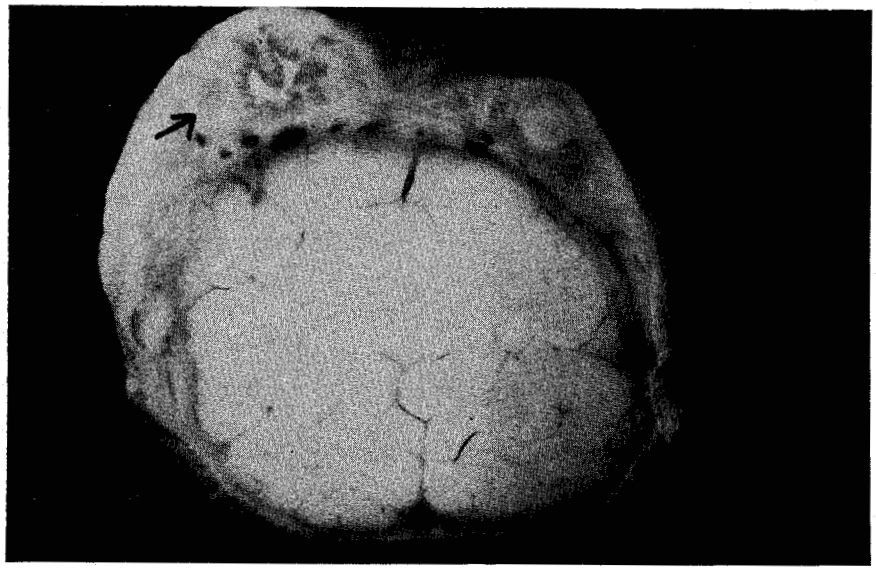

Figure 7. Type 4 corpus albicans above a corpus luteum.

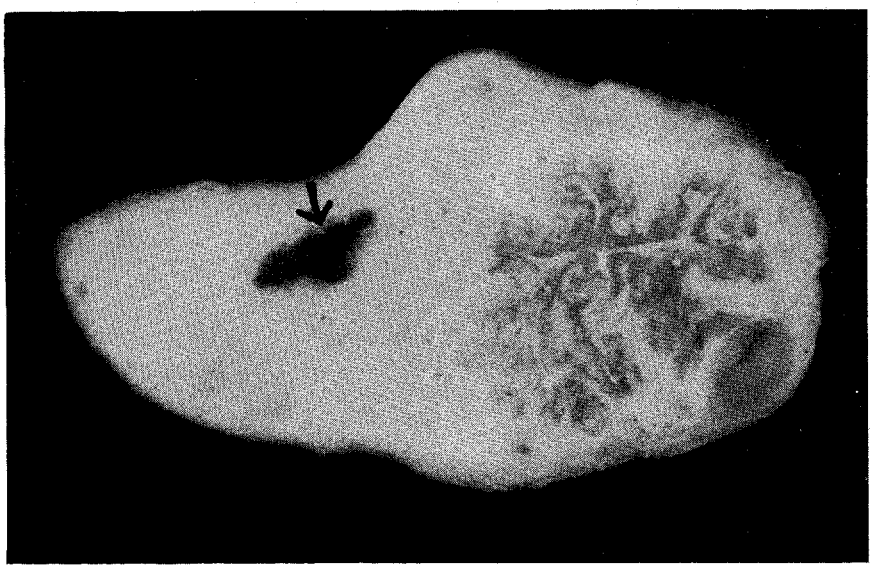

Figure 8. Type 5 corpus albicans.

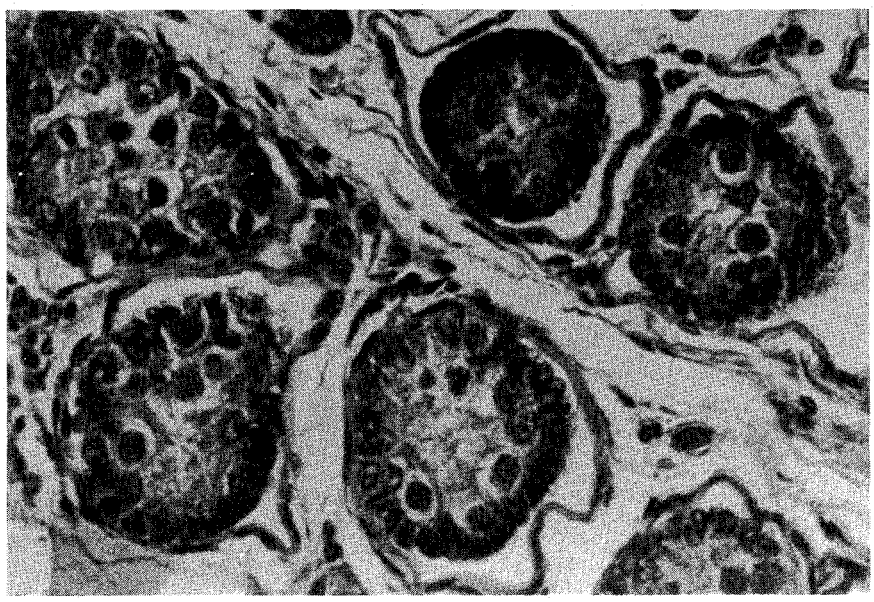

Figure 9. Stage 1 of sexual maturity of testes: immature stage with narrow tubules and abundant interstitial tissue.

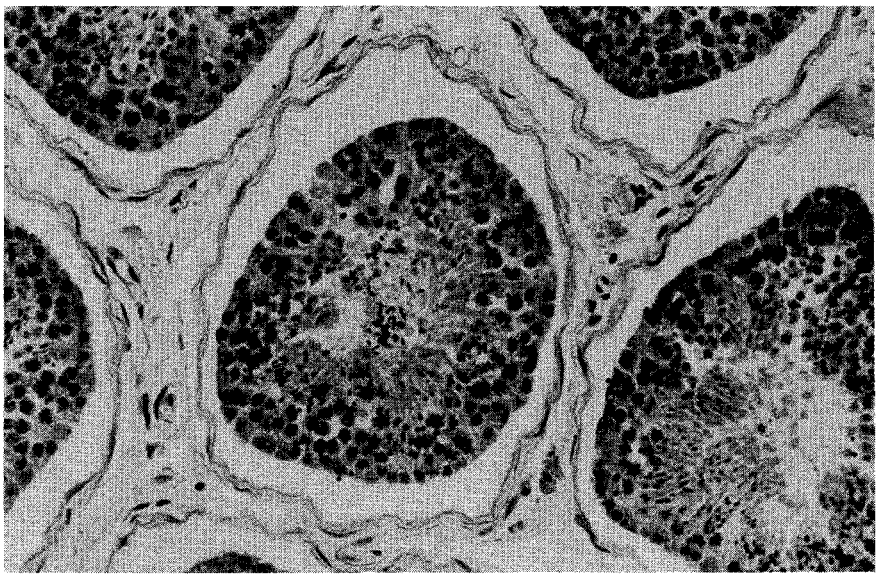

Figure 10. Stage 2 of sexual maturity of testes: maturing stage with larger tubules and reduced interstitial tissue.

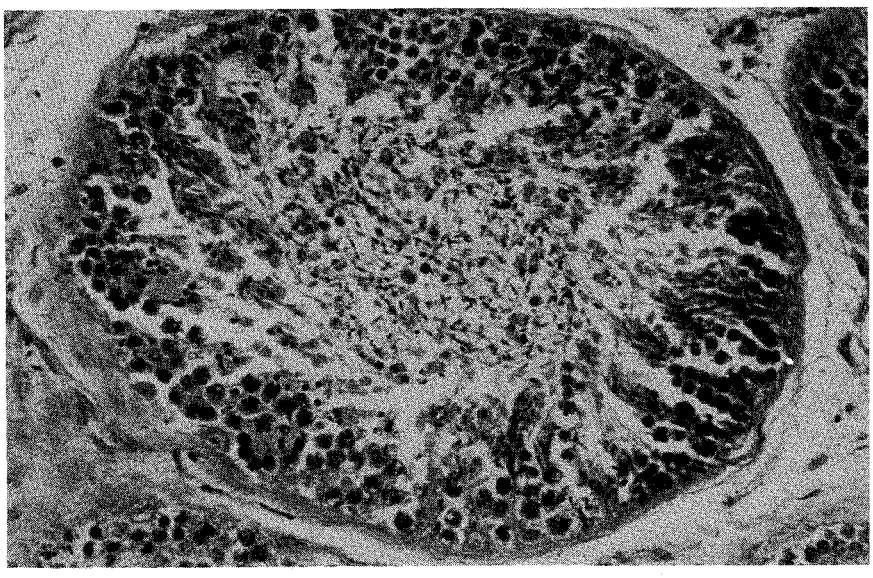

Figure 11. Stage 3 of sexual maturity of testes: Mature stage with large tubules, little interstitial tissue and spermatozoa present

APPENDIX

CODING FORMAT FOR CETACEAN LIFE HISTORY DATA 1990

Card Number 1

Column 1-4	Cruise Number
Column 5-11	Specimen Number (Initials and Serial Number)
Column 12	Number of the Card
Column 13-14	Year of Capture
Column 15-16	Month of Capture
Column 17-18	Day of Capture
Column 19-21	Set Number
Column 22-25	Position of Capture (Latitude)
Column 26	Hemisphere of Capture - Coded: 1 = North 2 = South
Column 27-31	Position of Capture (Longitude)
Column 32	Hemisphere Pre-set E/W (Coded 2)
Column 33-34	Species/Stock (See attached Code Table)
Column 35	Sex - Coded: 1 = male 2 = female
Column 36-39	Total Length (cm)
Column 40-43	Girth (cm)

Column 44	Condition of Mammary Glands - Coded: 1 = Lactating 2 = Not Lactating
Column 45	Fetus Sex - Coded: Blank = Unknown 1 = Male 2 = Female
Column 46-49	Fetus Length (Nearest 0.1 cm)
Column 50	Teeth Collected - Coded: 1 = Yes 2 = No
Column 51	Testis Collected - Coded: 1 = Yes 2 = No
Column 52	Ovaries Collected - Coded: 1 = Yes 2 = No
Column 53	Fetus Collected - Coded: 1 = Yes 2 = No
Column 54	Stomach Collected - Coded: 1 = Yes 2 = No
Column 55	Head Collected - Coded: 1 = Yes 2 = No

Column 56 — Carcass C[illegible] Coded:

Column 57 — Phot[illegible]ed - Coded:

Yes
No

Column 58 — [illegible]llected - Coded:

1 = Yes
2 = No

Column 59 — Coloration (S. attenuata; only) - Coded:

1 = Neonatal
2 = Two-tone
3 = Speckled
4 = Mottled
5 = Fused/Adult

Column 60 — Predominant Morphotype (S. longirostris; only) - Coded:

1 = Eastern
2 = Whitebelly
3 = Costa Rican
4 = Undetermined

Column 61 — Cape (S. longirostris) - Coded: 1 or 2
(See LH Form For Description)

Column 62 — Fin (S. longirostris) - Coded: 1,2, or 3

Column 63 — Belly (S. longirostris) - Coded: 1,2,3,4, or 5

Card Number 2

Column 1-11	Same as Card Number 1
Column 12	Number of the Card
Column 13-18	Total Weight (gm)
Column 19-24	Left Gonad Weight w/Epi (Nearest 0.1 gm)
Column 25-30	Left Gonad Weight w/o Epi (Nearest 0.1 gm)
Column 31-36	Right Gonad Weight w/Epi (Nearest 0.1 gm)
Column 37-42	Right Gonad Weight w/o Epi (Nearest 0.1 gm)
Column 43-45	Right Testis Length (mm)
Column 46	Degree of Testis Development - Coded: 0 = No Spermatogenesis Present 1 = Mature, Spermatogenesis Present
Column 47	Condition of Epididymis - Coded: 0 = No Sperm Present 1 = Some Sperm Present 2 = Copious Sperm Present
Column 48-50	Tubule Diameter (μm)
Column 51- 53	Diameter of Largest Follicle (Nearest 0.1 mm)
Column 54	Ovary in which Corpus Luteum was Found - Coded: 0 = Left Ovary, Fetus in Left Horn 1 = Right Ovary, Fetus in Right Horn 2 = Left Ovary, Fetus in Right Horn 3 = Right Ovary, Fetus in Left Horn 4 = Left Ovary, No Fetus Found 5 = Right Ovary, No Fetus Found

Column 55-56	Greatest Diameter of Corpus Luteum (mm)
Column 57-58	Second Diameter of Corpus Luteum (mm)
Column 59-60	Third Diameter of Corpus Luteum (mm)
Column 61-62	Number of Stage 1 Corpora Albicantia, Left Ovary (See attached description of Corpora Albicantia Stage Criteria)
Column 63-64	Number of Stage 2 Corpora Albicantia, Left Ovary
Column 65-66	Number of Stage 3 Corpora Albicantia, Left Ovary
Column 67-68	Number of Stage 4 Corpora Albicantia, Left Ovary
Column 69-70	Number of Stage 5 Corpora Albicantia, Left Ovary
Column 71-72	Number of Stage 6 Corpora Albicantia, Left Ovary
Column 73-74	Number of Stage 1 Corpora Albicantia, Right Ovary
Column 75-76	Number of Stage 2 Corpora Albicantia, Right Ovary
Column 77-78	Number of Stage 3 Corpora Albicantia, Right Ovary
Column 79-80	Number of Stage 4 Corpora Albicantia, Right Ovary

Card Number 3

Column 1-11	Same as Card 1
Column 12	Number of Card
Column 13-14	Number of Stage 5 Corpora Albicantia, Right Ovary
Column 15-16	Number of Stage 6 Corpora Albicantia, Right Ovary
Column 17-18	Total Number of Corpora Albicantia on Left Ovary
Column 19-20	Total Number of Corpora Albicantia on Right Ovary
Column 21-22	Total Number of Corpora Albicantia on Both Ovaries Plus Corpus Luteum

Column 23	Pregnant - Coded: 1 = Yes 2 = No
Column 24-30	Total Fetus Weight (gm)
Column 31	Measuring Device
Column 32-34	Growth Layer Group (μm)

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