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The Prenatal Development of the Dog: Preimplantation Events[1]

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Prenatal development of purebred beagle dogs was studied from the time of ovulation to the time of implantation. Ovulation occurred 1–2 days after the bitch's first acceptance of coitus. Primary oocytes were shed from the ovary, and the first evidence of polar body formation was observed 3 days after breeding. The embryos entered the uterus as morulae of 16 cells or more, 8–12 days after breeding, and they became blastocysts shortly thereafter. During the free-floating blastocyst stage, which lasted about 1 week, the blastocysts grew to 2.6 mm in diameter and migrated through the uterus. Implantation, first marked by local endometrial edema at the definitive site of each embryo, began an average of 17–18 days after breeding. Shortly after the occurrence of local endometrial edema, differentiation of the primitive streak and three primary germ layers began in the embryo.

The age of embryos was assessed on the basis of the time elapsed from breeding, the time elapsed from refusal to breed, and the time after the vaginal smear showed a change from predominantly cornified to predominantly noncornified epithelial cells. The calculated age of embryos at a given stage of development varied as much as 7 days when either postbreeding or postrefusal ages were used. The variation was reduced to 1–2 days when age was determined according to characteristics of the vaginal smear. The early embryonic development in the dog appears to be more closely associated with hormonal events near the end of estrus than with the time of ovulation. The vaginal smear is the best clinical index of these events.

Current knowledge of the early stages of prenatal development in the dog rests largely on the classical studies of Bischoff (1845), Duval (1893), Bonnet (1897, 1901), and Van der Stricht (1923b). These studies provide two kinds of information: a rather detailed picture of the morphologic features of the early conceptus and certain facts about specific developmental processes, including ovulation, ovum maturation, tubal transport, and implantation. However, they fail to relate specific developmental stages to prenatal age. More recently, various aspects of the estrous cycle have been studied (Evans and Cole, 1931; Arenas and Sammartino, 1939; Griffiths and Amoroso, 1939; Hancock and Rowlands, 1949), but such studies have included only minimal information on the development of the conceptus and its relation to the maternal cycle.

Certain aspects of reproduction in the dog appear to be unusual when compared with other species. For example, a wide range in postbreeding age has been observed for any specific stage of embryonic development. Both ova and sperm may remain viable for several days, creating uncertainty about the exact time of fertilization. Also there is controversy concerning the time of ovulation. Some investigators believe that ovulation occurs a day or two after the onset of estrus (Bischoff, 1845; Evans and Cole, 1931), while others believe that it occurs late in estrus (Whitney, 1940b; Griffiths and Amoroso, 1939; Gier, 1950, 1960). The length of time that a bitch is receptive to breeding is

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also variable, with an average of 9 days (Evans and Cole, 1931). Tubal transport is exceptionally prolonged in the dog, with 8–10 days required for passage through the oviduct (Bischoff, 1845; Andersen, 1927).

In the absence of any correlated study of maternal and fetal aspects of reproduction in the dog, these unusual features have led to confusion and uncertainty. Our studies involving *in utero* irradiation of the beagle have made further study of normal prenatal development necessary. An attempt is made in this investigation to relate the development of the embryo to events in the estrous cycle and to describe the temporal sequence of events occurring during the preimplantation development of the dog.

MATERIALS AND METHODS

The dogs used in the study were purebred beagles from the research colony at the Collaborative Radiological Healta Laboratory in Fort Collins, Colo. The dogs were raised in the colony from birth and were housed outdoors in graveled pens. The dogs received no medication during the experiment and were free of clinical signs of disease.

At least three mature adult bitches were assigned at random to each of the following collection times: 0, 1, 3, 5, 8, 11, 13, 15, 16, 17, 18, 19, 20, and 21 days after breeding. Specimens were collected from three additional animals 1 day after refusal of coitus, without regard to the number of days postbreeding. A total of 54 bitches were used in this study.

The bitches were examined twice weekly for signs of proestrus, including vulvar swelling and bleeding. When a bitch was observed to be in proestrus, she was placed once a day with a proven fertile male until she permitted breeding. Only one mating was allowed. This was on the first day of acceptance, considered to be the first day of estrus. Beginning the second day of estrus, the bitch was tested daily with a vasectomized male until she refused to be bred on two consecutive days. The first day of refusal was noted for purposes of timing development.

In order to determine the possibility of conception when breeding occurs late in estrus, five additional bitches were tested with a vasectomized male to determine the first day of acceptance. Attempts were then made to breed these bitches to a fertile male on the seventh and ninth days of estrus. These females were allowed to whelp and raise their litters.

Vaginal smears were prepared from the day after breeding through the second day of refusal. The smears were fixed in methyl alcohol and stained with Giemsa's stain.

On the day scheduled for embryo collection, the bitch was killed with an overdose of sodium pentobarbital. The reproductive tract was removed, and the numbers of corpora lutea, implantation sites, embryos, and ova were recorded, as were the positions of the conceptuses. Uterine embryos were recovered by dissection and those in the oviducts by flushing with physiological saline solution or observation of serial microscopic sections.

All tissues were fixed after preliminary examination in 10% buffered formalin or in Bouin's fixative. Detailed observations and measurements were made after placing material in fixative solution. Measurements included the diameter outside the corona radiata, the diameter inside and outside the zona pellucida, the diameter of the inner cell mass, and the dimensions of the embryonic shield. Sections of ovaries, oviducts, uterus, and embryos were cut at 5 μm from paraffin-embedded material and stained with Harris' hematoxylin and eosin.

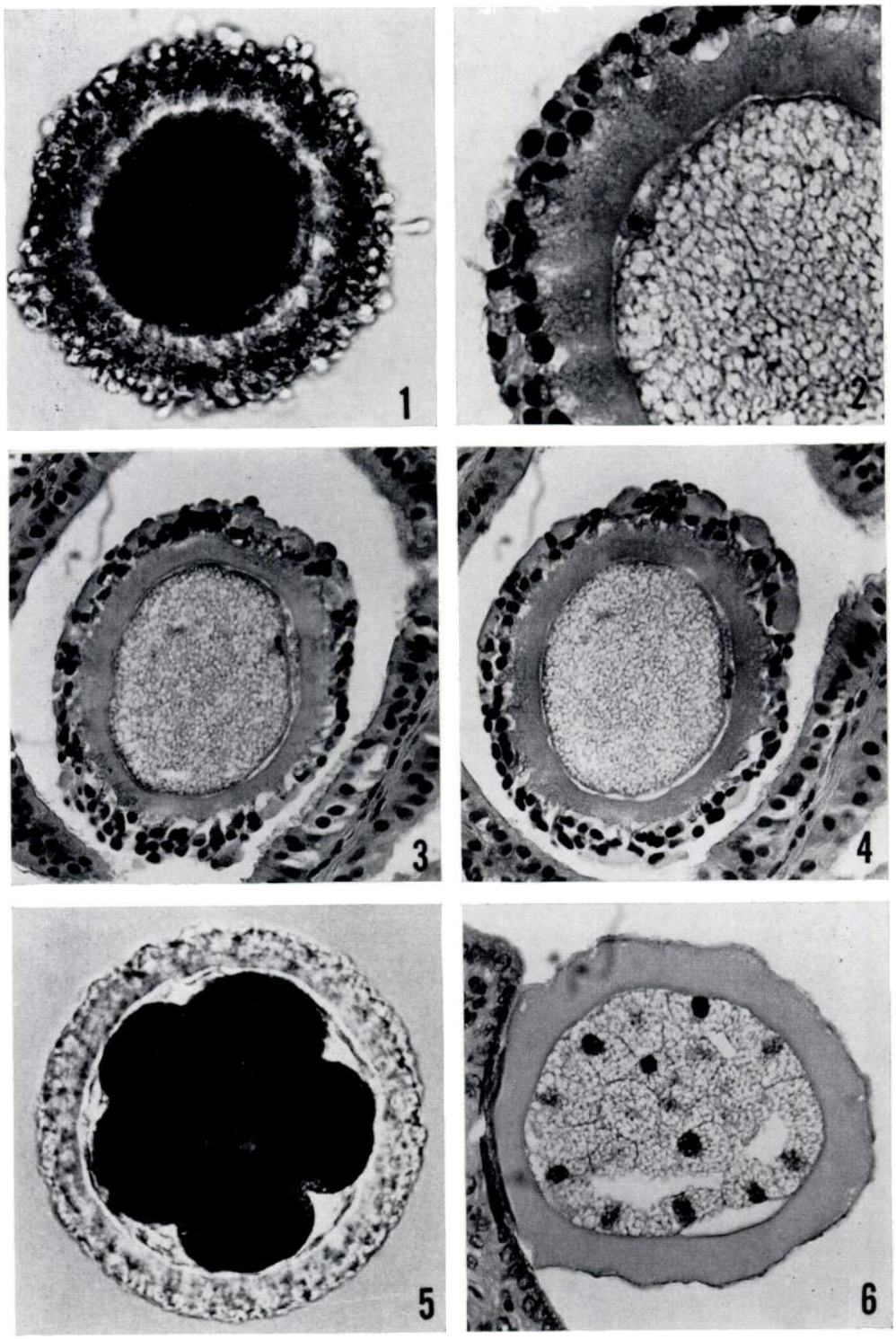
RESULTS

In 41 of the bitches examined sufficient time postcoitus had elapsed to determine conception. Thirty-two of these were pregnant, giving a conception rate of 78%. The conception rate was 79–87% for the colony as a whole during the time span of this investigation. Conception could not be determined in 13 bitches with uncleaved ova. The mean length of estrus among all bitches examined was 8.8 days (SD = 4.0, n = 39). The bitches which conceived were in estrus 8.0 days (SD = 3.8, n = 31), and those which did not conceive 12.0 days (SD = 2.9, n = 8). Of the five bitches assigned to be bred on the seventh and ninth days of estrus, one refused to breed and the other four conceived and whelped.

In general, members of a given litter were at the same stage of development, so each litter will be considered as a unit. Reported measurements are litter averages.

Development of Embryos

Precleavage Ova. Ovulation had not occurred in three bitches observed on the day



FIGS. 1–6.

of breeding and in one bitch observed one day after breeding, whereas all other bitches had ovulated. Nine bitches at 1, 3, 5, and 8 days postbreeding had uncleaved ova in the oviducts (Fig. 1). The ova were spherical with an overall diameter, including corona radiata, of 230–240 μm and a diameter inside the zona pellucida of 118–135 μm. (The dimensions of sectioned specimens were consistently 10–20 μm smaller than fresh specimens due to shrinkage which occurred during processing.) Microscopic sections of 41 uncleaved ova in the oviducts were studied. Oviducal ova at 1 day postbreeding were located in the ovarian and middle thirds of the oviduct. At 3 days one ovum from a single bitch was found in the middle third, while 13 ova in three bitches were fully two-thirds of the distance toward the uterus. At 5 days all ova were in the uterine third of the oviduct (Table 1).

TABLE 1

THE POSITION OF UNCLEAVED OVA IN THE OVIDUCT[a]

Age post-breeding	Proximal oviduct	Middle oviduct	Distal oviduct
1 day	00	0 00	
1 day		0000000	
1 day		000000	
3 days		0	00
3 days			00000
3 days			000000
5 days			00 00 00
5 days			0 00

[a] Each circle represents one ovum in a litter. Migration through the oviduct is from left to right, with the distance from the left side indicative of the distance traveled toward the uterus.

The nucleus of each ovarian primary oocyte was a pale, homogeneous body at the periphery of the cell and contained several basophilic globular masses. By 3 days after breeding the nucleus was flattened and chromatin appeared as fine short strands. Polar body formation was observed in ova recovered from two bitches 3 days after breeding (Figs. 2–4).

All uncleaved ova had similar vacuolated cytoplasm which stained lightly with eosin. The zona pellucida appeared as a homogeneous, slightly refractile, eosinophilic layer, and the corona radiata consisted of several layers of small radially arranged cells. The heads of several spermatozoa were embedded in the zonae of some ova located in the distal portion of the oviduct.

Morulae. Twelve oviducal morulae in the 8–16-cell stage were collected from three bitches at 5 and 12 days postbreeding. The morulae measured 127–135 μm in diameter inside the zona pellucida and 191–197 μm outside the zona (Fig. 5). Individual blastomeres were polyhedral, and each had a round, centrally located nucleus which stained lightly with hematoxylin and contained one or two nucleoli. The vacuolated cytoplasm of the cells was similar to that of

FIG. 1. Oviducal ovum. Central dense cytoplasm of the ovum is surrounded by the zona pellucida and corona radiata. Unstained whole preparation. Three days postbreeding. ×220.

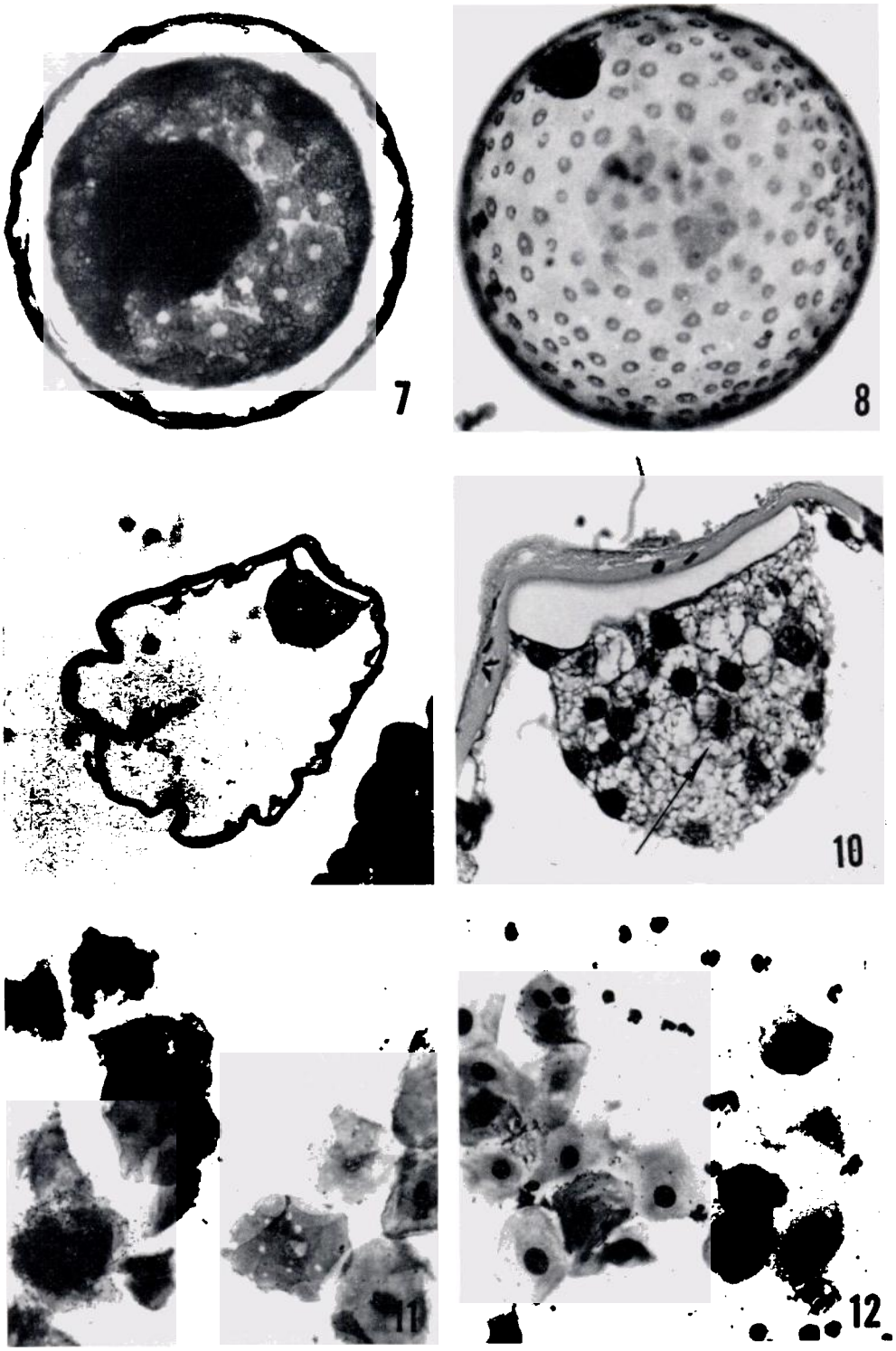
FIG. 2. Oviducal ovum with one polar body visible in the perivitelline space. Microscopic section, hematoxylin and eosin. Three days postbreeding. ×450.

FIG. 3. Oviducal ovum during polar body formation. Short strands of chromatin are oriented parallel to the cell boundary. Another section of the same cell is pictured in Fig. 4. Microscopic section, hematoxylin and eosin. Three days postbreeding. ×280.

FIG. 4. Oviducal ovum during polar body formation. At least one polar body is located in the perivitelline space. Microscopic section, hematoxylin and eosin. Three days postbreeding. ×280.

FIG. 5. Eight- to 10-cell morula. Individual cells appear approximately equal in size. The morula is encased within the zona pellucida. Unstained whole preparation. Twelve days postbreeding, 3 days postcornification. ×280.

FIG. 6. Blastocyst 215 μm in diameter. The blastocoel is seen as a space which partially separates one layer of cells along the periphery from the main mass of cells. Microscopic section, hematoxylin and eosin. Thirteen days postbreeding, 4 days postcornification. ×310.



FIGS. 7–12.

uncleaved ova, as was the zona pellucida, but the corona was absent. The heads of several spermatozoa were embedded in the zona pellucida of each morula examined.

Blastocysts. Free-floating blastocysts ranging in size from 215 μm to 2.8 mm in greatest dimension were observed in 16 litters 8–20 days after breeding. Blastocysts 2.1–3.1 mm in greatest dimension from four litters were in definitive implantation sites.

The smallest blastocysts were found in a cluster at the tubal extremity of the uterine horn. These appeared grossly as many-celled morulae, but microscopically a narrow, roughly crescent-shaped blastocoel divided one layer of cells along the periphery of the mass from the rest of the cells (Fig. 6).

Blastocysts 275 μm to 2 mm in diameter consisted of a single-layered trophoblast of squamous cells, the cytoplasm of which was thin, vacuolated strands between flattened nuclei (Figs. 7 and 8). In the smaller blastocysts, the inner cell mass consisted of a spherical group of polyhedral cells with light staining nuclei and vacuolated cytoplasm (Figs. 9 and 10). In the larger blastocysts, the cell mass was more flattened, and the cytoplasm stained more deeply. The zona pellucida remained intact in all free-floating blastocysts.

The site of recovery of large blastocysts indicated a progressive migration through the uterine horns. Approximately 3 days were required for the blastocysts to reach 1 mm in diameter. During this time they remained spherical and were found spaced along the horn corresponding to the ovary of their origin. Blastocysts larger than 1 mm in diameter assumed an ovoid shape, and in 4 additional days they expanded to 2.6 mm in greatest dimension. At the end of this time the blastocysts were evenly spaced along both uterine horns, regardless of the distribution of corpora lutea between the two ovaries.

The endoderm first became recognizable as a single squamous layer of cells beneath the inner cell mass in blastocysts 2 mm in diameter. Endometrial edema first marked the sites of implantation when the blastocysts were 2.5 mm in diameter. At this same time the zona pellucida was shed and the blastocyst lost its turgidity, so that removal of an embryo from its site resulted in rupture and collapse. After the zona had been shed, the endoderm extended peripherad from the inner cell mass.

Primitive Streak. Differentiation of the primitive streak began shortly after local uterine swelling had occurred. The embryonic shield was oblong to pyriform, and the streak was an opaque line along the midline of the shield. The primitive streak appeared microscopically as a slight thickening of the superficial layer of the shield.

FIG. 7. Blastocyst 275 μm in diameter. The inner cell mass appears as a solid dense area around which individual trophoblast cells with vacuolated cytoplasm are seen. The blastocyst is surrounded by a prominent zona pellucida. Unstained whole preparation. Eight days postbreeding, 5 days postcornification. ×230.

FIG. 8. Blastocyst approximately 600 μm in diameter. The inner cell mass is dense and globular in form, and individual trophoblast cells are visible. Unstained whole preparation. Eleven days postbreeding, 7 days postcornification. ×90.

FIG. 9. Blastocyst—500 μm in diameter—within the uterus. The zona pellucida is a distinct band surrounding the embryo. Microscopic section, hematoxylin and eosin. Eleven days postbreeding, 6 days postcornification. ×290.

FIG. 10. Blastocyst—500 μm in diameter—inner cell mass. A single mitotic figure is visible (arrow), and several spermatozoan heads are embedded in the zona pellucida. Microscopic section, hematoxylin and eosin. Eleven days postbreeding, 6 days postcornification. ×460.

FIG. 11. Vaginal smear on the last day that cornified epithelial cells were dominant. Bacteria are also seen, but no leukocytes are present in the field. Smear collected from the same bitch as that pictured in Fig. 12, but 1 day earlier. Giemsa's stain. ×290.

FIG. 12. Vaginal smear on the first day that noncornified epithelial cells were dominant. Many leukocytes and a few cornified cells are also seen. Smear collected from the same bitch as in Fig. 11, but 1 day later. Giemsa's stain. ×290.

TABLE 2
DEVELOPMENTAL STAGES FROM OVULATION THROUGH PRIMITIVE STREAK FORMATION RELATED TO AGE OF EMBRYOS

Stage of development	Mean diameter outside zona pellucida (μm)	Mean diameter of inner cell mass (μm)	Age of litter[a]		
			a	b	c
Ovum in oviduct	152	—[b]	1	—	—
	157	—	1	—	—
	160	—	1	—	—
	142	—	3	—	—
	171	—	3	—	—
	167	—	3	—	—
	156	—	5	—	—
	163	—	5	—	—
	170	—	8	—	1
Morula in oviduct	197	—	5	—	0
	191	—	5	4	1
	195	—	12	1	3
Free-floating blastocyst	215	—	13	1	4
	275	88	8	1	5
	500	84	11	8	6
	530	91	15	5	7
	544	75	8	6	7
	558	85	15	5	7
	593	88	11	10	N.O.[c]
	715	114	11	5	7
	921	122	13	5	7
	1144	135	13	9	N.O.
	1190	124	16	2	7
	1230	210	19	10	11
	1490	185	16	6	8
	2000	N.O.	15	9	N.O.
	2250	360	20	6	11
	2270	490	18	6	11
Implanted blastocyst	2500	250	18	11	11
	—	380	20	10	11
	2600	460	17	6	11
	—	590	16	9	11
Primitive streak	—	1.1 mm	19	9	9
	—	1.3 mm	17	6	13
	—	2.0 mm	17	9	13
	—	2.4 mm	21	11	12

[a] a—number of days after breeding; b—number of days after refusal to breed; c—number of days after the loss of vaginal cornification.

[b] —measurement did not apply or change had not occurred. [c] N.O.—not observed.

The endoderm became a discontinuous layer of attenuated cells inside the blastocyst, and formation of the mesoderm began as the primitive streak took form. By the time the embryonic shield was an elongated tear-drop-shaped plate 2.4 mm long, the endoderm was a continuous layer lining the entire blastocyst, and the mesoderm was two to five cells thick under the embryonic shield.

No structural attachments were observed between the trophoblast and the endometrium in primitive streak stage embryos, although it is probable that there was physical contact between the two layers after the zona was shed. The blastocysts, however, shrank or collapsed during processing, and no such contact was observed.

The Vaginal Smear. During early estrus the vaginal smear had a uniform appearance among all bitches (Fig. 11). Cornified epithelial cells were abundant, with variable numbers of erythrocytes intermixed. The cornified cells had sharp angular outlines and pyknotic nuclei. Some cells contained cytoplasmic granules. The smear contained little cellular or other debris. A few noncornified epithelial cells were commonly present but in insignificant numbers compared with the abundant cornified cells. Bacteria were often present, and their numbers usually increased gradually during estrus. Leukocytes were rarely seen.

In late estrus an abrupt change occurred in the vaginal smear, with the cornified cells being replaced by noncornified cells in approximately 1 day (Fig. 12). The cornified cells of late estrus were apparently susceptible to rapid destruction, as they were often seen in a state of partial decomposition surrounded by bacteria and neutrophilic leukocytes. Neutrophils were generally present during the last day or two on which the smear consisted mainly of cornified epithelial cells, but their numbers usually increased after the cornified cells disappeared. The number of neutrophils and the time of their appearance were variable in relation to the

time of replacement of cornified cells by noncornified cells. The day on which the change from mainly cornified to mainly noncornified vaginal epithelial cells was first observed in each bitch was used as a means of timing development (Table 2).

Mating Behavior. The first day that a bitch would stand for service was determinable by an obvious change in behavior at this time. Most bitches would readily stand, whereas on previous days they had refused the advances of the male and had not allowed him to mount. Males were attracted to the females at this time without exception.

Accurate determination of the first day that a bitch refused to be bred was more difficult. Instead of showing clear acceptance on one day followed by refusal the following day, acceptance of males by most bitches showed a gradual decline over several days. Near the end of the period a bitch often appeared reluctant, but after a time would allow copulation if the male was aggressive and persistent. The problem was complicated by the fact that some males showed a lack of interest in a bitch when she was in late estrus. A male would occasionally show no interest in a bitch on one day, and on the following day would be interested and succeed in breeding her. Similarly, a bitch would seem either passively or aggressively unwilling to breed one day yet would stand for service on the following day.

Determination of Embryonic Age

An age for each litter was determined on the basis of the time from breeding, the time from first refusal to breed, and the time from the first day of a vaginal smear with predominantly noncornified epthelial cells (Table 2). The three timing methods compared indicate the variation encountered for successive stages of development (Table 3).

A range in age of 7 days was regularly observed among litters at any specific stage of development when postbreeding and postrefusal times were used to determine age. The range was reduced to 4 days when age was calculated on the basis of vaginal cytology, with the 4-day range observed in only two instances, and a 1–2-day range most commonly encountered. Specific stages of prenatal development were more closely correlated with the loss of cornification than with either the first or last day of acceptance.

Most bitches continued to accept the male for 1–5 days after the vaginal smear contained mainly noncornified cells. At the extremes, one bitch first refused the male 3 days before cornified cells disappeared from the smear, and another continued to accept until 7 days after noncornified cells became

TABLE 3

AGE OF EMBRYOS DETERMINED BY THREE TIMING METHODS

Stage of development	Number of litters observed	Postbreeding age in days; range (mean)	Postrefusal age in days; range (mean)	Postcornification age in days; range (mean)
Oviducal ovum	9	1–8 (4.2)	—	Cornified-1
Morula	3	5–12 (7.3)	1–4 (2.5)	0–3 (1.3)
Blastocyst 200–750 μm	8	8–15 (11.1)	1–10 (5.1)	4–7 (6.1)[a]
Blastocyst 750–1500 μm	5	13–19 (15.4)	2–10 (6.4)	7–11 (8.3)[b]
Blastocyst 1500–2500 μm	7	15–20 (17.6)	6–11 (7.6)	11 (11.0)[c]
Primitive streak	4	16–21 (18.3)	6–11 (9.0)	9–13 (12.0)

[a] Based on seven observations.
[b] Based on four observations.
[c] Based on six observations.

prominent. The mean number of days of acceptance was 2.3 days (SD = 2.4, n = 27) after the vaginal epithelium became noncornified.

DISCUSSION

Investigators and dog breeders are interested in knowing the optimum time to breed bitches in order to achieve a high conception rate. In general, there is little agreement on a single optimum point in the cycle at which conception is most likely (Griffiths and Amoroso, 1939; Hancock and Rowlands, 1949; Rowlands, 1950; Newberry and Gier, 1952). Whitney (1940a) has reported a reduced rate of conception among bitches bred only once on the first day of acceptance. However, a high conception rate (78%) was achieved in this study in which all bitches were bred once on the first day of acceptance. Bitches are routinely bred on the first and third days of acceptance in this colony, and the conception rate for more than 2000 breedings has been approximately 85%. It is further evident that the period of fertility extends over several days. The fact that all of the bitches bred on the seventh and ninth days of acceptance conceived and whelped litters confirms the speculation of Evans and Cole (1931) and others that the bitch has an extended period of fertility.

Rowlands (1950) reported reduced fertility among bitches with estrous periods longer than 13 days, supporting the results of this study. The conception rate in this study among 25 bitches in estrus 10 days or less was 96%. Among 15 bitches in estrus 11 days or more (maximum 17 days) the conception rate was 47%. Bitches with prolonged estrus may be experiencing a long period of elevated estrogen levels. Estrogen is known to prevent pregnancy in bitches if given soon after a misalliance (McDonald, 1969). The excess estrogen may delay tubal passage long enough to initiate degeneration, or the effect may be directly on the embryo or on the fluid environment of the genital tract.

Ovulation and Fertilization

Our observations support the original views of Bischoff (1845), Ancel and Bouin (1908), and Evans and Cole (1931) that ovulation takes place 1–2 days after the onset of estrus in the bitch. More recently, however, other workers have asserted that ovulation occurs later in estrus (Griffiths and Amoroso, 1939; Whitney, 1940b; Gier, 1950, 1960; Newberry and Gier, 1952). These latter authors generally have based their conclusions on indirect evidence. Whitney, for example, stated that ovulation occurs on the fifth day of estrus, basing this on evidence derived from the time of whelping.

Those who have supported the idea of late occurring ovulation have generally made the assumption that ovulation and fertilization occur at approximately the same time (Whitney, 1940b; Griffiths and Amoroso, 1939; Gier, 1960; Newberry and Gier, 1952). Van der Stricht (1923b) made extensive studies of ovum development, ovulation, and fertilization in various mammalian species. He observed that, in the dog, ova are shed before either maturation division has taken place. The polar bodies are thus not shed, and fertilization is not possible, until some time after ovulation. Among mammals this situation is unique to the dog, fox, and possibly the horse (Austin, 1961, 1969). Evans and Cole (1931) reported that ova with the first polar body in the process of formation were observed in the ovarian and middle thirds of the oviduct. Other workers have subsequently supported the view that ova are shed as primary oocytes in the dog (Andersen, 1927; Asdell, 1964) and in the fox (Pearson and Enders, 1943). A similar conclusion is drawn from the results of this study, where no evidence of polar body formation was seen until ova were in the distal portion of the oviduct 3 days after breeding.

The canine reproductive cycle is unusual in that ovulation occurs near the beginning of

estrus. In general ovulation in most domestic animals occurs near the end of estrus (Austin, 1969). From our study it appears that all follicles rupture at approximately the same time, an observation which has also been noted by others (Evans and Cole, 1931; Arenas and Sammartino, 1939). In every instance, all corpora lutea were at the same stage of development, and in those animals with newly formed corpora lutea, no mature unruptured follicles were present.

At the time of ovulation, the ova were reported by Evans and Cole (1931) to be without a zona pellucida. Pearson and Enders (1943), however, reported the presence of the zona at the time of ovulation in the fox, and in our study the zona was present surrounding ova in young as well as in maturing and atretic follicles. There appears to be sufficient evidence to state that the zona is established before ovulation.

The observed size of the uncleaved ova (118–135 μm) was within the size range reported for other domestic animals (120–180 μm) (Austin, 1969). Hartman (1929) estimated the average size of the dog ovum to be 135–145 μm from the work of Bischoff (1845) and Van der Stricht (1923b). However, the size of the dog ovum has also been reported as 77 $\times$ 90 μm by Asdell (1964), from the work of Evans and Cole (1931) in which Bouin's fixed rather than fresh specimens were measured.

Spermatozoa reach the oviduct in the bitch within 25 sec after ejaculation (Evans, 1933). The time of entry of the spermatozoa into the ovum appears to be variable. They commonly enter during the first maturation division and are regularly present before and the second division is complete (Van der Stricht, 1923b). Austin (1961, 1969), citing Van der Stricht's work, stated that spermatozoa commonly enter the primary oocyte. Van der Stricht, however, mentioned only one instance from his work in which a spermatozoan head was seen in a primary oocyte, and he considered this exceptional.

There is apparently a period of delay of several days between the time of ovulation and the times of fertilization, pronuclei formation, and cleavage in the dog. Evans and Cole (1931) observed pronuclear stages 8 days after breeding. During this delay the ovum, which is shed in an immature stage of development, undergoes maturation and preparation for fertilization. In most other mammals, maturation begins before ovulation, and ova are shed late in estrus in a condition ready to be fertilized immediately.

Tubal Transport

According to Bischoff (1845), the ova move rapidly through the proximal oviduct, traversing the first half of the oviduct in a few hours, and reaching the uterine end in a day or less. He reported a total time in the oviduct of 8–10 days, thus indicating that the ova are held for a week or more at the uterine end of the oviduct. In the present study viable uncleaved ova as well as morulae were located in the uterine portion of the oviduct, indicating that this is the site of pronuclei formation and cleavage. The total length of time in the oviduct is unusually long in the dog as compared with other mammalian species, most of which require only 3–4 days for tubal passage. Estrogens reportedly delay the movement of ova from the oviduct into the uterus (Chang, 1968; Tausk, 1969). As long as estrogen has a dominant influence, the ova are "locked" in the oviduct, and when progesterone becomes dominant, the embryos are transferred into the uterus.

Little has been reported on the temporal sequence of events in the early development of the dog ovum. According to Gier (1950) the first cleavage division is complete 6–7 days after ovulation, the second 24 hr later, and embryos enter the uterus in the two-cell to four-cell stage. According to Bonnet (1897) embryos are not present in the uterus while the bitch is accepting the male. Evans and Cole (1931) observed most embryos

to be in the uterus on the first day of refusal. We observed morulae still in the oviduct 1 and 4 days after first refusal, and it is probable that the time of entry of the conceptus into the uterus is variable with regard to the time of first refusal. Embryos entered the uterus between the 16-cell stage and the youngest blastocyst stage. This observation is in agreement with the statement of Andersen (1927) that the embryos are in the 16-cell to 32-cell stage when they enter the uterus, as well as that of Van der Stricht (1923a) that the blastocyst is formed as soon as the conceptus enters the uterus.

The Blastocyst

The dog embryo is known to exist in a free-floating blastocyst form for some time before implantation. In the present study, the length of this time was observed to be approximately 7 days. Tietz and Seliger (1967) observed blastocysts from the 10th to the 16th day after breeding. Gier (1950) stated that the blastocyst stage begins on the 10th day and lasts until the 18th day. The corresponding time in other species is extremely variable, ranging from 3 days in rodents to 7 weeks in the mare (Austin, 1969). At some time during the free blastocyst stage, probably when the conceptus measures about 1200 μm in diameter, embryos migrate from one uterine horn to the other and become established in approximately equal numbers in each horn regardless of the numbers of corpora lutea in each ovary. This phenomenon has been observed in other species, and experimental proof exists for the cat (Markee and Hinsey, 1933) and for the pig (Miller and Dzuik, 1968).

Implantation

Implantation in the dog is central and is characteristically a gradual process which proceeds in stages over a period of time. Various authors seem to regard different parts of the process as the beginning of implantation, and a commonly accepted definition of the initial events has not been made. Enders and Schlafke (1967) described implantation in the rat as follows: "implantation can be considered to start when a fixed position of the blastocyst in relation to the uterus is established." Such a definition would seem to apply to the situation which is seen in the dog. When the blastocysts assume definitive positions within the uterus, endometrial edema indicates these positions. This is the first easily observable change during implantation in the dog, and it serves as a good point to begin a description of the process. The blastocysts are no longer free floating when they are enclosed within edematous implantation sites. After localized endometrial edema has occurred, the blastocysts shed their zonae pellucidae and differentiation of the embryonic disc into the primitive streak begins. These observations are not in agreement with those of Gier (1950), who concluded that canine embryos are in a free-floating blastocyst stage until the initial somites are formed.

Comparison of Three Methods of Determining Age

Two principal observations stand out in this study concerning the correlation between embryonic development and events in the estrous cycle. One is the lack of correlation between developmental stages and the times of first and last acceptance of coitus by the bitch. The other is the close correlation between developmental stages and the time of the end of vaginal cornification.

A wide range in postbreeding age of embryos at any particular stage of development has been encountered by all who have studied canine reproduction (Evans and Cole, 1931; Gier, 1950; Tietz and Seliger, 1967). Nevertheless, the first day on which a bitch will breed appears to be a good indicator of hormonal and histologic events in the estrous cycle because this day bears a consistently close relationship to the time of ovulation.

TABLE 4

TEMPORAL SEQUENCE OF EVENTS IN THE PREIMPLANTATION DEVELOPMENT OF THE BEAGLE

Days elapsed from the end of vaginal cornification	Stage of development
0–1	Cleavage, morula formation
2–3	Growth to 16-cell morula
4–5	Entry into uterus and formation of blastocyst
6–7	Growth of blastocyst to 1200 μm, migration through uterus
8–11	Growth to 2500 μm, elongation, establishment of definitive implantation sites
11–12	Local uterine edema and beginning primitive streak

Based on Bonnet's observation that embryos were not present in the uterus while the bitch was accepting the male, it has been suggested that the observed variability in developmental rate could be reduced if age were determined from the day of a bitch's first refusal (Tietz and Seliger, 1967). Tietz and Seliger postulated that development may be temporally controlled by events associated with the end of estrus, such as the onset of functional activity by the corpus luteum. A reduction in the variability was not observed in this study, however, and it appears that the last day of estrus as it is defined behaviorally is no more closely correlated with development of the conceptus than is the first day of estrus. The difficulty encountered in observing with assurance the day of first refusal makes this an unreliable indicator of hormonal and histologic events associated with development.

The close association between the developmental stage of the embryo and the condition of the vaginal epithelium near the end of estrus suggests that the vaginal smear is the best clinical indicator of developmental events. The end of vaginal cornification is easily observed, and the hormonal events which control vaginal cornification probably also help regulate the development of the embryo. A temporal sequence of events in the preimplantation development of the dog, based on the time elapsed from the end of vaginal cornification, is given in Table 4.

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