

## Some Comparative Aspects of Implantation

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### INTRODUCTION

Within the last decade studies of mammalian oviimplantation have burgeoned and become more diversified. The primarily morphologic focus of earlier studies has shifted rapidly to more sophisticated multidisciplinary analyses of the regulative mechanisms of implantation, and their integration with carefully defined morphologic variables. It is understandable, but in some respects unfortunate, that the bulk of recent studies have focussed on a mere handful of semidomesticated and laboratory species, mostly rodents. To be sure the ready induction of facultative delayed implantation in mice and rats provides a powerful and controllable model permitting detailed analyses to be made of endocrine and other basic parameters of the implantation sequence—in mice and rats! It is obvious, however, from the diversity of implantation patterns already recognized among mammals, that restriction of study to these few species will not permit us to distinguish with certainty between those regulatory mechanisms that are of wider significance and those that are novel and phylogenetically restricted. Given the selective pressures operative in evolution, variability in morphologic and temporal patterns presumably reflect variability in underlying regulatory mechanisms, perhaps even at quite fundamental levels. Hence a wider comparative approach in oviimplantation studies is both desirable and necessary.

This paper, intended as a general introduction to the more detailed and topically restricted presentations to follow in this symposium will sketch from a comparative viewpoint certain chronological events in the implantation sequence and in addition some more unusual adaptations which are perhaps

less familiar. The coverage will not be exhaustive, and is intended to be more informative than analytical.

### PREIMPLANTATION PERIOD

#### *Natural superovulation and wastage of ova*

Arbitrarily we may say that the preimplantation sequence begins with ovulation and the release of ova into the oviduct—where they are fertilized. Only slightly prior to this time too, the preconditioning of the uterus for the reception of the germ is initiated by ovarian hormones. In most higher mammals the number of ova shed at ovulation bears a proximate relationship to the number of young produced per pregnancy, and substantial wastage of ova is thus prevented. However, such parsimony is lacking in some lower mammals and there is at least one notable exception among hystricomorph rodents. Table 1 summarizes the situation in a few representatives from several orders of mammals, and for comparison, in some closely related species which display more conventional patterns. Note that among marsupials the common opossum (*Didelphis virginiana*) and the native cat (*Dasyurus viverrinus*) shed many more eggs than will ultimately produce viable offspring (Hartman, 1928; McCrady, 1938; Hill, 1910). The number of small embryos that could be accommodated in the uterus for the short time they reside there is probably potentially greater than the number of offspring eventually brought to term, for this is strictly limited by the number of mammae available in the marsupium. It appears, however, that in these two species most losses occur via a failure of fertilization (opossum) or because of cleavage abnormalities (native cat). In contrast the brush possum

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(*Trichosurus vulpecula*) is monovular and there is normally no wastage of ova (Von der Borch, 1963), and the same is true of various macropod marsupials (Sharman et al, 1966; Tyndale-Biscoe, 1973).

Among insectivores the tenrec (*Hemicentetes semispinosus*) and the elephant shrew (*Elephantulus myurus*) are notable natural superovulators. In the tenrec more than 40 ova may be shed, but litters larger than 10 fetuses have not been recorded (Bluntschli, 1938). The elephant shrew is even more extravagant in that between 60 and 120 ova are ovulated, but only two are destined to be brought to term (van der Horst and Gillman, 1941; Tripp, 1971). Interestingly the patterns of egg loss differ in the two species. In *Elephantulus* there is some wastage due to nonfertilization and cleavage abnormalities, but all except two of the many normally developing ova eventually degenerate in utero for lack of suitable implantation sites. In the tenrec, however, as many as 40 blastocysts have been observed to implant and develop successfully to approximately midgestation. Later three quarters of them more or less are resorbed presumably due to overcrowding. In contrast to *E. myurus* most elephant shrews do not superovulate. For example, *E. fuscipes*, a member of the same genus, sheds only one or two eggs which usually develop without loss, so the situation in *E. myurus* is novel even within its own family (Tripp, 1971).

In bats a more or less 1:1 relationship between ova shed and litter size prevails. The majority are monovular and give birth to single progeny, as exemplified by *Myotis lucifugus*, *Desmodus rotundus* and *Glossophaga soricina* (Wimsatt, 1944, 1945; Quintero and Rasweiler, 1973, 1974; Rasweiler, 1972, 1973). But there are exceptions involving a low level superovulation accompanied by some egg wastage. The American vampire bat *Eptesicus fuscus* and *Pipistrellus subflavus* normally deliver two young, but very frequently they ovulate several ova in excess, up to 7 having been found. All ova are fertilized, become implanted, and develop

normally for a time, but those beyond two are eventually resorbed (fig. 1) (Wimsatt, 1945).

It is among rodents, however, that we find the most remarkable example of superovulation and egg wastage yet recorded in mammals. The plains viscacha *Lagostomus maximus*, a hystricomorph from South America, releases at ovulation between 200 and 800+ eggs (Weir, 1971). Somewhere between 95 to 99+ percent of these remain unfertilized and degenerate, while the seven or eight that alone are fertilized become implanted in the uterus. Only two young are delivered, however, and the excess are resorbed (fig. 1). Contrastingly, the related mountain viscacha (*Lagidium peruanum*) of Peru is monovular and delivers a single fetus (Pearson, 1949). Wild caught striped hamsters *Cricetulus griseus* from China apparently fertilize and implant most eggs ovulated, but there is a progressive loss of embryos between days 6 and 16 of pregnancy. Among 29 pregnant females examined by Droogleever Fortuyn (1929), which contained an aggregate of 193 "egg chambers", 74 (38+%) possessed degenerating embryos.

Among artiodactyls the pronghorn antelope (*Antilocapra americana*) displays a unique pattern of "programmed" egg wastage (O'gara, 1969). The species normally produces two young, but four or five ova are released, fertilized, and develop into elongating blastocysts within the uterus. These are eventually distributed between two locations in each horn, in a distal dilated portion of the lumen nearer the oviduct and in a larger proximal dilatation near the corpus uteri (fig. 2a). The chorionic vesicles all develop a necrotic tip at their oviductal ends only, and the proximal pair of vesicles elongate more rapidly than the distal pair. As the proximal vesicles expand their necrotic tips invaginate, and eventually penetrate completely the smaller distal vesicles and destroy them (fig. 2b) leaving only the pair of proximal vesicles to complete development. In contrast the Uganda kob (*Adenota kob*) and several other true antelopes are typically monovular, and deliver single progeny (Buechner et al, 1966).

It is evident from these examples that

IMPLANTATION: COMPARATIVE ASPECTS

Table 1  
Natural Superovulation and Prenatal Loss of Embryos

Species	Ova Shed (number)	Ova Lost		Authority
		preimplantation stages	postimplantation stages	
<b>Marsupials</b>				
opossum ( <i>Didelphis</i> )	22(56) *	approx. 30% unfertilized	?	Hartman, 1925; 1928
native cat ( <i>Dasyurus</i> )	20-25(35)*	30 ± % during cleavage	?	Hill, 1910
brush possum ( <i>Trichosurus</i> )	1 **	none	none	Von der Borch, 1963
<b>Insectivores</b>				
elephant shrew ( <i>E. myurus</i> )	120	"Some" unfertilized;	98±%, as 4-cell "blastulas"	van der Horst & Gillman, 1941
elephant shrew ( <i>E. fuscipes</i> )	98	"some" during cleavage		Tripp, 1971
tenrec ( <i>Hemicentetes</i> )	1-2 **	none	none	Tripp, 1971
	40+	"probably some loss"	up to 75% resorbed	Bluntschli, 1938
<b>Chiroptera</b>				
big brown bat ( <i>Eptesicus fus.</i> )	3-7	unlikely	33-75% resorbed	Wimsatt, 1945
pipistrelle ( <i>Pipistrellus subfl.</i> )	2-7	unlikely	0-70% resorbed	Wimsatt, 1945
little brown bat ( <i>Myotis lucif.</i> )	1 **	none	none	Wimsatt, 1944; 1945
vampire bat ( <i>Desmodus</i> )	1 **	none	none	Wimsatt & Trapido, 1954
long-tongued bat ( <i>Glossophaga</i> )	1 **	none	none	Quintero & Rasweiler, 1973; 1974
				Rasweiler, 1973
<b>Rodents</b>				
plains viscacha ( <i>Lagostomus</i> )	200-800	96-99±% unfertilized	up to 75% of implanted embryos	Weir, 1971
mt. viscacha ( <i>Lagidium</i> )	1 **	none	none	Pearson, 1949
striped hamster ( <i>Cricetulus griseus</i> )	6.7+ **	probably some	approx. 38±%	Droogleever Fortuyn, 1929
<b>Antiodactyls</b>				
Pronghorn ( <i>Antilocapra</i> )	4-5	50±%, at blastocyst stage	occasionally	O'gara, 1969
uganda kob ( <i>Adenota kob</i> )	1 **	none	none	Buechner et al., 1966

\* maximum number observed in a single individual in parentheses  
 \*\* related oligoovulating species included for comparison

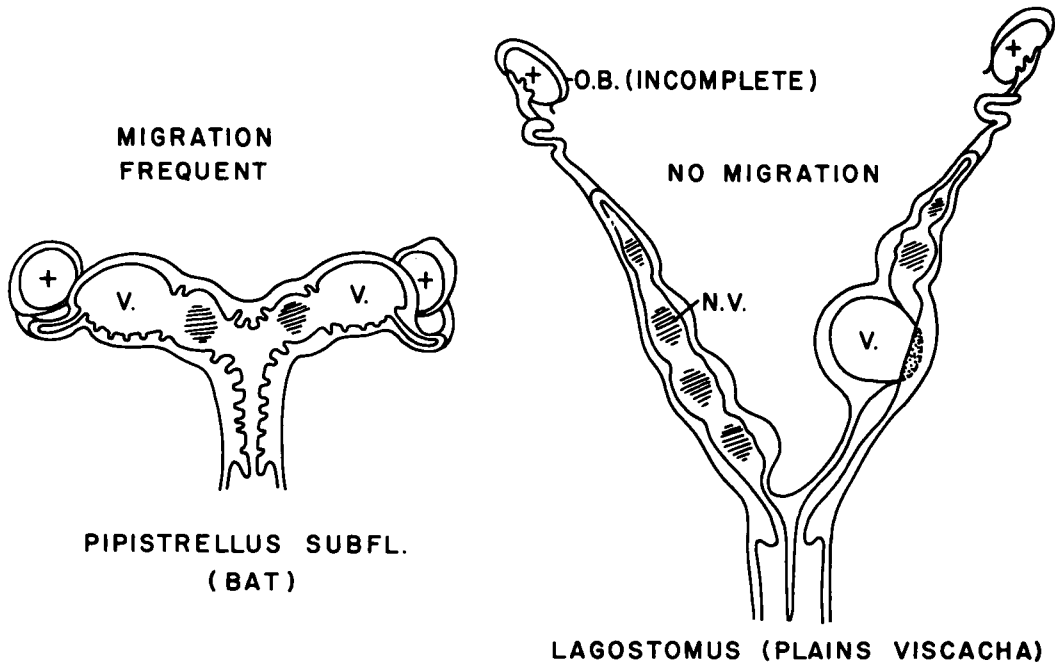


FIG. 1. Resorption patterns in a bat and a viscacha. A plus (+) sign on an ovary indicates that it has ovulatory competence. The hatched lines (n.v.) indicate embryo resorption sites. "Migration" designates transuterine migration of ova. V., viable embryos.

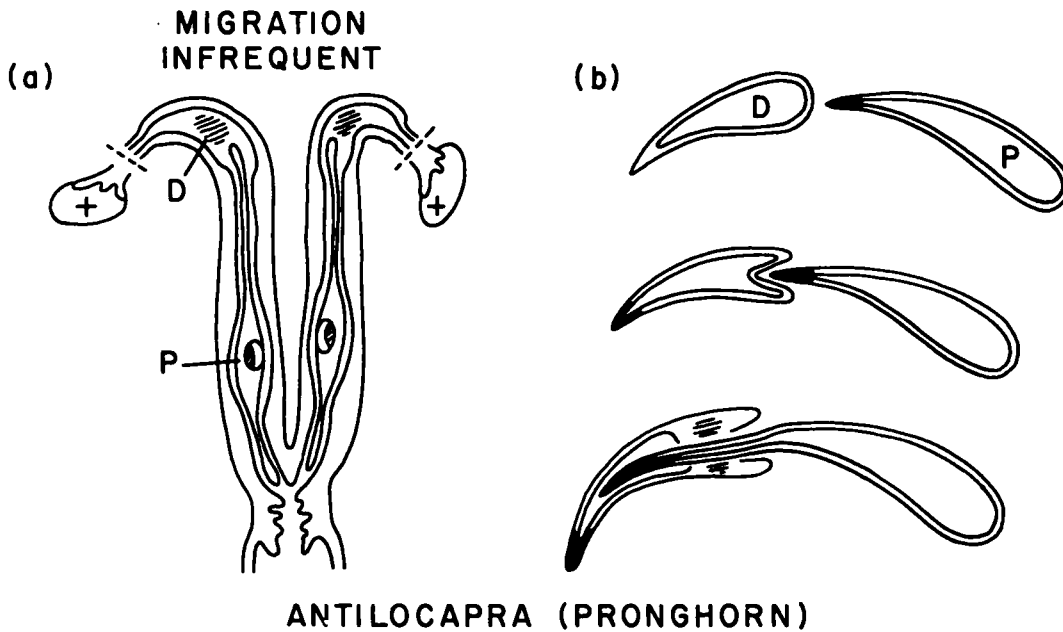


FIG. 2. The placement of blastocysts in the uterus of the pronghorn is shown in (a); the site of destruction of the distal embryo in each horn is indicated at D. (b) The mode of destruction of the distal choric vesicle (D) by the proximal one (P) is shown (redrawn from O'gara, 1969).

superovulation and wastage of eggs are a normal occurrence during early development in at least a few mammals. It is also clear that they are not restricted to any one group, or to more "primitive" as opposed to "higher" mammals, for both superovulation and egg loss patterns vary within, as well as between major taxons. The adaptive significance (if any) of superovulation in mammals remains obscure, but surely the differing patterns observed raise interesting questions for future study concerning their regulation and control.

*Comparative organization and development of the blastocyst*

It is generally held that all mammalian blastocysts are similarly organized and comprised of three principal parts, an outer investing layer of trophoblast cells (presumptive ectoderm of fetal membranes and placenta), an eccentrically placed inner cell mass (presumptive embryo proper), and a fluid filled space, the blastocyst cavity (presumptive yolk-sac cavity in whole or in part). That this is not universally true we shall see presently, but to stay with the above structure for a moment we should note that most authors still call it a "blastocyst" after it has been converted to a bilaminar vesicle by the outgrowth of the primitive endoderm, and by some authors even after the extraembryonic mesoderm has achieved its definitive arrangement and the vesicle has become tri- or multilaminar. This invites confusion so perhaps we should agree that "chorionic vesicle" would be a more appropriate designation for the germ from at least the trilaminar stage onward. If these definitions are adhered to, it will become clear that it is not always a "blastocyst" that implants in the uterus, even though this is commonly alleged. In ungulates such as horse, bovids, deer and antelopes, in which the free living period of the developing ovum in utero is relatively prolonged, there is a corresponding increase in size and differentiation of the conceptus before implantation occurs, and it is clearly a chorionic vesicle

that implants, not a blastocyst. At the other end of the scale the ovum of the elephant shrew (*E. myurus*) initiates implantation while still a "preblastocyst" consisting of only four cells (van der Horst and Gillman, 1941; van der Horst, 1942). Nevertheless in most higher mammals implantation does involve a true blastocyst. At this time it may be unilaminar as in anthropoidea and the guinea pig (Luckett, 1974; Blandau, 1949a), bilaminar as in the rabbit, insectivores, numerous bats and prosimian primates (Böving, 1963; Wimsatt and Wislocki, 1947; Wimsatt, 1944; Luckett, 1974), or intermediate as in mouse, rat and some bats (Snell, 1941; Rugh, 1968; Enders and Schlafke, 1967; Wimsatt, 1954; Rasweiler, 1973).

Generally there is a rough correspondence between the post-fertilization age of the blastocyst at implantation and its relative size and degree of differentiation, but this is by no means precise or constant from one species to another. Developmental rates and the time of implantation vary in accordance with inherent genetic constitution, variations in the availability and utilization of metabolic materials, the preparative state of the endometrium when developing ova first enter the uterus, and probably many other parameters. Variability in growth rates of the ovum may also be observed among individuals of the same species in response to a variety of intrinsic or extrinsic influences. The latter include genetic strain differences (in mice; McLaren, 1968), altered energy stages (that can prolong facultative delayed implantation in mice; Shapira et al, 1974), and even natural or contrived fluctuations of ambient temperature which in heterothermic bats can accelerate or retard embryonic growth and shorten or prolong gestation (Pearson et al, 1952; Racey, 1973). Some examples of developmental rate differences in selected mammals may be gleaned from Table 2.

Other important differences observed during the earlier stages of development of mammalian ova involve the external coats deposited about the egg, and some fundamen-

tal differences in developmental pattern between the eggs of lower insectivores and marsupials on the one hand and those of higher mammals on the other. Figure 3 schematically portrays the external coats of mammalian blastocysts. The innermost, the zona pellucida, is formed about the egg within the ovary, characterizes the eggs of all mammals, and is the sole external investment of the egg in the vast majority of them. While it is a relatively homogeneous structure in most mammals the zona of the diapausing blastocyst of the fur seal reveals an additional component, a dense inner "subzonal layer", which according to Enders (1971) is absent

before ovulation but is subsequently deposited from within during the early free period in the egg's development. Among higher eutheria the rabbit is unusual in that a thick mucoid coating is deposited about the zona as the ovum traverses the oviduct and uterus, (Enders, 1971) and the marsupials go a step further, laying down about the zona both an albuminous coat and an outer dense shell membrane during the passage of the egg (Hill, 1910; Enders and Enders, 1969; Hughes, 1974). These external coats are known to be rich in glycoproteins but a precise chemical characterization is still lacking for any species. The permeability proper-

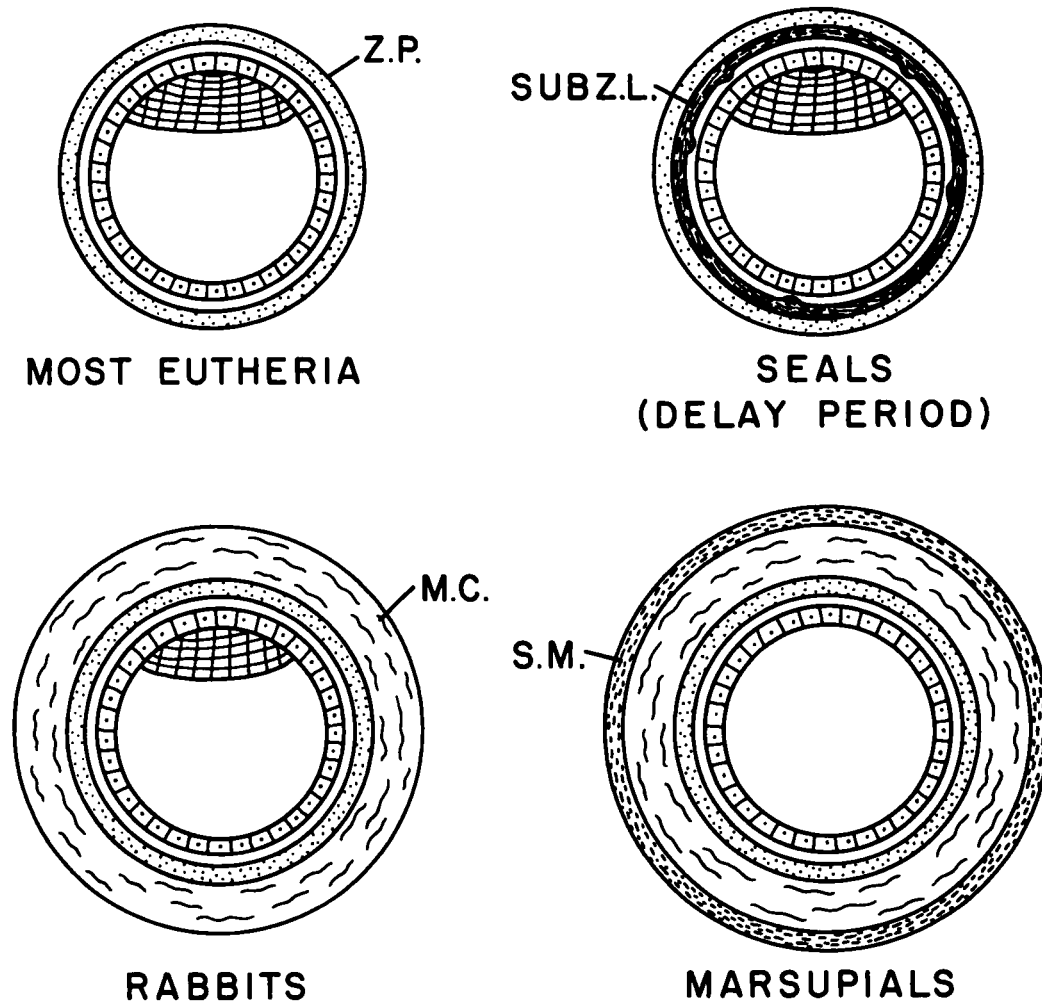


FIG. 3. External coats of mammalian blastocysts. M.C., mucoid coat; S.M., shell membrane; SUBZ.L., subzonal layer of zona pellucida; Z.P., zona pellucida.

ties of the external coats have been discussed in a review by Enders (1971), and the different ways in which they are disposed of at implantation by Enders, and by Dickmann (1969).

Figure 4 depicts the four major patterns of development of the mammalian egg, and also the developmental stage of the ovum when

implantation begins in selected mammals. The left column portrays the familiar eutherian pattern and is self-explanatory. The two center columns illustrate the quite different patterns of blastocyst formation observed in primitive insectivores. The last portrays the development of the ovum of the opossum,

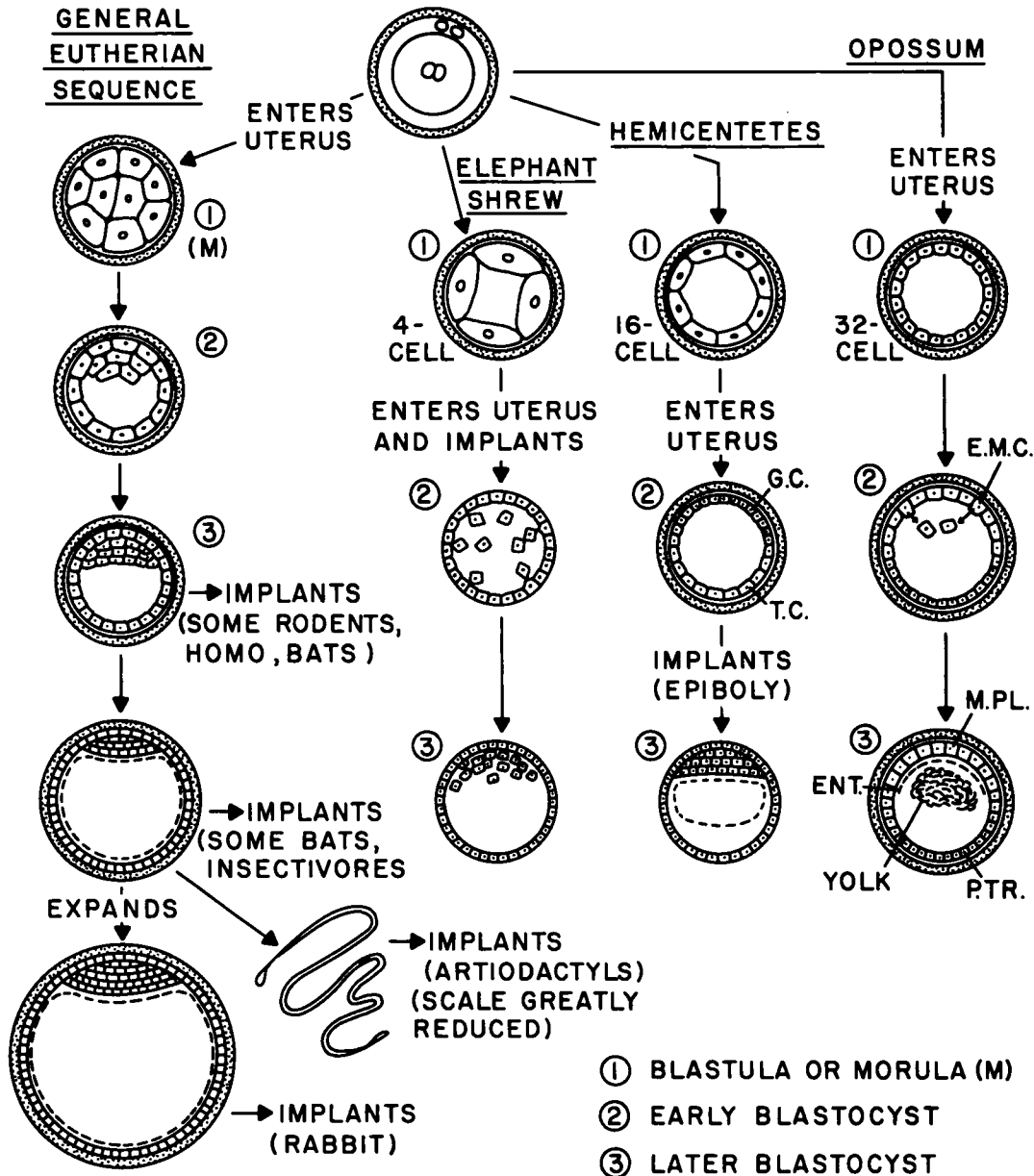


FIG. 4. Patterns of blastocyst development in mammals. E.M.C., entodermal mother cell; ENT., entoderm; G.C., germinal cells; M.P.L., medullary plate cells; P.T.R., primitive trophoblast cells.

which in most respects is representative of the marsupial condition, and is quite different from any of the eutherian patterns. Basically, the elephant shrew, tenrec and opossum are similar in that a morula stage is never achieved, for a hollow unilaminar vesicle is directly formed very early in development, specifically at the 4-cell stage in *Elephantulus* (van der Horst, 1942; Tripp, 1971), by the 16-cell stage in the tenrec (Bluntschli, 1938; Goetz, 1937; 1938), and at approximately the 32-cell stage in the opossum (Hartman, 1928; McCrady, 1938). These young vesicles of the tenrec and opossum have no inner cell mass and generally resemble the blastulas of lower chordates such as *Amphioxus*. The small 4-cell vesicle of *Elephantulus*, after arriving at the implantation site also eventually develops into a blastula-like structure of  $100 \pm$  cells. But here the resemblance to lower chordates ends, for gastrulation leads as in other mammals to the differentiation of embryonic and extraembryonic components, albeit in different ways in the three species under consideration (fig. 4).

In the elephant shrew and tenrec a typical blastocyst with inner cell mass and investing trophoblast is eventually formed, whereas in the opossum the embryonic component remains from the outset an integral part of the wall of the vesicle. In both tenrec and opossum the cells of the presumptive embryonic pole of the hollow vesicle early become recognizably different from those of the presumptive abembryonic pole. In the tenrec they are eventually displaced into the interior and overgrown by the rapidly proliferating cells of the abembryonic hemisphere, so that the inner cell mass is formed secondarily as it were. In the opossum the animal pole cells are never overgrown by abembryonic trophoblast cells but differentiate directly into an exposed embryonic plate. In the tenrec entodermal cells derive from the inner cell mass as in most higher mammals. In the opossum they are budded off into the interior by "entoderm mother cells", but these are located among the other cells of the embryonic pole of the

hollow vesicle. Despite these differences in pattern it is clear that in the tenrec and opossum there is an early differentiation of the hollow vesicle into presumptive embryonic and extraembryonic regions which are readily homologized with the inner cell mass and peripheral trophoblast respectively of the typical eutherian blastocyst.

The situation in *Elephantulus* is unique (van der Horst, 1942). Differentiation into presumptive embryonic and extraembryonic regions is deferred to a later stage of development than in the opossum, tenrec and higher eutheria. The vesicle remains a hollow "blastula" up to the time it contains  $100 \pm$  cells, and all of these retain an equal potential for differentiation into embryonic cells. The inner cell mass and primitive entoderm are formed by amoeboid cells that bud off into the interior from any point about the inner circumference of the vesicle. The earlier migrating cells secondarily aggregate to form a germinal node, and the later ones form the entoderm. Only after the inner cell mass has been thus formed does the polarity of the germ become recognizable.

The early developing eggs of these primitive mammals would make interesting experimental models for comparative studies of regulative mechanisms in the early differentiation of the egg. The egg bypasses the morula stage so the "inner vs. outer" position effect which in many higher mammals seemingly determines which cells will become trophoblast and which become embryonic cells (McLaren, 1968, 1969) is lacking, and other determinants must be involved.

#### *Oviductal phase of development.*

Some marked variations occur in the length of time the ova remain in the oviducts, and as might be expected, in the degree of development they achieve before entering the uterus. In a few mammals the oviductal phase is quite prolonged and most of the preimplantation development of the egg is accomplished therein. In most others the oviductal phase is relatively short and most



of the preimplantation development of the germ occurs while it lies free in the uterus. Presumably these differences relate not so much to interspecific differences in the developmental rate of the ovum per se as to the time required in different species for adequate preparation of the uterus, and to the differential capacities of the oviduct and uterus to sustain ovular development during the preimplantation period.

Representative examples from various orders of mammals are presented for comparison in Table 2. The ovum remains in the oviduct for the shortest time, only one day, in marsupials, and by far for the longest period, 12–16+ days in certain bats. The average is 3–4 days in other mammals, except in carnivores it is somewhat longer, 4–7 days. In marsupials the egg has scarcely begun to develop when it leaves the oviduct, for it enters the uterus as a pronuclear zygote. In the elephant shrew, only slightly more advanced, two and four cell stages are discharged. Among ungulates the pig and sheep have the shortest tubal transport time, 2.5–3+ days, and development has proceeded only to the 3–8 cell stage when the eggs enter the uterus. In tree shrews (Tupaiaidae) and certain shrews of the genus *Sorex* the ovum apparently enters the uterus as a blastocyst (Luckett, 1974; Brambell and Perry, 1945), but there is no information concerning the length of the tubal journey. Either it is prolonged, or the development of the egg is much more rapid than in other mammals; the former seems more likely. In the bats *Desmodus*, *Glossophaga* and *Carollia* (all members of the same superfamily, Phyllostomatoidea) the excessive length of the oviductal phase is accompanied by a precocious development of the ovum within the oviduct. Blastocyst formation and shedding of the zona both occur within the isthmus and the eggs are ready to implant when they enter the uterus. In these species at least peculiarities of the reproductive cycle would seem to provide an explanation for the long oviductual phase; in all of them a menstrua-

tion occurs close to the time of ovulation and most uterine growth (preparative) is hence deferred until after ovulation.

#### *Uterine phase of development*

Table 2 also presents some comparative data on the total elapsed time from fertilization to implantation, and the developmental stage of the ovum at implantation. The duration of the free period of the embryo in utero can be approximated by subtracting the figures for length of tubal transport from those representing the total elapsed days to implantation.

The shortest elapsed time between fertilization and implantation is about six days (mouse, rat, man), and the longest, except in ungulates, is 16+ days (vampire bat). In general, bats and carnivores have appreciably longer preimplantation periods than other non-ungulates. While the period in ungulates is relatively long there is a lot of variability from one species to another. In the pig preimplantation is shorter than in some carnivores, although the size of the chorionic vesicle at attachment is appreciably greater in the pig. In the sheep the preimplantation period is roughly comparable to that of carnivores and bats, but again the implanting chorionic vesicle is much larger in the sheep. The cow and the horse present the longest known preimplantation phases, 40+ and 50–60 days respectively, and as in other ungulates the chorionic vesicles are very large at the time of attachment.

Finally, it should be stressed that while the length of the preimplantation period is roughly comparable between certain phyllostomid bats (and possibly Tupaiaidae and shrews) and some ungulates, such as sheep, virtually all of the development of the egg takes place in the oviduct in the bats, whereas in ungulates most of it occurs within the uterus. This indicates that the oviduct in bats plays a supportive role in the earlier development of the ovum which in other mammals is delegated to the uterus—unless

Table 2  
Chronology of Preimplantation Egg Transport and Development

Species	Tubal transport (days)	Develop. stage entering uterus	Implantation (days post fertil.)	Develop. stage at implantation	Authority
<b>Marsupials</b>					
opossum ( <i>Didelphis</i> )	1	1-cell, pronuclear	none, strictu sensu		Hartman, 1928 McCready, 1938
native cat ( <i>Dasyurus</i> )	1 (?)	1-cell, pronuclear	?	probably late pr. streak stage	Hill, 1910
<b>Insectivores</b>					
elephant shrew ( <i>E. myurus</i> )	1-2 (?)	1,2 and 4-cell stages	?	early blastocyst	van der Horst & Gillman, 1941 van der Horst, 1942
tree shrew ( <i>Urogale everetti</i> )	?	unilaminar blastocyst	?	bilaminar blastocyst	Luckett, 1968
tree shrew ( <i>Tupaia longipes</i> )	5-6 (est.)	bilaminar blastocyst	?	bilaminar blastocyst	Luckett, 1968
shrew ( <i>Sorex</i> sp., <i>Blarina</i> brev.)	?	small blastocyst	?	bilaminar blastocyst	Brambell & Perry, 1945 Wimsatt & Wislocki, 1947
<b>Chiroptera</b>					
vampire bat ( <i>Desmodus</i> )	16+	blastocyst, some endoderm diff.	not less than 16 days	Blastocyst, bilaminar partially	Quintero & Rasweiler, 1973 Wimsatt, 1954
long-tongued bat ( <i>Glossophaga</i> )	12-14	blastocyst	15	Blastocyst, bilaminar partially	Rasweiler, 1974 Rasweiler, 1972
short-tailed bat ( <i>Carollia</i> )	13-16	blastocyst	?	?	Bonilla & Rasweiler, 1974
little brown bat ( <i>Myotis lucif.</i> )	?	morula	not less than 10 days	Blastocyst, bilaminar	Wimsatt, 1944
free-tailed bat ( <i>Tadarida brasili.</i> )	?	adv. morula	?	Blastocyst, bilaminar	Stephens, 1962
<b>Primates</b>					
rhesus monkey ( <i>Macacus</i> )	4	16-cells	8-9	Blastocyst	Heuser & Streeter, 1941 Lewis & Hartman, 1941
baboon ( <i>Papio</i> sp.)	4-5	32 ± cells	8-9	Blastocyst	Hendricks, & Kraemer, 1968, Hendricks, 1971

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Table 2

Species	Stage	Cell Count	Days	Implantation Site	References
man (Homo)	3-4	?	6-7	blastocyst	Hafer, 1973 Hertig and Rock, 1945 Hertig et al, 1956
<b>Rodents</b>					
rabbit	3-3½	20+ cell morula	7	bilaminar blastocyst	Anderson, 1927 Boving, 1959
mouse	3-3½	16+ cell morula	6	blastocyst, some entoderm	Lewis & Wright, 1935; Snell, 1941 Humphrey, 1968
rat	4	morula	6	blastocyst, some entoderm	Enders & Schlafke, 1967 Psychoyos, 1973
hamster (Cricetus auratus)	3½	4-8 cells	4-1/3	blastocyst, some entoderm	(Orsini) Ward, 1948
guinea pig	3½	8 cells	6	unilaminar blastocyst	Blandau, 1949a Squier, 1932
mt. viscacha (Lagidium)	?	?	less than 11	blastocyst	Pearson, 1949
plains viscacha (Lagostomus)	3-4	1-2 cells	?	blastocyst	Weir, 1971
<b>Carnivores</b>					
dog	4-5	16-cell to young blastocyst	11-12*	bilaminar blastocyst, 2500 µm diam.	Holst & Plemister, 1971
cat	6-7	28-30 cell morula	13-14	bilaminar blastocyst	Anderson, 1927 Amoroso, 1952
ferret	5-6	32+ cells	12 †	bilaminar blastocyst	Hamilton, 1934 Anderson, 1927 Enders, 1971
<b>Ungulates</b>					
horse	?	?	49-63	elongated chorionic vesicle w. allantois	Hamilton & Day, 1945 Amoroso, 1952
pig	2½ - 3-3/4	3-8+ cells	11-20 (progressive)	elongated chorionic vesicle w. devel. allantois	Oxenreider & Day, 1965 Amoroso, 1952
sheep	2½ - 2-3/4	?	15-17 (progressive)	15-19 cm chorionic vesicle w. allantois	Holst, 1974; Chang & Rowson, 1965; Boshier, 1969
cow	4-4½	8-16 cells	40+ (progressive)	elongated chorionic vesicle w. allantois	Hamilton & Lasinge, 1946 Chang, 1952

\* elapsed time from end of vaginal cornification (within 1-2 days of ovulation)

we are prepared to accept that the development of the bat ovum up to the blastocyst stage is wholly independent of its milieu. This appears unlikely, however, for Rasweiler (1972, 1973), Quintero and Rasweiler (1974), and de Bonilla and Rasweiler (1974) have shown that in *Desmodus*, *Glossophaga* and *Carollia* there is a differential stimulation of the oviducts prior to and following ovulation involving a marked accentuation of secretory activity in the oviduct containing the newly released egg. This is perhaps most logically interpreted as a special adaptation to provide a supportive environment for the egg during its protracted stay in the oviduct.

#### *Spacing of ova in utero*

In polyovular species the tendency toward equidistant spacing of ova in utero before implantation helps prevent overcrowding and attendant loss of embryos by resorption. The regularity of spacing varies however from one species to another, possibly in relation to variations in the relative sizes of the blastocyst during spacing (McLaren, 1968). In rabbits, for example, spacing is more precise than in rat, mouse or guinea pig (Böving, 1971). These are virtually the only species incidentally in which the subject has been investigated by rigorous quantitative methods.

Spacing involves much more than the mere physical propulsion of blastocysts through the uterine lumen. It involves the participation of biomechanical factors in arresting the ova at suitable implantation sites, physiological interactions between embryo and uterus leading to attachment, possible additional spacing adjustment after implantation, and endocrine and other physiological mechanisms which control and coordinate these events. Fetal losses due to spacing irregularities are probably not uncommon given the number of variables involved, but they are often difficult to detect since early-lost embryos may leave no trace. Bent Böving through his imaginative experiments has been the major contrib-

utor to a better understanding of the biomechanics of spacing in laboratory rodents, especially the rabbit, and he has also been the field's most prolific reviewer (Böving, 1959, 1963, 1971, 1972; Böving and Larsen, 1973). No attempt will be made here to discuss spacing mechanisms in depth; only some high points will be sketched to provide a sense of what is involved, plus the identification of some additional factors, not often emphasized, but which are relevant if spacing is to be viewed in a wider comparative context.

Böving (1971) has shown that the propulsive force for movement of ova in the rabbit, and presumably in all mammals, derives from coordinated circumferential contractions of the myometrial smooth muscle, presumably under the influence of estrogens. He discussed various ways in which the muscle activity might propel the ova, but favored the view that the propagated contractions cause the egg to be squeezed by the endometrium and thereby moved along. He has shown that the proximate stimulus for spacing in the rabbit is blastocyst expansion, sufficient to distend the uterus, presumably by an active pumping of fluid into the blastocyst interior under the influence of progesterone. It is possible that a similar stimulus is operative in carnivores, whose blastocysts also expand before implantation (e.g. dog, Holst and Phemister, 1971; bear, Wimsatt, 1974). But since comparable blastocyst expansion does not occur in mice, rats, guinea pigs and numerous other mammals different mechanisms must be involved in these species. Böving dismisses the possibility of preformed implantation sites in the rabbit on the grounds that multiple blastocysts always space equidistantly despite variations in their number, and that a single blastocyst implants medially in the horn, presumably having passed favorable sites enroute.

It has been suggested by DeFeo and Kleinfeld (cited by Hamilton, 1973) that in the rat some spacing is accomplished after implantation by appositional growth in the myometrium adjacent to the implantation sites.

Hamilton (1973) tested this hypothesis in mice by using a combined pontamine blue and colchicine technique on day 5 postfertilization. She found that mitotic rates were higher in the stroma adjacent to the implanting blastocysts than between them and that most of those adjacent to the blastocysts were nearer the myometrium, while those between sites were nearer the lumen. She also noted that sites identified by pontamine blue on day 5 were spaced more evenly than random in 87 percent of cases. This evenness was unchanged on day 6, when the sites were visible macroscopically, and was unaffected by the number of blastocysts implanting. She concluded that growth of the uterus after implantation is not a major spacing mechanism, at least in the mouse. It is unfortunate that Hamilton did not extend her observations beyond the sixth day for it might still be argued that displacement of embryos by differential growth of the uterus could occur well after implantation was completed.

Bidirectionality of movement of ova within the uterus in conjunction with spacing has seldom been emphasized. Böving (1971) has shown that in the rabbit contraction waves stimulated by a distending object move in both directions along the horn, and while these do not move the object itself they do move (in opposite directions) its neighbors. Restall and Bindon (1971) reported that in the mouse before spacing occurred the zygotes tended to be grouped together in the middle of each horn, and that subsequent spacing occurred equally in both directions from the center. A final example is provided by Dutourne and Canivenc (1971) in their study of migration of diapausing blastocysts and glass beads the size of blastocysts in the uterus of the European badger. They found that both blastocysts and beads tended to remain grouped together and were frequently moved back and forth between the two horns of the uterus without loss as they passed through the corpus above the cervical canal. Their study casts no light on the spacing mechanism per se in the badger, but it does demonstrate the potentialities

for bidirectional movement of blastocysts within the uterus. It seems reasonable to assume that random bidirectional movements of eggs in utero normally occur before and during the final spacing phase in all polyovular species and perhaps monovular ones also.

The question of blastocyst arrest as a component of spacing is a difficult one, for the mechanism probably varies from one species to another and as between species with preformed implantation sites (whether highly localized or not) and those lacking such sites. Böving (1971) has indicated that the rabbit blastocyst eventually expands and distends the uterus to such a degree that it can no longer be moved. Thus, the expansion stimulus which initiated spacing movements in the first place eventually inhibits it; blastocyst arrest is essentially a biomechanical process in the rabbit. Further expansion of the rabbit blastocyst causes a muscular relaxation at the antimesometrial side and a ballooning of the wall to form the implantation "dome". Concurrent constriction of the muscles in the non-distended segments adjacent to the blastocyst appear to lock it in place.

The arrest mechanism in other species, perhaps even some with expanding blastocysts, may involve other mechanisms than merely the effects of blastocyst expansion on uterine muscular activity. Holst and Phemister (1971) believe that blastocyst arrest in the dog is effected by endometrial swelling from a localized edema at the presumptive implantation site. Edematous swelling has been described at the specialized implantation sites of the elephant shrew (van der Horst and Gillman, 1941) and of tree shrews (Luckett, 1968), but has not been specifically implicated in blastocyst arrest in these species. Nevertheless, localized or more generalized edematous swelling alone or in conjunction with muscular constriction could well be an important arresting factor in other species.

In those species (e.g. man) in which the dimensions of the uterine cavity seemingly

preclude a "clasping" mechanism whether by edematous swelling of the endometrium or otherwise, adhesiveness of the blastocyst wall (after loss of zona) may be a factor in the final arrest of the egg; this mechanism has been discussed recently by Enders (1972). The possibility that in some species at least, the uterus may cooperate in such a mechanism is suggested by work of Mintz (1972) with mice. She describes an "implantation initiating factor" (IIF) derived from the uterus which promotes "fixation" (arrest?) by causing zona-free blastocysts to adhere to the uterine wall. The factor also lyses the zona. She attributes facultative delayed implantation in mice to a deficiency of IIF, the production of which is hormonally controlled (by estrogens). Correction of the deficiency allows implantation to proceed in mice.

If spacing phenomena are to be considered from a broad comparative viewpoint additional factors besides those discussed become relevant. A partial listing of these includes 1) the extent to which the lumina of the uterine horns are separate or confluent, 2) the equality of ovulation between the two ovaries especially in relation to the degree of separation of the uterine horns, 3) the possibilities for transuterine migration of ova, 4) the existence of specialized implantation sites as opposed to a more generalized competence of the endometrium, and 5) the effects of an earlier pregnancy in rendering the former implantation sites refractory to a subsequent implantation at the same site.

If the uterine horns are fully separated then spacing mechanisms presumably operate independently in each. Significant inequalities in ovulation rates on opposite sides could lead to an excess of ova in one horn with attendant spacing problems, overcrowding, and egg loss. Fortunately, in most known instances there is a tendency for ovulations to be divided more or less equally between the two ovaries (e.g. opossum, rat). Confluence of the uterine horns makes transuterine migration of ova a possibility (but not necessarily a probability), and if migration can occur an

equal distribution of embryos presumably requires a coordination of the spacing mechanisms of the two horns. Something of this sort must be involved in the bat *Pipistrellus subflavus*, a species in which ovulatory inequality often exists, and transuterine migration of ova can definitely occur in both directions, but in which the implantation sites tend to be equally distributed between the horns (Wimsatt, 1945). In common ungulates such as the sheep and cow the bicornuate uterus is septate; that is the cornual lumina are separated most of their length, and communication occurs between them only via a very constricted corpus just above the cervical canal. This anatomical configuration would theoretically permit transuterine migration of ova, and yet there is a very strong tendency in both species, when a single ovum is released (the usual condition), for the conceptus to remain in the horn of the ovulating side. A study of 1030 pregnant ewes (Reimers et al, 1973) revealed that of 959 which had only one corpus luteum, migration of ova to the contralateral horn occurred in only 38, whereas 31 of 32 animals with two corpora lutea in the same ovary carried twins, and in all of them one fetus had migrated to the contralateral horn. Scanlon (1971) made similar observations on 659 pregnant cows and 1063 pregnant ewes. In 643 cows, monovular and carrying a single fetus, the percentage showing transuterine migration was zero; the corresponding figure for 834 ewes in the same condition was 8.0 percent. Of 10 cows with two corpora lutea in the same ovary and carrying two fetuses transuterine migration had occurred in only 20 percent; the corresponding figure for 120 ewes was 87.5 percent. In five cows that had two corpora lutea in one ovary but carried only one fetus migration had occurred in none of them; out of 108 ewes, however, the migration rate was 13.9 percent. In one cow and one sheep in which there was a single ovulation but monozygotic twins were present, transuterine migration of one embryo had occurred in each. The regulative nature of the spacing mech-

anisms implicit in these results is something to be marvelled.

The subjects of preformed implantation sites and refractory areas (to implantation) in the endometrium have relevance for spacing problems but they will be discussed later in another context.

## IMPLANTATION PERIOD

### *Patterns of implantation*

Three major forms of implantation commonly referred to today to indicate the degree of penetration of the uterine mucosa by the implanting blastocyst were already recognized by embryologists before the turn of the century. They were then and still are designated "central", "eccentric" and "interstitial" respectively (the term "superficial" is often used today as a synonym for "central"). To these three was later added another type of implantation designated "partially interstitial". All four are depicted schematically in figure 5. The addition of a fifth type, which might be called "secondarily interstitial", would be useful to signify those situations in which an originally eccentrically implanted blastocyst is later excluded from the uterine cavity by an overgrowth of the surrounding endometrial tissues—such as occurs in rats, mice, hamsters and numerous other small mammals. Arrows in the second drawing depict this type of situation.

### *Orientation of blastocysts*

Mossman (1937) first called attention to, and stressed the importance of the non-random nature of blastocyst orientation to the uterus, specifically to the plane of symmetry of the organ defined by its mesometrial—antimesometrial (i.e. mid-sagittal) axis. Blastocyst orientation involves two major components: the directionality of the germ disc, and of the point of first trophoblastic attachment to the defined plane of symmetry. The orientation of the disc is designated "antimesometrial" or "mesometrial" according to whether it faces away from or toward the mesome-

trium; if it faces somewhere between it is designated "lateral" orientation (fig. 5). The importance of disc orientation is that it constitutes a primary determinant of the later position of the various fetal membrane components, "... subject to modification by differences in relative growth rate and final size of the allantoic vesicle and amnion" (Mossman, 1971). The orientation of the point of first attachment of the trophoblast to the uterus like the orientation of the disc is fairly constant within major taxonomic groups (orders and suborders); it is important in that it generally marks the presumptive position of the chorioallantoic placenta, although this is not invariably true (fig. 7). The drawings in figure 6 illustrate the variable orientation of first trophoblastic attachments (indicated by the heavier lines) and the terminologies employed. The figure is self-explanatory, except it should be noted that 1) the "cotyledonary" type is a special example of a "diffuse" orientation and has usually been included in that category by earlier authors, and 2) many "circumferential" types have often been designated "bilateral" on the basis of their apparent duplicity when observed in sectioned material. There are, of course, true bilateral types (e.g. in tupaiids) in which attachments are made at two places on opposite sides of the uterus, but in instances where blastocysts occupy cylindrical implantation chambers (e.g. shrews and glossophagine bats, 2nd and 3rd figures of bottom row) the attachment is equatorial (i.e. lateral *and* circumferential) not bilateral. In forms with two separate attachment points a bidiscoidal placenta may result (e.g. macaque, tupaiids, natalid bats), while multiple attachments can give rise to multiple placental areas (e.g. placentomes of ruminants). Table 3 summarizes the implantation types and blastocyst orientations observed in members of some representative orders of mammals. It is essentially an updated version of similar tables published previously.

As emphasized by others (e.g. Mossman, 1971) the constancy of orientation of the

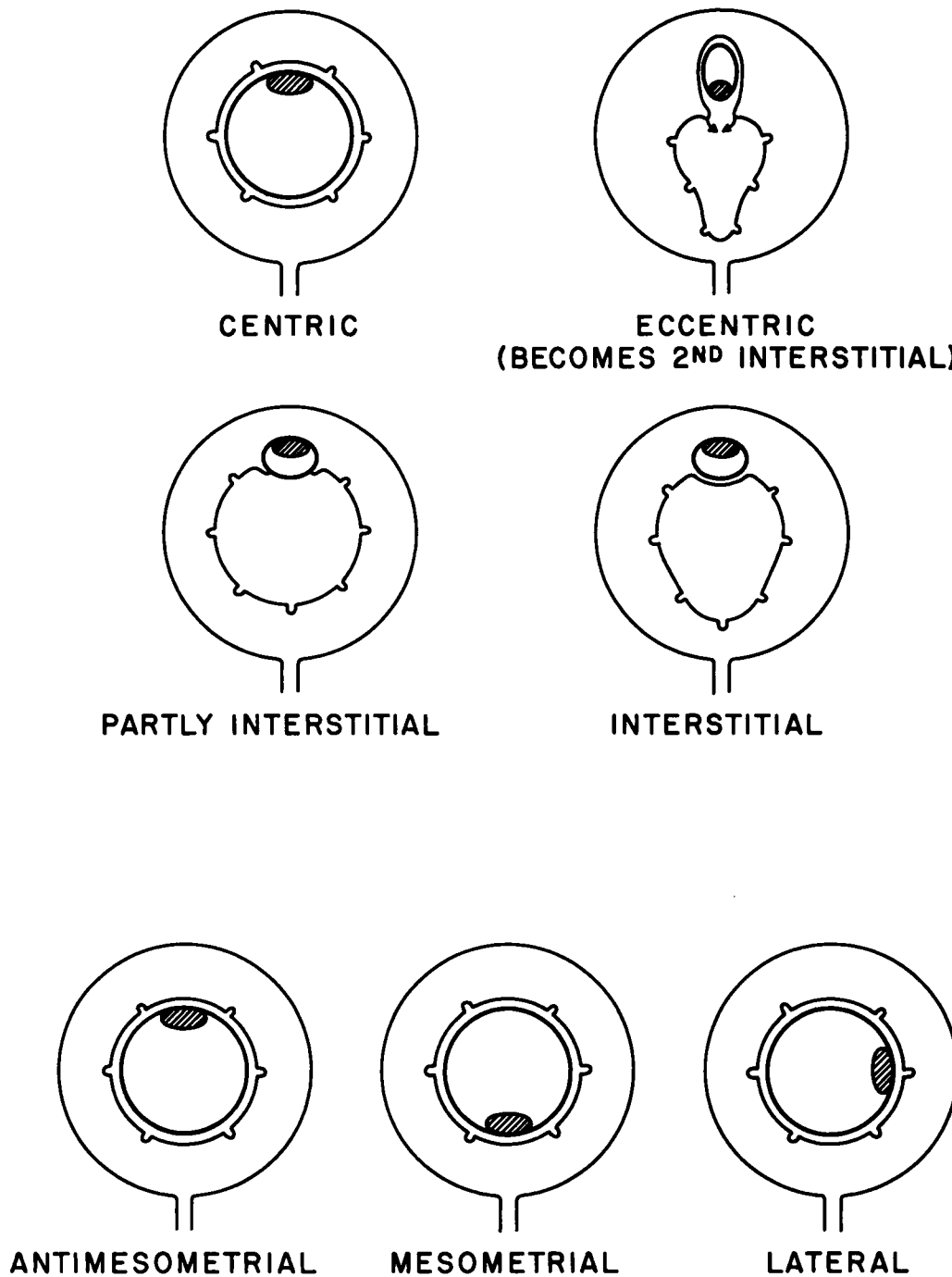


FIG. 5. The upper four drawings illustrate the common implantation patterns observed in mammals and terminologies. The lower three drawings illustrate various orientations of the embryonic cell mass. The mesometrium is directed toward the bottom of all figures.



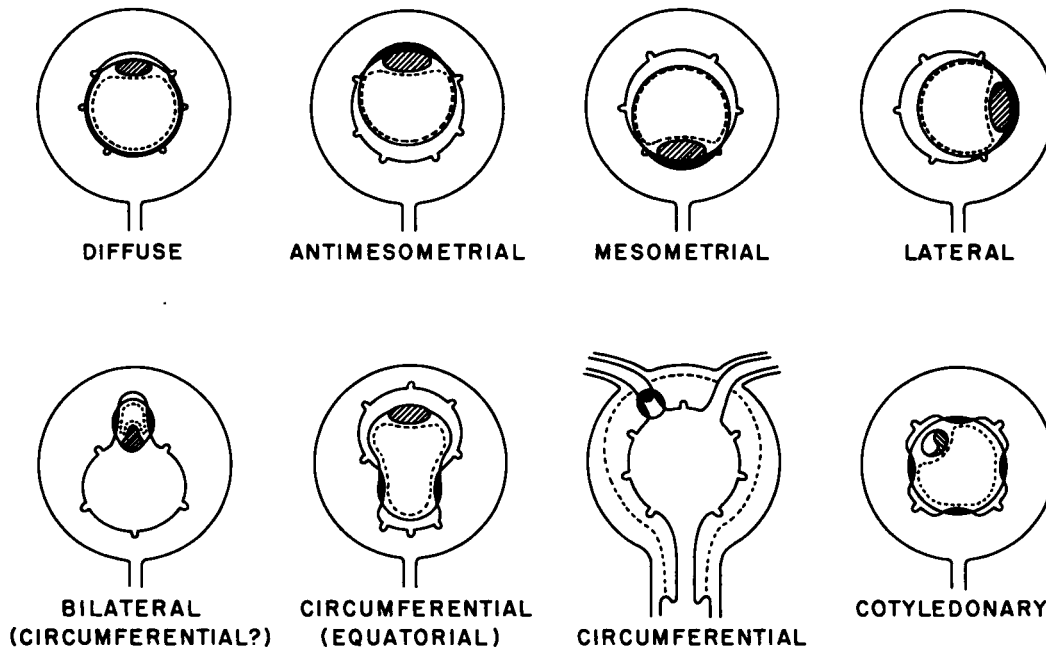


FIG. 6. Diagrams showing variations in the points of first trophoblastic attachment observed in mammals. The points of first attachment are indicated by a thickening of the line designating trophoblast. The mesometrium is toward the bottom of all figures.

germ disc to the sagittal plane of the uterus observed within most higher taxonomic groupings is lacking in a few of them, most notably in insectivores and Chiroptera. At least four different orientation patterns (of embryonic disc and first attachment site) are known in insectivores and no less than three in bats; these are represented schematically in figures 7 and 8. The stippled areas enclosed by a dotted line mark the site of the definitive placental disc. The variability displayed within these orders is perhaps not too surprising for they are both very ancient groups, and have clearly experienced great evolutionary radiation. For comparison, figures 9 and 10 demonstrate the more constant orientation patterns observed in primates and rodents. The rodents too are an ancient group, but as far as these characters are concerned evolutionary radiation in them has apparently followed a more conservative trend.

Mossman (1971) seems to be the only one who has called attention to the broader significance of specific blastocyst orientations in terms of their providing biological advan-

tages to the species involved. He posed the question, but could define no clues as to the possible functional advantage of any specific orientation. He reminds us, however that to accept them as purely fortuitous "... would assure continued ignorance ... and delay discovery of possible important principles of uterine-embryo interrelationships."

#### *Orientation mechanisms*

In considering orientation mechanisms it is important to keep in mind that "orientation" has two aspects. One concerns the blastocyst as a whole and involves the location of the attachment site within the uterus; it seems likely that this is largely determined by uterine influences. The second involves the specific orientation of the inner cell mass, and this is a much more complicated problem. In any event we may ask, to what extent are the mechanisms responsible for a constant orientation inherent in the blastocyst, in the uterus, or dependent on their mutual interaction? A related question is, do these mechanisms vary from one species to another? Many have at-

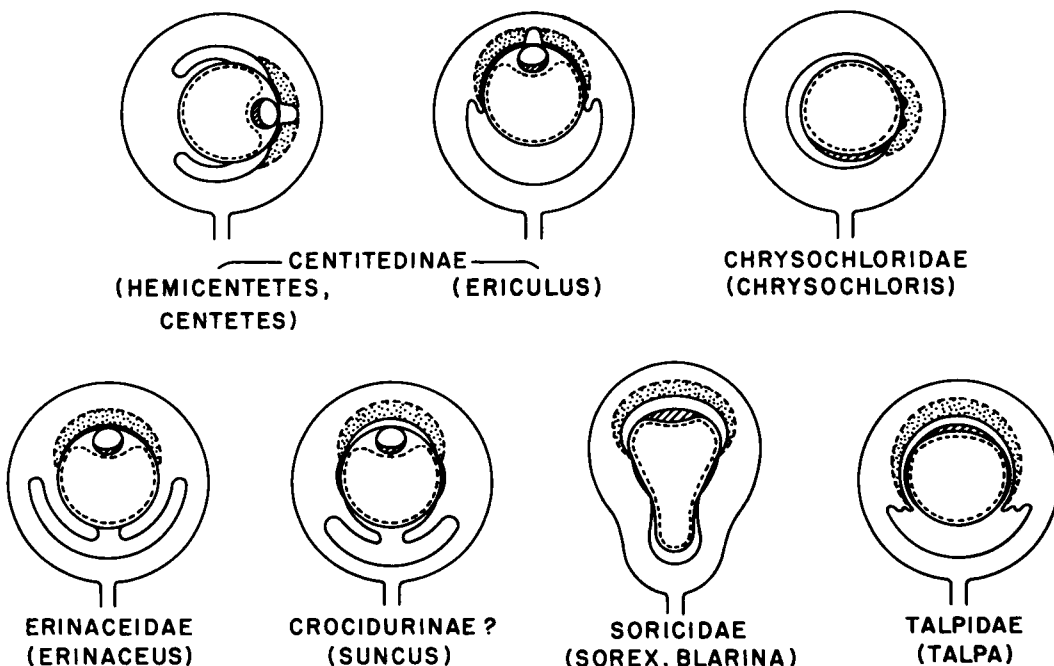


FIG. 7. Variations in the orientation of first trophoblastic attachment and placental disc location in Insectivora.

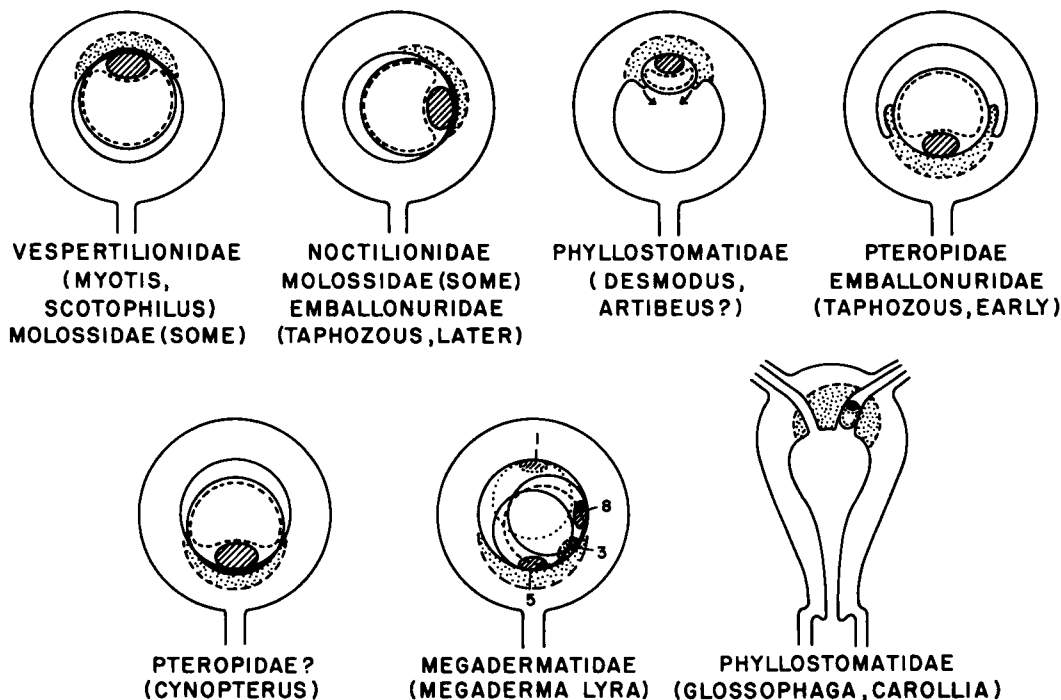


FIG. 8. Variations in the orientation of first trophoblastic attachment and placental disc location in Chiroptera. In middle drawing of bottom row four different orientations described in *Megaderma* by Gopalakrishna and Karim (1971) are shown. The numbers indicate the instances of each orientation pattern observed.

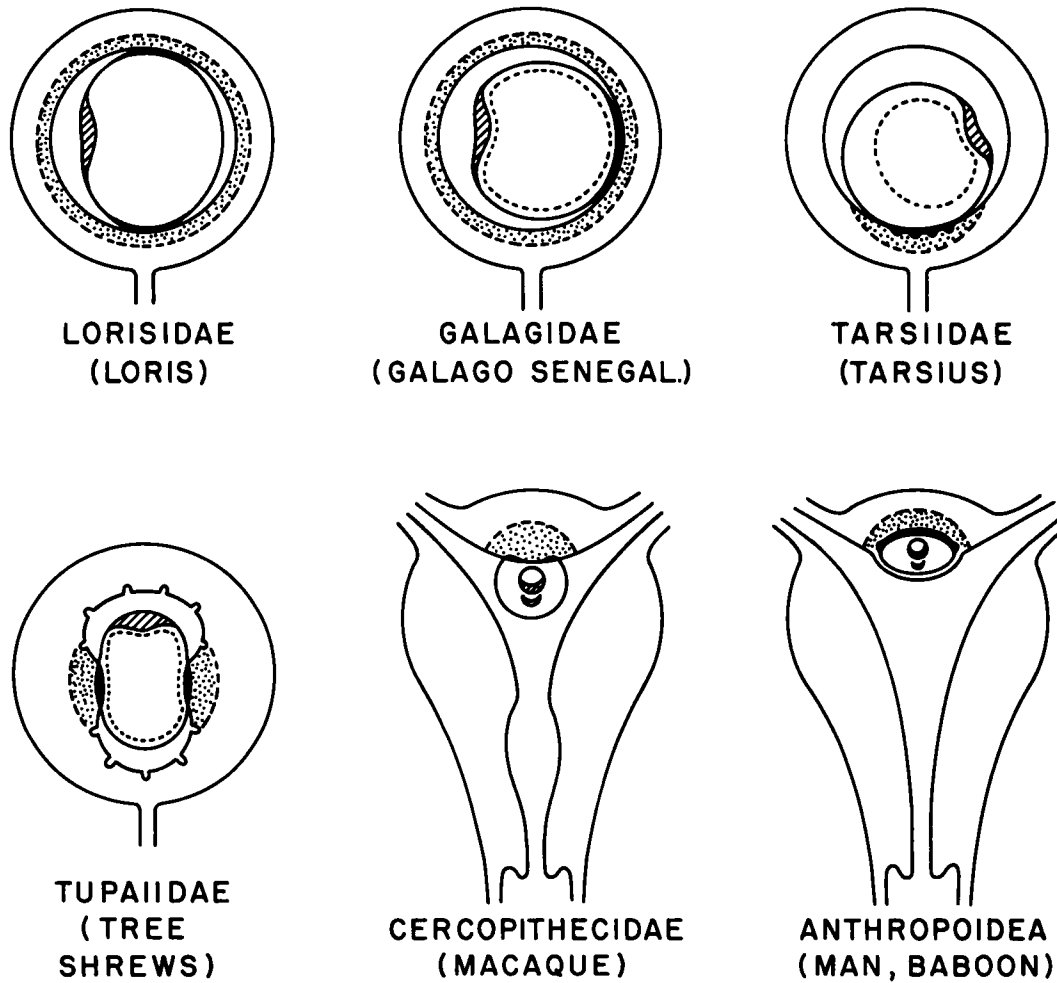


FIG. 9. Constancy of orientation pattern of first trophoblastic attachment and placental disc location in Primates. The diagram depicting Tupaiidae (lower left) does not fit the primate pattern, but most modern authors would place the tupaids with the Insectivora, so this diagram should be viewed in conjunction with those of other Insectivora in fig. 7.

tempted to solve the first question, but unambiguous answers have yet to be found for even a single species. In respect to the second question there is as yet no reason to suppose that orientation mechanisms are the same or even similar in unrelated groups of mammals. In comparative perspective we observe many more variables that could directly or indirectly influence orientation dynamics than are found within the context of single species. As far as the blastocyst is concerned these may include qualitative differences in the nature of trophoblast-inner cell mass interactions, variations in the size and shape of

implanting blastocytes in relation to spacing mechanisms, and the varying degrees of cellular and physiological differentiation that blastocysts achieve by the time they are ready to implant. For example, orientation mechanisms that might involve a "migration" of inner cell mass components within the trophoblastic shell are far more conceivable in procociously implanting small unilaminar blastocysts such as those of mice and rats (a possibility suggested by the work of Gardner, 1970; Gardner et al, 1973 with microdissected mouse blastocysts and subsequently adduced by Kirby et al, 1967 and Kirby, 1971 to be the

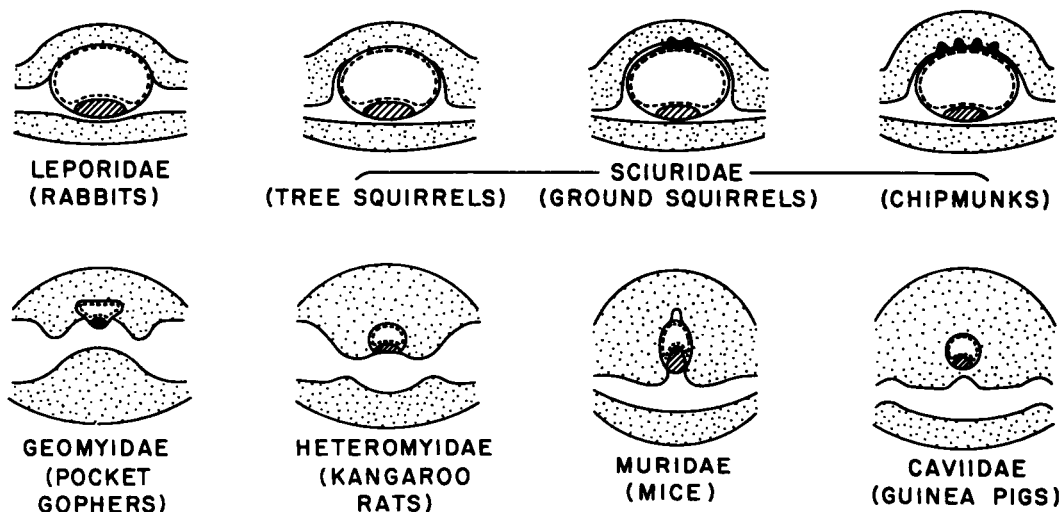


FIG. 10. Patterns of implantation in rodents. The figure also shows the effect of differences in blastocyst size on the implantation mode. The antimesometrial side is toward the top of the figure in all cases (redrawn from Mossman, 1937).

most likely orientation determinant in mice) than in the later implanting, larger and more differentiated bilaminar blastocysts of such animals as rabbits and carnivores; yet these too show constancy of orientation. Regulative interactions between trophoblastic and embryonic components of the blastocyst and any influence these might have on the establishment of orientation patterns with the uterus have scarcely been explored as yet.

Uterine adaptations of significance for the orientation of attachment sites (and indirectly perhaps for disc orientation if uterine influences can be transmitted to the interior of blastocysts) also vary enormously. Some that appear relevant include differences in luminal configurations related to the presence or absence of rugae, folds or other species-specific endometrial formations (Mossman, 1971), the presence in some species of specialized or preformed implantation sites (e.g. in tupaiids, elephant shrew, ruminants), and preparative modifications of uterine vascular patterns or localized edemas and epithelial changes that appear prior to the actual arrival of blastocysts (e.g. in pteropid bats).

It is generally asserted on the basis of a great deal of experimental evidence that implantation of the blastocyst is a mutual affair,

the result of a harmonious interaction between blastocyst and uterus. As far as specific orientations are concerned there is experimental evidence that underscores the importance of the uterus in determining the site of implantation, at least in the rat and mouse (Alden, 1945; Blandau, 1949b; Wilson, 1960), but little is yet known with certainty concerning the possible contributions of the blastocyst to its own orientation.

#### *Uterine adaptations for implantation*

*Total vs. restricted competence of uterus.* In the common laboratory rodents, many of their wild allies, and among numerous other mammals in all of the major taxonomic groupings the microscopic organization of the endometrium looks much the same throughout the length of the uterine horns, except perhaps at their extremities (oviductal end, and corpus in cases where the horns are confluent). This suggests that virtually the whole endometrial surface is potentially competent for implantation purposes. The evidence for this in most wild mammals is observational—the alternating use of both horns for pregnancy in monotocous species, and their simultaneous use in polytocous species—but it has a solid experimental basis

among laboratory rodents and domesticated carnivores and ungulates. In the hormonally conditioned uteri of rats, mice and hamsters for example the entire endometrium is known to be capable of decidual transformation (deciduoma formation) in response to appropriate artificial stimulation, and in rabbits and guinea pigs the progestational reaction of the endometrium appears more or less uniform throughout. Further it has been shown in rabbits that equidistant spacing of embryos occurs irrespective of their number: the distance between implantation sites merely diminishes as their number increases (Böving, 1954, 1963). The same has been noted in rats and mice, in which spacing of embryos is not quite so regular when their numbers are low, but becomes more so as embryo numbers increase and implantation sites crowd closer together (Böving, 1971). These results can only reflect an overall competence of the endometrium for implantation in these species.

There are several mammals however in which the endometrium is not everywhere competent, species in which implantation is restricted to selective sites; they include both monovular and polyovular forms. In some of them (certain antelopes and bats, see later) the restriction is only coarsely expressed, in that one horn is preferentially (or exclusively) utilized over the other. In other species (elephant shrew, tupaiids, viscachas, glossophagine bats) the restriction is more precise, implantation being localized to specific sites in one or both horns.

The elephant shrew *E. myurus* (fam. Macroscelididae) is perhaps the most unusual (van der Horst and Gillman, 1942). Implantation is restricted to a very small area localized at the mesometrial side of each horn just above its junction with the median uterus (Fig. 11). Moreover menstruation occurs only at these sites in *E. myurus*; the rest of the uterus remains wholly unaffected. As mentioned earlier *E. myurus* releases 50–120 ova at ovulation, most of which develop to a 4-cell stage in oviduct or uterus, but only two of

these which reach the small implantation site in each horn, “turn on” and develop into viable embryos. The vast remainder degenerate without passing the 4-cell stage. Not all elephant shrews superovulate, but all except *E. fuscipes* show the same localized single implantation sites as *E. myurus*; *E. fuscipes* sometimes implants two to the horn (Tripp, 1971). A somewhat similar situation to that of *E. myurus* is found in the tree shrews of the family Tupaiidae (Meister and Davis, 1956, 1958; Luckett, 1968). Tupaiids do not superovulate, but they too carry only two fetuses, one in each horn. Unlike *E. myurus*, however, implantation in tupaiids is bilateral, that is the blastocyst attaches simultaneously to two gland-free endometrial cushions lying laterally at opposite sides of the cornual lumen; in consequence a bidiscoidal placenta is eventually formed (fig. 9). These specialized cushions are clearly recognizable in virgin uteri, but they extend the entire length of the uterine horn and hence are less circumscribed than the minute implantation sites of *E. myurus*. According to Conaway and Sorenson (1966) the post-estruual menstruation of tupaiids is largely restricted to these cushion areas.

Glossophagine bats of the genera *Glossophaga* and *Carollia* show a thus far unique localization of the implantation site, (Rasweiler, 1974; deBonilla and Rasweiler, 1974) which is depicted schematically in figures 8 and 11. In these bats (unlike most) the uterus is simplex, but the oviducts enter cranially rather than laterally as in the human. Implantation occurs in a narrow tubular segment, the “intramural uterine cornu” (Rasweiler, 1974), interposed between the end of the oviduct and the main uterine cavity (fig. 8). Thus the egg implants without ever entering the cavity of the uterus proper. Rasweiler suggests that the “intramural uterine cornu” is probably homologous to the cranial end of a horn in a bicornuate uterus. These bats are also unusual in that a menstruation ensues at or shortly after ovulation (Rasweiler, 1972). Another example of

Table 3  
Implantation Characteristics of Eutheria

Classification	Implantation type		Orientation of disc		Orient. 1st. attach.		Depth				
	Centric	eccentric	Mesom.	Antimes.	Lateral	Mesom.	Antimes.	Lateral	Superf.	Part. Interst.	Interst.
<b>Insectivora</b>											
Tenrecidae	+								+		
Chrysochloridae	+		+						+		
Erinaceidae				+					+		+
Macrocecididae		+				+					+
Soricidae	+			+				circumf.	+		
Talpidae (Talpa)	+			+					+		
Talpidae (Scalopus)	?			?					+		
Tupaidae	+			+				bilateral	+		
<b>Chiroptera</b>											
Pteropidae		?									
Megadermatidae	+		+								+
Noctilionidae	+		+						+		
Phyllostomatidae					+				+		
(Glossophaga)											secondary
(Desmodus)											+
Vespertilionidae	+								+		
Molossidae	+								+		
<b>Primates</b>											
Lemuridae	+										
Lorisoidae	+										
Tarsiidae	+										
Cercopithecoidea	+										
Hominoidea											+
<b>Edentata</b>											
Dasybus novemc.											
"fundic"											

Table 3

Lagomorpha									
Leporidae	+	+							+
Rodentia									
Sciuromorpha		+							+
Geomyoidea		+							+
Myomorpha		+							
Hystricomorpha		+							
			+						secondary some
Carnivora									
Fissipedia									
Pinnipedia									
Perissodactyla									
Equidae									
Artiodactyla									
Suiformes									
Cervoidea									
Bovoidea									

\* Luckett (1974) has proposed that the anterior and posterior walls of the (simplex) uterus are homologous to the lateral walls of a bicornuate uterus. If this is true then disc orientation in higher primates would be comparable to the lateral orientation of prosimians.

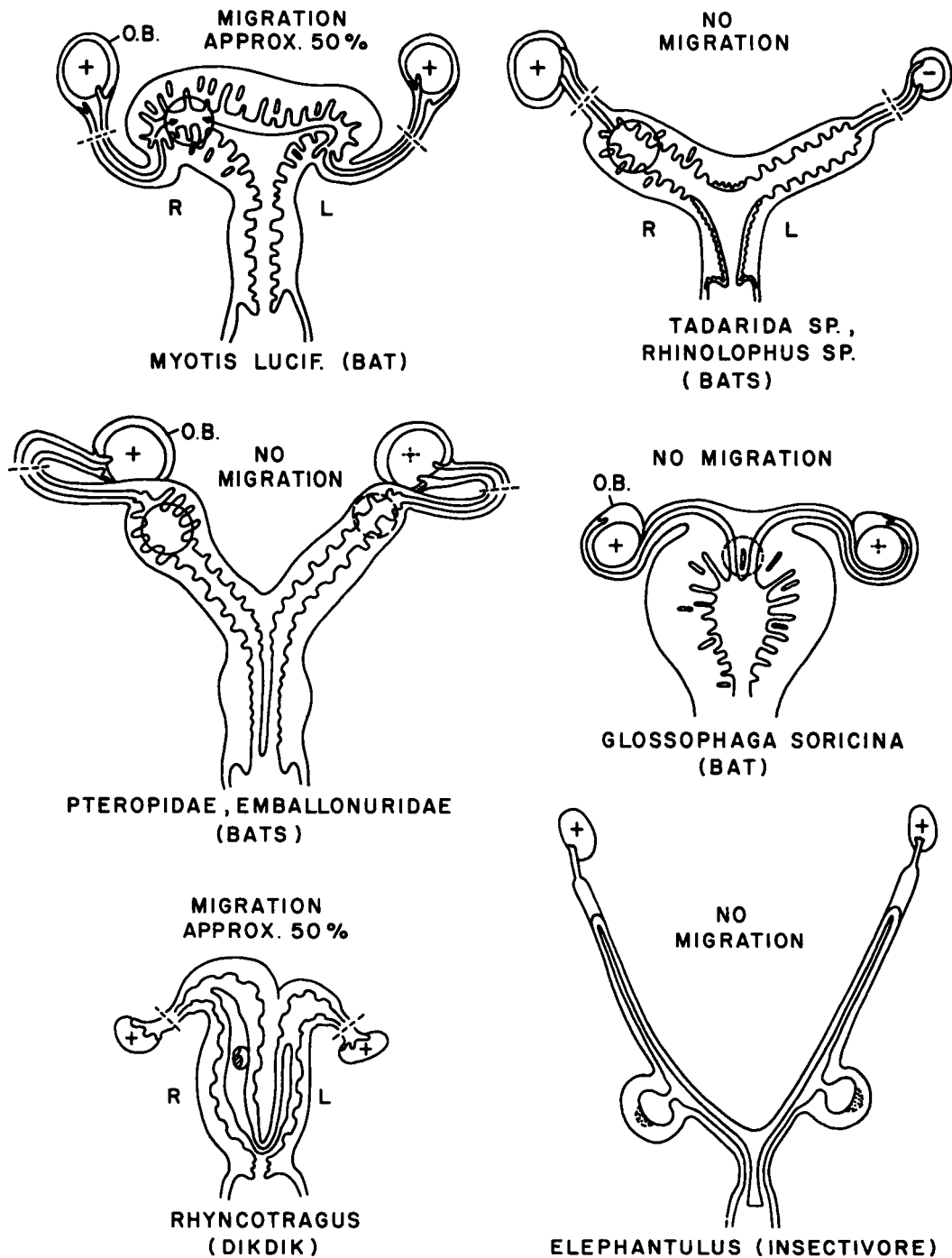


FIG. 11. Some examples of specialized implantation sites in mammals. Solid circles within the diagrams indicate the site of implantation. Dashed circles indicate the site of implantation in a succeeding pregnancy in those cases where right and left ovaries ovulate alternately. The plus and minus signs (+, -) on the ovaries indicate their competence for ovulation; a dashed "plus" sign indicates alternating ovulation between ovaries. The right side lies to the left of the figure in all cases.



localized implantation is provided by the mountain viscacha (*Lagidium peruanum*) studied by Pearson (1949). The species is monovular, and while the uterine horns are quite long, implantation invariably occurs mesometrially just a short distance above the cornual junction (fig. 12): this suggests the presence of a physiologically specialized receptive site for implantation low in the horn. Pearson apparently did not examine the uterus histologically so whether the endometrium here shows structural modifications as well is unknown. The caruncles of ruminants (and probably the placental "stripes" of pangolins, fig. 12, Heath and Amachree, 1967) are also examples of preformed implantation sites but the ruminant condition is so generally familiar that it requires no special comment.

*Endometrial refractoriness.* While in most

mammalian species the entire endometrial surface along the uterine axis appears competent to accept an implanting blastocyst (assuming in all cases adequate hormonal preparation of the uterus), it does not follow that this is true in respect to providing support during the later stages of pregnancy. Fetal resorption presumably can occur in any polytocous species if embryos become excessively overcrowded. In a few species, however, a well established gradient of decreasing competence to support later stages of development is apparent, and it may extend in either direction along the horn in different species. In the pipistrelle bat, implantation normally occurs at two sites, proximally and distally in each horn. The distal pair of embryos survives, whereas the proximal pair (nearer the corpus) is resorbed (fig. 1). An-

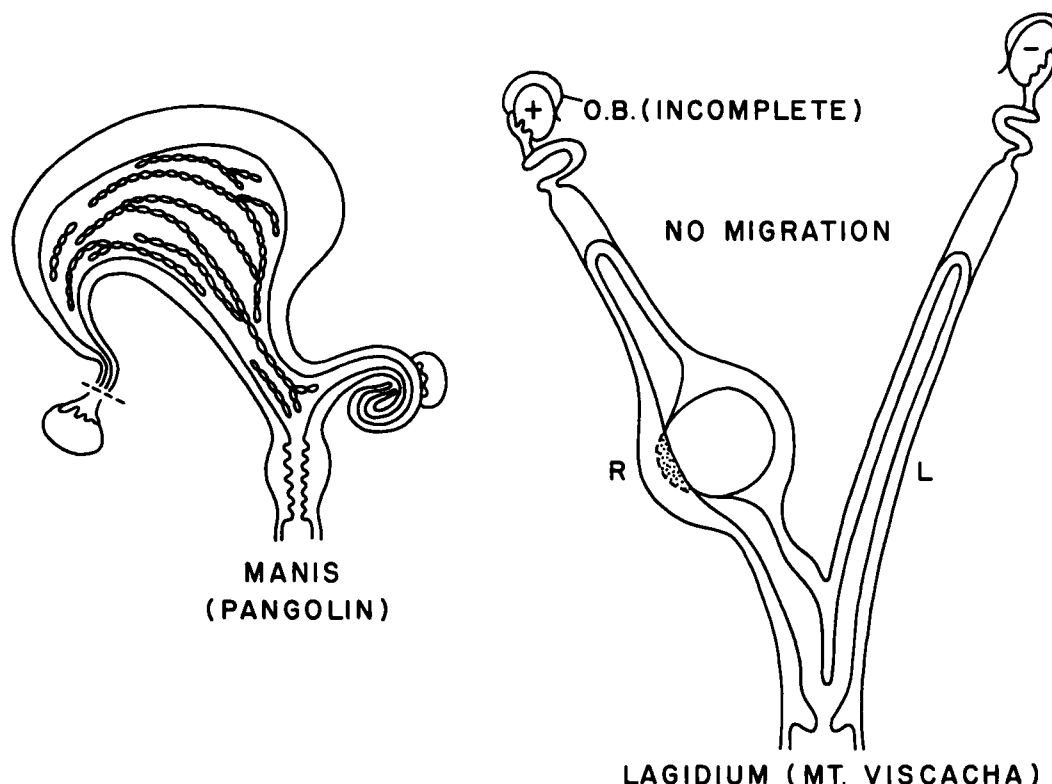


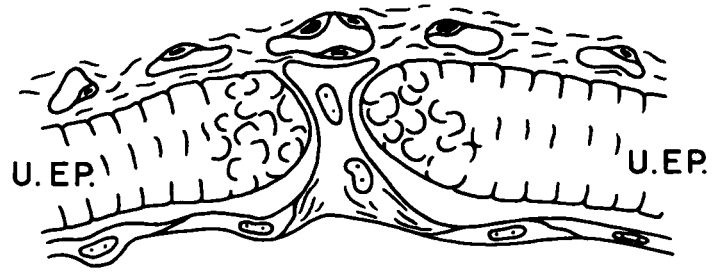
FIG. 12. Specialized implantation sites in the pangolin and the mountain viscacha. The beaded strips on the uterine wall in the pangolin indicate the location of the placental "stripes" of this species. The right horn lies to the left side of the figures. Note that the left ovary is non-functional in the mountain viscacha (compare with plains viscacha in fig. 11).

other example, and one in which the gradient extends in the opposite direction is provided by the plains viscacha (*Lagostomus maximus*). In this superovulating species seven or eight blastocysts implant, in both horns, but all except one in each horn are resorbed by day 90 of the 155 day long gestation period (fig. 1). Resorption always involves the embryos situated distally in the horns, the viable embryos being retained just above the cornual junction (Weir, 1971).

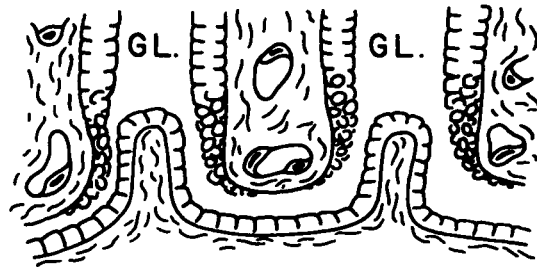
Another aspect of endometrial refractoriness involves the question of whether or not repeated pregnancies can occur at the same uterine site. Obviously this does occur in those species such as the elephant shrew and glossophagine bats in which the implantation sites are highly localized. In tree shrews the bilateral implantation cushions are elongated areas, so successive implantations could occur at different points along their surface. In the bat *Myotis lucifugus* (Fig. 11) implantation always occurs at the same antimesometrial location near the middle of the right horn. The species is monovular, ovulation occurs from either ovary, and the horns are short. When ovulation occurs in the left ovary the ovum migrates to the right horn to implant (Wimsatt, 1944, 1945). In this bat, however, a full year elapses between pregnancies which permits adequate time for site restoration. It is in species such as smaller rodents with rapidly recurring pregnancy cycles that the problem of endometrial refractoriness becomes more acute. It is well known in the case of microtines, rats, mice, hamsters, etc. that the previous pregnancy sites are marked for a time by accumulations of pigmented cells, the so-called "placental scars". Normally implantations for a successive pregnancy occur before these scars disappear, and in hamsters and microtines at least the new implantation sites are generally situated between the former sites (Orsini, 1962; Droogleever Fortuyn, 1929; K. Martin, pers. comm.). This suggests that so long as the scars are present these segments of the endometrium remain relatively refractory to new implantations. That they are not necessarily

completely so, however, is indicated by the observations of Droogleever Fortuyn on prenatal death in the striped hamster. In the animals studied prenatal losses approximated 38 percent, practically all of them occurring before day 16 of pregnancy. While not all losses were attributable to an unfavorable location of the egg chambers in relation to placental scars, in most cases where death had occurred at very early stages implantation had taken place above or very close to the placental scar tissue. In these cases he states "... the germ-vesicle moves toward the periphery of the decidua and ... this movement is in all cases away from the group of brown cells." Embryonic death in these instances was attributed to the displacement of the vesicle from a central position in its decidua.

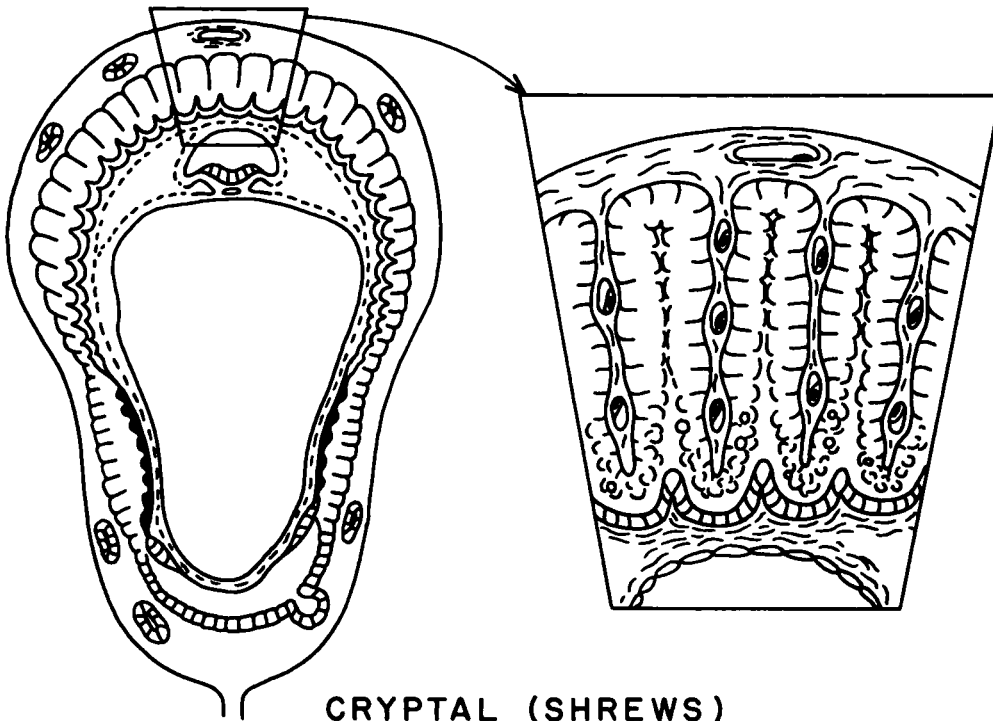
*Endometrial targets of the trophoblast.* In deciduate mammals the initial "adhesive" phase of implantation is rapidly followed by the "penetration" phase in which the attached parts of the trophoblast begin an active invasion of the endometrium (Enders, 1972). The endometrial components (i.e. "targets") that elicit this trophoblastic response, and in some cases subsequently "direct" (in a topographic sense) the pattern of trophoblast growth, clearly differ in various groups of mammals; so too probably do the physiological mechanisms involved. Three examples will be presented merely to illustrate the sort of diversity that is encountered. In some mammals (bat, Wimsatt, 1944; rabbit, Böving, 1962) localized areas of vascular congestion develop in the subepithelial tissues at the presumptive implantation site (either before or after arrival of the blastocyst), and are presumed to be important in influencing at least the initial pattern of trophoblastic invasion. Böving has shown in the rabbit that the points of origin of invasive trophoblastic sprouts correspond in a striking way to the distribution of subepithelial capillaries such that the invasive sprouts are aimed at individual vessels, and penetrate between the surface epithelial cells to reach them (fig. 13). The extent to which the vascular architecture



VASCULAR (RABBITS)



GLANDULAR (CARNIVORES, TENRECS)



CRYPTAL (SHREWS)

FIG. 13. "Targets" of trophoblastic invasion of the endometrium. Description in text.

specifically directs later growth patterns of the trophoblast in the rabbit is unknown, but the pattern of intravascular trophoblast invasion observed by Enders (1960) in the armadillo suggests that it may be important in this species.

Epithelial targets of two sorts are characteristic of other mammalian groups. Among carnivores and some insectivores (e.g. tenrecs, Bluntchli, 1938) the uterine glands provide the primary invasion route (fig. 13). Primary trophoblastic villi are formed in those parts of the chorion which overlie the mouths of the glands and as they elongate they penetrate the gland ducts. This is accompanied by symplasmic degeneration of the contiguous glandular epithelium. The later massive formation of invasive trophoblastic syncytium and destruction of the interglandular stromal tissues is a distinctly secondary phenomenon (Wimsatt, 1974). In shrews of the genera *Blarina* and *Sorex* a much more elaborate epithelial target is provided for trophoblast penetration (Brambell and Perry, 1945; Wimsatt and Wislocki, 1947). It takes the form of elongated cylindrical crypts which arise as special proliferations of the surface epithelium previous to trophoblastic attachment (fig. 13). Initial attachment in shrews is lateral and circumferential, but the allantoic placenta develops later at the antimesometrial side of the implantation chamber. Epithelial crypt formation begins in the circumferential field of primary attachment, but rapidly extends to the antimesometrial side. Degeneration and rearrangement of cells within the antimesometrial crypts gradually opens a pathway for the penetration of trophoblastic villi into the crypts.

In neither carnivores nor shrews is anything known concerning the physiological interactions involved in the "selection" of these epithelial pathways by the primary trophoblastic villi. It is clear, however, that they somehow exert a localized influence on the trophoblast which induces the formation of villi at the appropriate spots.

### *Reproductive asymmetry and unilateral implantation*

In the preceding sections several examples have been cited of mammals that display some degree of asymmetry in their reproductive functions including implantation phenomena. It appears useful at this time to put them together in a single context so that the various patterns may be more easily visualized. This has been attempted in Table 4, which while it probably does not include all known cases of asymmetric function in mammals, certainly lists the principal species for which reliable data are presently available. The first seven species listed do not display reproductive asymmetries; they belong to taxonomic groupings (Chiroptera, Ungulata), however, in which some members do display asymmetry and are included for comparison. It is noteworthy that of the nineteen remaining species showing some degree of asymmetry, ten are bats. The Chiroptera as a group seem to show a higher incidence of reproductive asymmetry, and carry it farther, than any other order of mammals. It should also be noted that all nineteen of the species listed are monovular.

Asymmetry is expressed as a unilateral dominance of the ovary, the uterus or both. The simplest pattern is essentially functional, and involves a non-random alternation of ovulations between left and right ovaries in successive cycles. In the giant fruit bats (*Pteropus* and *Rousettus*) this is accompanied by a localized endometrial reaction at the distal end of the uterine horn on the side of the ovulating ovary, and it is here that implantation subsequently takes place (fig. 11). In the bat *Glossophaga*, in which the uterus is simplex, a differential stimulation of the oviduct occurs on the ovulating side, and implantation ensues in the intraendometrial segment of this same oviduct (fig. 8). In all the remaining species on the list, except the brush possum, functional asymmetry is combined with a permanent differential enlarge-

Table 4  
Some Examples of Functional Reproductive Asymmetry and Unilateral Implantation

Condition	Remarks	Authority
<b>1. Cornua equal</b>		
<b>a. Ovaries equal</b>		
<i>Myotis austroriparius</i> (bat)	2 young, in opposite horns	Sherman, 1937
<i>Eptesicus fuscus</i> (bat)	2 young, in opposite horns	Wimsatt, 1945
<i>Pipistrellus subflavus</i> (bat)	2 young, distally in opposite horns	Wimsatt, 1945
<i>Pipistrellus pipistrellus</i> (bat)	1 young, either horn (England); 2 usual in Europe	Deanesley & Warwick, 1939
<i>Antilocapra americana</i> (pronghorn)	2 young, medial ends of opposite horns	O'gara, 1969
cow, ewe	1 young, either horn; if 2 young, opposite horns	Kelmers et al, 1973; Scanlon, 1971
<b>b. Ovaries equal but alternate</b>		
<i>Pteropus giganteus</i> (bat)	1 young, in horn of ovulating side	Marshall, 1948, 1949, 1953
<i>Rousettus leschenaulti</i> (bat)	2 pregnancies in quick succession (1 young), in opposite horns	Gopalakrishna & Karim, 1971
<i>Desmodus rotundus</i> (bat)	1 young, probably in either horn	Quintero & Rawweller, 1972
<i>Glossophaga soricina</i> (bat) - uterus simplex	1 young, ovulation spontaneous, cyclic, alternate	Rawweller, 1972
<i>Betongia cuniculus</i> (rat kangaroo)	uteri probably have equal potentialities	Flynn, (Pearson 1949)
<b>2. Right cornu dominant</b>		
<b>a. Ovaries equal</b>		
<i>Myotis lucifugus</i> (bat)	1 young, in right cornu nearly 100%	Wimsatt, 1944, 1945
<i>Adenota kob</i> (antelope)	1 young, one ovum released from either ovary	Buechner et al, 1966
<i>Sylvicapra grimmia</i> (antelope)	1 young; one ovum released from either ovary	Child & Hosman, 1965
<i>Rhinoceros kirkii</i> (dikdik)	1 young; one ovum released from either ovary	Symington & Paterson, 1970
<i>Aepyceros melampus</i> (impala)	1 young; one ovum released from either ovary	Kellas, 1955
<i>Muntiacus muntjac</i> (deer)	1 young; one ovum released from either ovary	Hosman & Hosman, 1962
<b>b. Right ovary dominant</b>		
<i>Miniopterus schr.</i> (bat)	1 young; in right horn	Courrier, 1927
<i>Rhinolophus sp.</i> (bats)	1 young; ovulation from right ovary 100%	Matthews, 1937
<i>Tadarida cynocephala</i> (bat)	1 young; ovulation from right ovary 100%	Sherman, 1937; Stephens, 1962
<i>Molossus ater</i> (bat)	1 young; ovulation from right ovary 100%	Wimsatt, (unpublished)
<i>Lagidium peruanum</i> (mt. viscacha)	1 young; ovulation from right ovary 100%	Pearson, 1949
<b>c. Left ovary dominant</b>		
<i>Miniopterus natalensis</i> (bat)	8 cases, all preg. in right horn; all ovulations from left ovary	Matthews, 1941
<i>Kobus defassa</i> (waterbuck)	92% preg. in right horn; left ovary more active than right (approx. 70%)	Spinage, 1969
<b>3. Left cornu dominant</b>		
<i>Trichosurus vulpecula</i> (brush possum)	monovular, from either ovary; left uterus heavier, with greater hyperplastic potential than right.	Von der Borch, 1963.

ment of the right horn of the uterus; in the brush possum the left horn is dominant. In the bat *Myotis lucifugus* and all of the antelopes shown, the ovum may derive from either ovary, but implantation occurs in the larger right horn. Left horn pregnancies have been reported among some of the antelopes listed, but the incidence is very low, and in the bat *Myotis lucifugus* it has been observed only twice among more than 2000 pregnant females examined over a period of years (personal observations). Note that another bat (*M. austroriparius*) belonging to the same genus is included among the initial group on the list which display no reproductive asymmetry whatever.

The next group down the list (4 bats and 1 rodent) is of special interest because in these all functions are restricted to the right side, i.e. ovulation occurs only from the right ovary and implantation occurs only in the right horn. The four bat species represent three separate families of Chiroptera; Vespertilionidae (*Miniopterus*), Rhinolophidae (*Rhi-*

*nolophus sp.*) and Molossidae (*Tadarida* and *Molossus*). All members of the last two families, as far as known, display complete dextral dominance in their reproductive functions (fig. 11), whereas among vespertilionid bats many "symmetric" species are known. The mountain viscacha, the only rodent on the list, normally displays complete dextral asymmetry, but if the right ovary is removed the left becomes functional and implantation then occurs in the left horn (Pearson, 1949).

Some of the smaller species listed if adaptable to laboratory regimes would presumably make excellent models for studies of local humoral interactions between ovary and uterus, although the seasonally restricted nature of their reproductive cycles might cause some difficulties. Local utero-ovarian relationships have most recently been reviewed by Ginther (1967).

#### Delayed implantation

That embryonic development in a mammal can be arrested or slowed down for a period

of time thereby prolonging gestation has been known since the middle of the last century when Ziegler (1843) and Bischoff (1854) first described the phenomenon in the roe deer. A few decades later Fries (1880) demonstrated the occurrence of embryonic diapause in the European badger, and Lataste (1891) postulated the possible occurrence of embryonic delay during lactation in several species of small rodents, including the rat and the mouse, but discovery of a quiescent phase in the development of the armadillo blastocyst was not made until the earlier years of the present century (Patterson, 1913; Hamlett, 1929). However, our current awareness of the widespread occurrence of delayed implantation in mammals, of its variable patterns, and of the multitude of influences involved in its regulation is of recent origin, mostly gained within the last twenty years. Thus far the subject has inspired one major conference (Enders, 1963), a number of shorter reviews (e.g. Daniel, 1970) and an excellent analysis by Sadleir (1969) of bio-ecological parameters affecting delay patterns in wild-living species. The readily inducible facultative delay of implantation associated with lactational stress in a few laboratory rodents has been extensively used in recent years as a model for analytical studies of endocrine and other factors involved in blastocyst development, and of the interactions between blastocyst and uterus at implantation. This work has been frequently reviewed (e.g. Psychoyos, 1973) and will not be covered here; rather, emphasis will be placed on patterns of delayed implantation as they are encountered in mammals outside the laboratory in the natural environment, and on some of the regulatory mechanisms thought to be involved.

*Perspectives.* The terms "delayed implantation" and "embryonic diapause" have sometimes been used interchangeably by authors to designate the same general phenomenon, but neither of them when used in such a general context necessarily characterizes the conditions which may prevail in individual cases of retarded development in mammals.

The first term for example fails to cover those situations where the blastocyst implants on schedule, but in which its development then slows down for a period of time. Such a condition is definitely known in two species of bats, and probably occurs in a third. Bradshaw (1962) referred to it as "delayed development", but "retarded development" more accurately describes the condition. The term "embryonic diapause" is suitable in cases where there is an actual arrest of embryonic development at some stage (as in marsupials, Sharman and Burger, 1969), but it is misleading if applied to species like the roe deer and badger in which blastocyst development slows down but never actually ceases (Short and Hay, 1966; Canivenc, 1966a): "delayed implantation" is obviously the better term to use in these instances. Moreover the term "delayed implantation" conveys a temporal connotation, whereas "embryonic diapause" does not.

Leaving these ambiguities aside, two major types of delayed implantation have long been recognized. Formerly designated "spontaneous" and "induced" delayed implantation respectively, they are now more often called "obligate" and "facultative" delayed implantation. Obligate delay is the more familiar type observed in most higher mammals in which a quiescent period of the blastocyst prior to implantation is a normal, genetically fixed component of *every* pregnancy cycle. By this definition even the lactational delay of many Australian marsupials (Sharman et al, 1966; Sharman and Burger, 1969; Tyndale-Biscoe, 1973), while displaying some facultative components, is of the obligate type. Facultative delay defines the situation in which a quiescent period of the blastocyst may occur under certain stressful conditions, but is not a usual accompaniment of every pregnancy cycle. It is exemplified by the familiar "lactational delay" of some rodents which, after experiencing a post-partum estrus and fertilization, delay implantation of the blastocysts if subjected to an excessive suckling stimulus.

In a broader biological context delayed implantation must also be viewed in a temporal perspective. Sadleir (1969) has proposed a series of useful definitions for this purpose. He distinguishes between "seasonal" and "aseasonal" delayed implantation, and in respect to the first, he further delineates "synchronous" and "asynchronous" types. In synchronous seasonal delay, mating, implantation and birth occur at fixed times during the year, although the times may differ as between species. Many of the obligate delay species shown in Table 5 demonstrate this pattern. In asynchronous seasonal delay mating and/or births may occur over longer periods within a fixed breeding season, but implantation does not occur at a fixed time of the year. An example is the facultative delay that probably occurs in some seasonally breeding wild rodent allies of laboratory rats and mice in response to lactational stress; Sadleir (1969) cites one known case involving the red tree mouse *Phenacomys*, which breeds repeatedly from spring to autumn but in which gestation length can vary between 28 and 42 days. Asynchronous seasonal delay is also demonstrated by a few seasonally breeding Australian marsupials such as the quokka (Main et al, 1959; cited by Sadleir, 1969) in which, if the joey is lost (or removed) from the pouch, implantation of the diapausing blastocyst present at this time in the uterus occurs sooner than it otherwise would.

Aseasonal delayed implantation refers to those situations in which a species breeds continuously so that the time of implantation is not fixed in reference to the time of year. The sea otter, for example, delivers pups at any time of year, but the high proportion of apparently diapausing blastocysts found in uteri suggests that an aseasonal delay pattern exists (Sinha, Conaway and Kenyon, 1966; cited by Sadleir, 1969). Aseasonal delayed implantation of a slightly different type may also occur in some macropod marsupials which have continuous breeding cycles (Sadleir, 1969). In response to loss of pouch young (as a result of nutritional stress brought on by

extended drought periods or other causes) implantation of the delayed blastocyst present in the uterus occurs ahead of time and independently of season.

*Patterns of obligate delayed implantation.*

Some representative patterns of pregnancy with obligate delayed implantation in species from several orders of higher mammals are depicted in Table 5. Included also are the special cases of so-called "delayed development" reported recently in two phyllostomid bats (*Macrotus* and *Artibeus*), and the pregnancy cycle of a natalid bat (*Natalus*) in which a similar condition also probably obtains. While the Table is self-explanatory, the form of notation and two of the patterns shown require brief comment. In several instances the horizontal bars depicting the major events of the seasonal cycle are double rather than single. This was done in order to show the temporal overlap in major phases of the cycle that has been reported to occur in the species concerned. In the Fisher *Martes pennanti*, for example, the cycle is a full year long and the known periods of conception and birth overlap to an appreciable degree.

However the double bars depicting the pregnancy cycles of the mink *Mustela vison* and the fruit bat *Artibeus jamaicensis* reflect specific peculiarities of the yearly cycles in these two species. In the mink the duration of the delay period varies according to the time at which copulation occurs. Copulations in early March (upper bar) are associated with a longer delay period of the blastocysts, while copulations in late March (lower bar) lead to a shortening of the delay period (Pearson and Enders, 1944; Dukelow, 1966). The situation in the bat *Artibeus* is if anything even more interesting. As reported by Fleming (1971) this species in Panama is seasonally polyestrous and young are born twice yearly, in March or April and in July or August. Blastocysts conceived after the summer parturition implant in the uterus but then become essentially dormant from September to mid-November, when a normal rate of development again ensues (upper bar). On the other





opment of the egg proceeds to the free blastocyst stage, at which point it slows down appreciably until late December and early January, when preimplantation elongation of the germ abruptly begins. Blastocyst reactivation is preceded (beginning in October) by an increasing hypertrophy of the uterine glands and a build up of secretion products. The sudden release of these in January coincides with elongation and implantation of the blastocyst. Aitken (1974) has analyzed the secretion and found it to contain uterine-specific and serum proteins, 23 different amino acids, protein-bound glucose and galactose, and possibly fructose, all of which he regards as being essential for normal blastocyst growth. Their absence during the delay period was held to be responsible for the developmental slow-down of the egg. The reactivation of the blastocyst in January was also correlated with a rise in plasma estrogen levels, which probably conditions the release of the growth-promoting gland secretions. Aitken noted, however, that the ovaries appeared active during the delay period and displayed no appreciable histological changes at the time of blastocyst elongation, which may imply the existence of an alternative source of the plasma estrogens at this time.

The cycle in the European badger *Meles meles* begins with a post-partum estrus in early February. Development to the free blastocyst stage proceeds normally, but as in the roe deer it abruptly slows down, and implantation is deferred until early December (Canivenc, 1966a, 1966b). The corpora lutea appear less active during the delay period, but implantation in December is associated with an obvious resumption of luteal activity (histological criteria). Injections of gonadotrophins and progesterone during delay failed to activate the corpora lutea or induce implantation however. Canivenc (1966a) hypothesized that whereas FSH and LH functions are presumably normal at the time of post-partum estrus and initial stages of corpus luteum formation, LTH levels were insufficient to carry their functional development farther, possibly due to

“exhaustion” resulting from the former pregnancy, but they are somehow restored in autumn and implantation ensues. We will return to the badger again a little later on.

The endocrine events involved in delayed implantation in the armadillo *Dasypus novemcinctus* present another enigma. This species breeds in July and August, but the free blastocyst remains dormant until late November, when implantation occurs. Enders and Buchanan (1959) and Enders (1966) found that bilateral ovariectomy during the delay period brought about precocious implantation of the delayed blastocysts within 18–24 days, but removal of a single ovary, whether or not it contained the single corpus luteum of pregnancy, did not interrupt diapause. Progesterone administered in smaller doses (10 mg/day) after bilateral ovariectomy did not reverse the effects of the operation, but larger doses (25 mg/day) inhibited implantation and caused loss of the blastocyst in some cases, as did 0.2 mg/day injections of estrogen. Histologically the corpus luteum appeared fully developed during the delay period and showed no striking changes at or following implantation. Labhsetwar and Enders (1969) subsequently showed that during the delay period the pituitary gland contained more LH than did the pituitaries of non-ovulated animals, and bilateral ovariectomy during delay resulted in a depletion of pituitary LH within 17 days. The authors also noted a progressive decline of pituitary LH following implantation.

It is possible of course that the endocrinological regulation of delayed implantation in the roe deer, badger and armadillo may involve synergisms between ovarian factors and other hormones that are not operative in the rat and mouse.

Little direct work has been done to date concerning the effects of nutritional factors on delayed implantation, but there is indirect evidence which suggests they may be important in at least a few species. Shapira et al (1974) recently demonstrated that implantation in lactating rats can be postponed at least until day 18 (post-fertilization) simply by

restricting food intake, and this was usually accompanied by a lower delivery rate. They concluded (as did Lataste, 1891) that the cause of delay in implantation was a decline in available energy supplies owing to excessive lactational stress on the mother. As pointed out by Sadleir (1969) nutritional effects on available energy levels could also be involved with delayed implantation in marsupials. In these, delay is directly related to lactation in that the blastocyst does not implant until lactation is terminated, either naturally or by experimental contrivance. However, experimental manipulation of dietary factors in marsupials to assess their capacity to alter the delay period has apparently not been attempted as yet.

The more or less precise timing of implantation in those species showing synchronous seasonal patterns of delay suggests that photoperiodic responses may be involved in their regulation. Experimental demonstration of photoperiodic effects has been attempted in only a few species, the marten, mink, spotted skunk, European badger and roe deer. Photoperiodic influences were demonstrable in all except the roe deer. Pearson and Enders (1944) exposed captive martens to increasing day length commencing in early fall, about a month after mating. This led to a shortening of the delay period by three or four months, the kits being born in winter rather than at the usual time in spring. The same authors exposed ranch mink to an increased day length of 1½ hours commencing in early February and mated the animals in late February and early March; a gestation period of just over 49 days resulted. Other females not exposed to increased day length before mating on the same dates, but subsequently exposed to increased photoperiod after mating, had gestation lengths of just over 50 days. Control animals which had no photoperiodic increase had gestation lengths of just under 55 days. Both treatments therefore shortened the delay period by approximately three days. Others have carried out similar experiments on mink and have obtained corresponding results (cf. Sadleir, 1969).

In the western spotted skunk (*Spilogale putorius*), but not the eastern species apparently, pregnancy is characterized by a relatively long period of preimplantational delay. Mead (1971) has shown that exposing intact pregnant females to a day length of 14 hours during the delay period hastened implantation so that it occurred at 169 days post-fertilization as compared with 218 days in the intact unlit controls. In blinded animals exposed to the same photoperiodic regime the delay period was prolonged; in one instance a viable unimplanted blastocyst was recovered after 316 days. Blinding also prevented the preimplantation rise in progesterone levels normally observed in intact controls. Mead concluded that photoperiod is responsible for the timing of implantation (i.e. termination of delay), but is probably not important in initiating delay or the luteal involution which is characteristic of the delay period.

The picture in the European badger is still clouded, but recent experiments by Canivenc et al (1971) suggest that photoperiod may be involved along with temperature in the regulation of delayed implantation in this species. It should be emphasized that in the badger, unlike the other species just considered, the delay period ends and implantation occurs in December, at a time when natural day length is still declining rather than increasing. Previous attempts to shorten the delay period by various hormonal manipulations were unsuccessful as mentioned earlier. Six pregnant females were placed in a climatic chamber in early May and subjected to a steadily declining photoperiod of 16 → 8 hours of light for one month and then a reversal from 8 → 16 hours of light for another month. During the second month the ambient temperature was also reduced by 10° (whether centigrade or Fahrenheit the authors did not state). Luteal biopsies at the end of six weeks indicated a reactivation of the corpora lutea, but implantation had not yet occurred. By the eighth week, however, implantation had been affected in four of the animals, some six months ahead of the normal time. This experiment did not of course distinguish between pho-

toperiodic and temperature influences in hastening implantation, but it does emphasize the importance of extrinsic factors in the regulation of seasonal delay patterns.

In contrast to the foregoing species, the roe deer may not respond to increased or decreased photoperiod by shortening the period of delay, but the available evidence derives from experimentation on a single animal. Lincoln and Guinness (1972) bred a female in early August, the normal breeding period. Beginning in mid-October the day length was artificially shortened to 6¾ hours until October 22 (this was the normal day length at the time of the winter solstice in December). Beginning October 22 the day length was gradually increased for 45 days until December 5. Thereafter the animal was exposed to normal daylight conditions for the balance of pregnancy. Birth occurred on May 12, only a few days before the normal kidding time, so the delay period was not significantly reduced by the experimental procedure. It is significant, however, that the moulting cycle was definitely accelerated, indicating the responsiveness of this parameter at least to photoperiod manipulation.

To the extent that photoperiod is involved in regulation of delayed implantation (or retarded development) patterns it is evident that not all species respond to it in the same way. In the European badger and most mustelids initiation of diapause occurs during a period of increasing daylength in the spring, while in the spotted skunk, roe deer and the bats *Artibeus* and *Miniopterus* it coincides with periods of decreasing daylength in the fall (Table 5). However, with the exception of the European badger and *Artibeus*, termination of diapause and implantation occur under conditions of increasing daylength in all species described to date. It seems obvious that other environmental factors besides/or in addition to photoperiod must be involved in the regulation of delay cycles in some species. Two parameters that have been implicated in a few of them are seasonal rainfall and temperature rhythms. The following examples are representative.

The pregnancy cycle of the African fruit bat *Eidolon helvum* (suborder Megachiroptera: fam. Pteropidae), living in Uganda almost precisely on the equator (0° 20' N), is characterized by a three-month period of delayed implantation. The species is monestrous and breeding is seasonal, but light and dark periods scarcely vary throughout the year (Mutere, 1967). Mating occurs from April to June, implantation is deferred to October or November, and parturition takes place in February and March. Breeding coincides with a higher rainfall peak in late spring, and implantation with a somewhat smaller peak in the fall; the period of delay is coextensive with a very dry period between rainfall peaks in summer and early fall. The availability of food fruits is maximized during the rainy seasons, so that post-implantation phase of pregnancy and the weaning of the young takes place when adequate energy sources are available. The same species was studied somewhat further north (7° 24' N) and at higher altitudes by Fayenuwo and Halstead (1974). Their observations differ from those of Mutere (1967) only in that the reproductive sequence was retarded by a month, and that implantation occurred at the beginning of the winter dry period, and births at the beginning of the spring wet season. In the American fruit bat *Artibeus* (suborder Microchiroptera: fam. Phyllostomatidae) the delay period of the blastocyst during the fall also assures that the young will not be born at an energetically less favorable time of year, for birth is deferred until the rains have begun in spring (Fleming, 1971). In the above instances rainfall patterns may constitute a proximate stimulus for timing of reproductive events, but the entrainment physiology involved is wholly unknown.

A final example, one in which ambient temperature has been implicated indirectly at least in the regulation of a delay cycle, is the bat *Miniopterus schreibersii* (fam. Vespertilionidae). In this species in Europe ( $\pm 45^\circ$  N lat.) the delay period, which extends from October to March, coincides almost exactly with the 5-month period of winter hibernation

(Peyre and Herlant, 1967). Implantation of the dormant blastocyst is only initiated after the bats arouse from hibernation in spring. Ambient temperatures in hibernacula range from 5°–8°C, and the body temperatures of the dormant bats would average no more than a degree or two higher than this; thus body metabolism is enormously depressed during hibernation. The same species also lives in New South Wales, Australia. At this latitude (30° S) the length of the hibernation period is appreciably shorter (3.5 mos. ±), and the period of blastocyst diapause is reduced correspondingly (see Wimsatt, 1969). Thus, it appears that in *Miniopterus* implantation delay is related directly to body temperature and/or metabolism levels, which in turn are dependent upon the ambient temperature. Whether developmental delay in this species is a purely passive response to cold and/or depressed metabolism, or, alternatively, involves an active regulatory influence for its initiation at least is unknown, but there are reasons for believing that the latter is more likely.

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#### BIBLIOGRAPHY

- AITKEN, R. J. (1974) Delayed implantation in roe deer (*Capreolus capreolus*). *J. Reprod. Fertil.* **39**, 225–233.
- AITKEN, R. J., BURTON, J. H. W., AND STEVEN, D. H. (1971) Ultrastructural changes in the uterine glands of the Roe deer during delayed implantation. *J. Physiol.* (London) **217**, 13P–15P.
- AITKEN, R. J., BURTON, J., HAWKINS, J., KERR-WILSON, R., SHORT, R. V. AND STEVEN, D. H. (1973) Histological and ultrastructural changes in the blastocyst and reproductive tract of the Roe deer, *Capreolus capreolus*, during delayed implantation. *J. Reprod. Fertil.* **34**, 481–493.
- ALDEN, R. H. (1945) Implantation of the rat egg. I. Experimental alteration of uterine polarity. *J. Exp. Zool.* **100**, 229–235.
- AMOROSO, E. C. (1952) Placentation. In "Marshall's Physiology of Reproduction," vol. 2, (A. S. Parkes, ed.), pp. 127–311, Longmans, Green, London.
- ANDERSON, D. (1927) The rate of passage of the mammalian ovum through various portions of the fallopian tube. *Am. J. Physiol.* **82**, 557–569.
- BISCHOFF, T. L. W. (1854) Entwicklungsgeschichte des Rehes. Giessen.
- BLANDAU, R. J. (1949a) Observations on implantation of the guinea pig ovum. *Anat. Rec.* **103**, 19–48.
- BLANDAU, R. J. (1949b) Embryo-endometrial interrelationship in the rat and guinea pig. *Anat. Rec.* **104**, 331–360.
- BLUNTSCHLI, H. (1938) Le développement primaire et l'implantation chez un Centétiné (Hemicentetes). *C.R. Assoc. Anat. Paris*, **44**, 39–46.
- BODLEY, H. D. (1974) The development of the chorionic-lantoic placenta in the bat *Macrotus waterhousi*. *Anat. Rec.* **178**, 313.
- DE BONILLA, H. AND RASWEILER, J. J. IV (1974) Breeding activity, preimplantation development, and oviduct histology of the short-tailed fruit bat, *Carollia*, in captivity. *Anat. Rec.* **179**, 385–403.
- BOSHIER, D. P. (1969) A histological and histochemical examination of implantation and early placentome formation in sheep. *J. Reprod. Fertil.* **19**, 51–61.
- BÖVING, B. G. (1954) Blastocyst-uterine relationships. Cold Spring Harbor Sympos. *Quant. Biol.* **19**, 9–28.
- BÖVING, B. G. (1959) Implantation. *Ann. N.Y. Acad. Sci.* **74**, 700–725.
- BÖVING, B. (1963) Implantation mechanisms. In "Conference on Physiological Mechanisms Concerned with Conception," (C. G. Hartman, ed.), pp. 321–396, Pergamon Press, London.
- BÖVING, B. G. (1971) Biomechanics of implantation. In "The Biology of the Blastocyst," (R. J. Blandau, ed.), pp. 423–442, Univ. Chicago Press, Chicago.
- BÖVING, B. G. (1972) Spacing and orientation of blastocysts in utero. In "Biology of Mammalian Fertilization and Implantation," (K. S. Moghissi and E. S. E. Hafez, eds.), p. 357–378, C. C. Thomas, Springfield, Illinois.
- BÖVING, B. G., AND LARSEN, J. F. (1973) Implantation. In "Human Reproduction," (E. S. E. Hafez and T. N. Evans, eds.), pp. 133–156, Harper and Rowe, Hagerstown, Md.
- BRADSHAW, G. V. R. (1962) Reproductive cycle of the California leaf-nosed bat, *Macrotus californicus*. *Science* **136**, 645–646.
- BRAMBELL, F. W. R. AND PERRY, J. S. (1945) The development of the embryonic membranes of the shrews, *Sorex araneus* and *Sorex minutus*. *Proc. Zool. Soc. Lond.* **115**, 251–278.
- BUECHNER, H. K., MORRISON, J. A. AND LEUTHOLD, W. (1966) Reproduction in Uganda kob with special reference to behavior. In "Comparative Biology of Reproduction in Mammals," (I. W. Rowlands, ed.), Symp. Zool. Soc. London no. 15, pp. 69–88, Academic Press, London.
- CANIVENC, R. (1966a) A study of progestation in the

- European badger (*Meles meles* L.). In "Comparative Biology of Reproduction in Mammals," (I. W. Rowlands, ed.) Sympos. Zool. Soc. London, no. 15, pp. 15-26, Academic Press, London.
- CANIVENC, R. (1966b) Progestational activity in the badger (*Meles meles* L.). In "Egg Implantation," Ciba Foundation Study Group no. 23, (G. E. W. Wolstenholme, ed.), pp. 46-55, Little, Brown, Boston.
- CANIVENC, R., BONNIN-LAFARGUE, M. AND LAJUS-BOUÉ, M. (1971) Réalisation expérimentale précoce de l'ovo-implantation chez le Blaireau européen (*Meles meles* L.) pendant la période de latence blastocyttaire. *C. R. Acad. Sci. Paris*. **273**, 1855-1857.
- CHANG, M. C. (1952) Development of bovine blastocyst with a note on implantation. *Anat. Rec.* **113**, 143-162.
- CHANG, M. C. AND ROWSON, L. E. A. (1965) Fertilization and early development of Dorset horn sheep in the spring and summer. *Anat. Rec.* **152**, 303-316.
- CHAPMAN, D. E. AND DANSIE, O. (1969) Unilateral implantation in muntjac deer. *J. Zool. London*, **159**, 534-536.
- CHILD, C. AND MOSSMAN, A. S. (1965) Right horn implantation in the common duiker. *Science*, **149**, 1265-1266.
- CONAWAY, C. H. AND SORENSON, M. W. (1966) Reproduction in tree shrews. In "Comparative Biology of Reproduction in Mammals," (I. W. Rowlands, ed.), Symposium Zoological Society of London no. 15, pp. 471-492, Academic Press, London.
- COURRIER, R. (1927) Etude sur le déterminisme des caractères sexuels secondaires chez quelques mammifères à activité testiculaire périodique. *Arch. Biol.* **37**, 173-334.
- DANIEL, J. C. (1970) Dormant embryos of mammals. *Bioscience* **20**, 411-415.
- DEANESLEY, R. AND WARWICK, T. (1939) Observations on pregnancy in the common bat (*Pipistrellus pipistrellus*). *Proc. Zool. Soc. Lond. A* **109**, 57-60.
- DICKMANN, Z. (1969) Shedding of the zona pellucida. In "Advances in Reproductive Physiology," vol. 4, (A. McLaren, ed.), pp. 187-206, Logos Press, London.
- DROOGLEEVER FORTUYN, A. B. (1929) Prenatal death in the striped hamster (*Cricetulus griseus*, M.-Edw.). *Arch. Biol.* **39**, 583-606.
- DUKELOW, W. R. (1966) Variations in gestation length of mink (*Mustela vison*). *Nature, Lond.* **211**, 211.
- DUTOURNE, B. AND CANIVENC, R. (1971) Dynamique de la migration blastocyttaire chez le Blaireau européen (*Meles meles* L.). *C.R. Acad. Sci. Paris* **273**, 2579-2582.
- ENDERS, A. C. (1960) Development and structure of the villous haemochorial placenta of the nine-banded armadillo (*Dasypus novemcinctus*). *J. Anat.* **94**, 34-44.
- ENDERS, A. C., ed. (1963) "Delayed Implantation." 318 pp., University of Chicago Press, Chicago.
- ENDERS, A. C. (1966) The reproductive cycle of the nine-banded armadillo (*Dasypus novemcinctus*), In "Comparative Biology of Reproduction in Mammals," (I. Rowlands, ed.), Symp. Zool. Soc. Lond. no. 15, pp. 295-310, Academic Press, London & New York.
- ENDERS, A. C. (1971) The fine structure of the blastocyst. In "The Biology of the Blastocyst," (R. J. Blandau, ed.), pp. 71-94, Univ. Chicago Press, Chicago.
- ENDERS, A. C. (1972) Mechanisms of implantation of the blastocyst. In "Biology of Reproduction; Basic and Clinical Studies," (J. Velardo and B. Kaspro, eds.), pp. 313-334, Pan American Congress of Anatomy, New Orleans, La.
- ENDERS, A. C. AND BUCHANAN, G. D. (1959) Some effects of ovariectomy and injection of ovarian hormones in the armadillo. *J. Endocrinol.* **19**, 251-258.
- ENDERS, A. C. AND ENDERS, R. K. (1969) The placenta of the four-eyed opossum (*Philander opossum*). *Anat. Rec.* **165**, 431-450.
- ENDERS, A. C. AND SCHLAFKE, S. (1967) A morphological analysis of the early implantation stages in the rat. *Am. J. Anat.* **120**, 185-225.
- FAYENUWO, J. O. AND HALSTEAD, L. B. (1974) Breeding cycle of straw-colored fruit bat, *Eidolon helvum*, at ILE-IFE, Nigeria. *J. Mammal.* **55**, 453-454.
- FLEMING, T. H. (1971) *Artibeus jamaicensis*: delayed embryonic development in a neotropical bat. *Science* **171**, 402-404.
- FRIES, S. (1880) Über die Fortpflanzung von *Meles taxus*. *Zool. Anz.* **3**, 486-492.
- GARDNER, R. L. (1970) Manipulations on the blastocyst. In "Schering Symposium on Intrinsic and Extrinsic Factors in Early Mammalian Development," (G. Raspé, ed.) *Adv. Biosci.* **6**, 279-296.
- GARDNER, R. L., PAPAIOANNOU, V. E. AND BARTON, S. C. (1973) Origin of the ectoplacental cone and secondary giant cells in mouse blastocysts reconstituted from isolated trophoblast and inner cell mass. *J. Embryol. exp. Morphol.* **30**, 561-572.
- GINTHER, O. J. (1967) Local utero-ovarian relationships. *J. Animal Sci.* **26**, 578-585.
- GOETZ, R. H. (1937) Studien zur Placentation der Centetiden. II. Die Implantation und Frühentwicklung von *Hemicentetes semispinosus* (Cuvier). *Z. Anat. Entwickl.* **107**, 274-318.
- GOETZ, R. H. (1938) On the early development of the Tenrecoidea (*Hemicentetes semispinosus*). *BioMorphosis* **1**, 67-79.
- GOPALAKRISHNA, A. AND KARIM, K. B. (1971) Localized progesterational endometrial reaction in the uterus of the Indian fruit-bat, *Rousettus leschenaulti* (Desmaret). *Curr. Sci.* **40**, 490-491.
- HAJEZ, E. S. E. (1973) Gamete transport. In "Human Reproduction," (E. S. E. Hafez and T. N. Evans, eds.), pp. 85-118, Harper & Rowe, Hagerstown, Md.
- HAMILTON, W. J. (1934) The early stages in the development of the ferret. Fertilization to the formation of the prochordal plate. *Trans. Roy. Soc. Edinburgh.* **58**, 251-278.
- HAMILTON, P. A. (1973) The spacing of the blastocysts in mice—a growth response of the uterus at the time of

- implantation. *J. Anat.* **114**, 309-310.
- HAMILTON, W. J. AND DAY, F. T. (1945) Cleavage stages of the ova of the horse, with notes on ovulation. *J. Anat.* **79**, 127-130.
- HAMILTON, W. J. AND LAING, J. A. (1946) Development of the egg of the cow up to the stage of blastocyst formation. *J. Anat.* **80**, 194-204.
- HAMLETT, G. W. D. (1929) Delayed implantation in the mammals, and its supposed relationship to polyembryony. *Anat. Rec.* **44**, 251.
- HARRISON, R. J. (1963) A comparison of factors involved in delayed implantation in badgers and seals in Great Britain. In "Delayed Implantation," (A. C. Enders, ed.), pp. 99-114, Univ. Chicago Press, Chicago, Illinois.
- HARTMAN, C. G. (1925) Observations on the functional compensatory hypertrophy of the opossum ovary. *Am. J. Anat.* **35**, 1-29.
- HARTMAN, C. G. (1928) The breeding season of the opossum (*Didelphis virginiana*) and the rate of intra-uterine and postnatal development. *J. Morph.* **46**, 143-215.
- HEATH, E. AND AMACHREE, M. (1967) Endotheliochorial placentation in a pangolin, *Manis tetradactyla*. *Acta Zool.* **58**, 309-315.
- HENDRICKX, A. G. ed. (1971) Embryology of the baboon. Univ. Chicago Press., Chicago.
- HENDRICKX, A. G. AND KRAEMER, D. C. (1968) Preimplantation stages of baboon embryos (*Papio sp.*). *Anat. Rec.* **162**, 111-120.
- HERTIG, A. T. AND ROCK, J. (1945) Two human ova of the previllous stage, having a developmental age of about seven and nine days respectively. *Contrib. Embryol., Carnegie Inst. Wash.* **31**, 65-94.
- HERTIG, A. T., ROCK, J., AND ADAMS, E. C. (1956) A description of thirty-four human ova within the first seventeen days of development. *Am. J. Anat.* **98**, 435-494.
- HEUSER, C. H. AND STREETER, G. L. (1941) Development of the macaque embryo. *Contrib. Embryol., Carnegie Inst. Wash.* **29**, 15-55.
- HILL, J. P. (1910) The early development of the Marsupialia, with special reference to the native cat (*Dasyurus viverrinus*). *Quart. J. Microsc. Sci.* **56**, 1-148.
- HOLST, P. J. (1974) The time of entry of ova into the uterus of the ewe. *J. Reprod. Fertil.* **36**, 427-428.
- HOLST, P. A. AND PHEMISTER, R. D. (1971) The prenatal development of the dog: preimplantation events. *Biol. Reprod.* **5**, 194-206.
- VAN DER HORST, C. J. (1942) Early stages in the embryonic development of *Elephantulus*. *S. Afr. J. Med. Sci.* **7**, Biol. Suppl., 55-65.
- VAN DER HORST, C. J. AND GILLMAN, J. (1941) The number of eggs and surviving embryos in *Elephantulus*. *Anat. Rec.*, **80**, 443-452.
- VAN DER HORST, C. J. AND GILLMAN, J. (1942) Preimplantation phenomena in the uterus of *Elephantulus*. *S. Afr. J. Med. Sci.* **7**, 47-71.
- HUGHES, R. L. (1974) Morphological studies on implantation in marsupials. *J. Reprod. Fertil.* **39**, 173-186.
- HUMPHREY, K. W. (1968) Observations on transport of ova in the oviduct of the mouse. *J. Endocrinol.* **40**, 267-273.
- JONKEL, C. J. AND WECKWORTH, R. P. (1963) Sexual maturity and implantation of blastocysts in the wild pine marten. *J. Wildl. Mgmt.* **27**, 93-98.
- KELLAS, L. M. (1954-55) Observations on the reproductive activities, measurements, and growth rate of the Dikdik (*Rhyncotragus kirkii thomasi* Neumann) *Proc. Zool. Soc. Lond.* **124**, 751-784.
- KIRBY, D. R. S. (1971) Blastocyst-uterine relationship before and during implantation. In "The Biology of the Blastocyst," (R. J. Blandau, ed.), pp. 393-412, Univ. Chicago Press, Chicago.
- KIRBY, D. R. S., POTTS, D. M. AND WILSON, I. B. (1967) On the orientation of the implanting blastocyst. *J. Embryol. Exp. Morph.* **17**, 527-532.
- LABHSETWAR, A. P. AND ENDERS, A. C. (1969) Pituitary LH content during delayed and post-implantation periods in the armadillo (*Dasyurus novemcinctus*) *J. Reprod. Fertil.* **18**, 383-389.
- LATASTE, F. (1891) Des variations de durée de la gestation chez les mammifères, et des circonstances qui déterminent ces variations. *Mem. Soc. Biol.* **43**, 21-31.
- LAWS, R. M. (1956) The elephant seal, III The physiology of reproduction. Falkland Is. Depend. Surv. Sci. implantation in the right uterine horn of the common Rep. no. 15, 66 pp.
- LEWIS, W. H. AND HARTMAN, C. G. (1941) Tubal ova of the rhesus monkey. *Carnegie Contrib. Embryol., Carnegie Inst. Wash.* **29**, 7-14.
- LEWIS, W. H. AND WRIGHT, E. S. (1935) On the early development of the mouse egg. *Carnegie Contrib. Embryol., Carnegie Inst. Wash.* **25**, 114-143.
- LINCOLN, G. A. AND GUINNESS, F. E. (1972) Effect of altered photoperiod on delayed implantation and moulting in Roe deer. *J. Reprod. Fertil.* **31**, 455-457.
- LUCKETT, W. P. (1968) Morphogenesis of the placenta and fetal membranes of the tree shrews (Family Tupaiidae). *Am. J. Anat.* **123**, 385-428.
- LUCKETT, W. P. (1974) Comparative development and evolution of the placenta in primates. *Contrib. Primat.*, vol. 3, pp. 142-234, Karger, Basel.
- MARSHALL, A. J. (1948) The breeding cycle of an equatorial bat (*Pteropus giganteus* of Ceylon). *Proc. Linn. Soc. London pt. 2*, **159**, 103-111.
- MARSHALL, A. J. (1949) Pre-gestational changes in the giant fruit bat (*Pteropus giganteus*), with special reference to an asymmetric endometrial reaction. *Proc. Linn. Soc. London*, **159**, 26-36.
- MARSHALL, A. J. (1953) The unilateral endometrial reaction in the giant fruit-bat (*Pteropus giganteus* Brännich). *J. Endocrinol.* **9**, 42-44.
- MATTHEWS, L. H. (1941) On the genitalia and reproduction of some African bats. *Proc. Zool. Soc. Lond. B* **111**, 289-346.
- MATTHEWS, L. H. (1937) The female sexual cycle in the

- British horse-shoe bats, *Rhinolophus ferrum-equinum insulanus* Barrett-Hamilton and *R. hipposideros minutus* Montagu. *Trans. Zool. Soc. Lond.* **23**, 224-267.
- MCCRADY, E. (1938) The embryology of the opossum. *Am. Anat. Memoirs* no. 16, 1-233. Wistar Inst. Anat. & Physiol. Philadelphia, Pa.
- MCLAREN, A. (1968) Fertilization cleavage and implantation. In "Reproduction in Farm Animals," 2nd ed., (E. S. E. Hafez, ed.), pp. 115-133, Lea & Febiger, Philadelphia.
- MCLAREN, A. (1969) Recent studies on developmental regulation in vertebrates. In "Handbook of Molecular Cytology." (A. Lima-de-Faria, ed.) pp. 639-655. North Holland, Amsterdam.
- MEAD, R. A. (1971) Effects of light and blinding upon delayed implantation in the spotted skunk. *Biol. Reprod.* **5**, 214-220.
- MEISTER, W. AND DAVIS, D. D. (1956) Placentation of the pigmy treeshrew *Tupaia minor*. *Fieldiana: Zoology* **35**, 73-84.
- MEISTER, W. AND DAVIS, D. D. (1958) Placentation of the terrestrial treeshrew (*Tupaia tana*). *Anat. Rec.* **132**, 541-554.
- MITCHELL, G. C. (1965) A natural history study of the funnel-eared bat, *Natalus stramineus*. Thesis, University of Arizona, Tucson.
- MINTZ, B. (1972) Implantation-initiating factor from mouse uterus. In "Biology of Mammalian Fertilization and Implantation," (K. S. Moghissi and E. S. E. Hafez, eds.), pp. 343-356, C. C. Thomas, Springfield, Illinois.
- MOSSMAN, H. W. (1937) Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Carnegie Contrib. Embryol. Carnegie Inst. Wash.* **24**, 129-246.
- MOSSMAN, H. W. (1971) Orientation and site of attachment of the blastocyst: a comparative study. In "The Biology of the Blastocyst." (R. J. Blandau, ed.), pp. 49-57. Univ. Chicago Press, Chicago.
- MOSSMAN, A. S. AND MOSSMAN, H. W. (1962) Ovulation, implantation, and fetal sex ratio in impala. *Science* **137**, 869.
- MUTERE, F. A. (1967) The breeding biology of equatorial vertebrates: reproduction in the fruit bat, *Eidolon helvum*, at latitude 0° 20' N. *J. Zool. Lond.* **153**, 153-161.
- O'GARA, B. W. (1969) Unique aspects of reproduction in the female pronghorn (*Antilocapra americana* Ord). *Am. J. Anat.* **125**, 217-225.
- (ORSINI) WARD, M. C. (1948) The early development and implantation of the golden hamster, *Cricetus auratus*, and the associated endometrial changes. *Am. J. Anat.* **82**, 231-276.
- ORSINI, M. W. (1962) Technique of preparation, study and photography of benzyl-benzoate cleared material for embryological studies. *J. Reprod. Fertil.* **3**, 283-287.
- OXENREIDER, S. L. AND DAY, B. N. (1965) Transport and cleavage of ova in swine. *J. Animal Sci.* **24**, 413-417.
- PATTERSON, J. T. (1913) Polyembryonic development in *Tatusia novemcincta*. *J. Morph.* **24**, 559-684.
- PEARSON, O. P. (1949) Reproduction of a South American rodent, the mountain viscacha. *Am. J. Anat.* **84**, 143-171.
- PEARSON, O. P. AND ENDERS, R. K. (1944) Duration of pregnancy in certain mustelids. *J. Exp. Zool.* **95**, 21-35.
- PEARSON, O. P., KOFORD, M. A. AND PEARSON, A. R. (1952) Reproduction of the lump-nosed bat *Corynorhinus rafinesquei* in California. *J. Mammal.* **33**, 273-320.
- PEYRE, A. AND HERLANT, M. (1967) Ovo-implantation différée et déterminisme hormonal chez le Minioptère, *Miniopterus schreibersi* K. (Chiroptère). *C. R. Seance Soc. Biol.* **161**, 1779-1782.
- PSYCHOYOS, A. (1973) Endocrine control of egg implantation. In "Handbook of Physiology," Sect. 7, Endocrinology, vol. 2, Female Reproductive System, Part 2 (R. O. Greep, ed.), pp. 187-215.
- QUINTERO, F. AND RASWEILER, J. J. IV. (1973) The reproductive biology of the female vampire bat, *Desmodus rotundus*. *Am. Zool.* **13**, 1284.
- QUINTERO, F. H. AND RASWEILER, J. J. IV. (1974) Ovulation and early embryonic development in the captive vampire bat, *Desmodus rotundus*. *J. Reprod. Fertil.* (in press).
- RACEY, P. W. (1973) Environmental factors affecting the length of gestation in heterothermic bats. *J. Reprod. Fertil. Suppl.* **19**, 175-189.
- RASWEILER, J. J. IV. (1972) Reproduction in the long-tongued bat, *Glossophaga soricina*. I. Preimplantation development and histology of the oviduct. *J. Reprod. Fertil.* **31**, 249-262.
- RASWEILER, J. J. IV. (1974) Reproduction in the long-tongued bat *Glossophaga soricina*, II. Implantation and early embryonic development. *Am. J. Anat.* **139**, 1-35.
- REIMERS, T. J., DZIUK, P. J., BAHR, J., SPRECHER, D. J., WEBER, S. K. AND HARMON, B. G. (1973) Transuterine embryonal migration in sheep, anteroposterior orientation of pig and sheep fetuses and presentation of piglets at birth. *J. Animal Sci.* **37**, 1212-1217.
- RESTALL, B. J. AND BINDON, B. M. (1971) The timing and variation of preimplantation events in the mouse. *J. Reprod. Fertil.* **24**, 423-426.
- RUGH, R. (1968) "The Mouse. Its Reproduction and Development." Burgess Publishing Co., Minneapolis.
- SADLEIR, R. M. F. S. (1969) "The Ecology of Reproduction in Wild and Domestic Mammals." 321 pp., Methuen & Co. Ltd., London.
- SCANLON, P. F. (1971) Incidence of transuterine migration of embryos in cattle and sheep. *J. Dairy Sci.* **54**, 786.
- SHAPIRA, N., KALI, J., AMIR, S., AND SCHINDLER, H. (1974) Delay in implantation in lactating rats in relation to energy consumption. *J. Reprod. Fertil.* **36**,

- 295-300.
- SHARMAN, G. B. (1963) Delayed implantation in marsupials. In "Delayed Implantation", (A. C. Enders, ed.), pp. 3-14, Univ. Chicago Press, Chicago.
- SHARMAN, G. B., AND BERGER, P. J. (1969) Embryonic Diapause in marsupials. In "Advances in Reproductive Physiology," vol. 4, (A. McLaren, ed.), pp. 211-240, Logos Press, London.
- SHARMAN, G. B., CALABY, J. H. AND POOLE, W. E. (1966) Patterns of reproduction in female diprotodont marsupials. In "Comparative Biology of Reproduction in Mammals," (I. W. Rowland, ed.), Sympos. Zool. Soc. London, no. 15, pp. 205-232, Academic Press, London.
- SHERMAN, H. B. (1937) Breeding habits of the free-tailed bat. *J. Mammal.* **18**, 176-187.
- SHORT, R. V., AND HAY, M. F. (1966) Delayed implantation in the Roe deer *Capreolus capreolus*. In "Comparative Biology of Reproduction in Mammals," (I. W. Rowlands, ed.), Sympos. Zool. Soc. London, no. 15, pp. 173-194, Academic Press, London.
- SNELL, G. D. (1941) The early embryology of the mouse. In "Biology of the Laboratory Mouse," (G. D. Snell, ed.), pp. 1-54, Dover Publications, New York (reprinted 1956).
- SPINAGE, C. A. (1969) Reproduction in the Uganda defassa waterbuck, *Kobus defassa ugandae* Neumann. *J. Reprod. Fertil.* **18**, 445-457.
- SQUIER, R. R. (1932) The living egg and early stages of its development in the guinea pig. *Carnegie Contrib. Embryol., Carnegie Inst. Wash.* **23**, 223-250.
- STEPHENS, R. J. (1962) Histology and histochemistry of the placenta and fetal membranes in the bat, *Tadarida brasiliensis cynocephala* (with notes on maintaining pregnant bats in captivity). *Am. J. Anat.* **111**, 259-286.
- SYMINGTON, R. B. AND PATERSON, N. J. (1970) A preliminary report on the phenomenon of unilateral implantation in the right uterine horn of the common duiker, *Sylvicapra grimmia*. *Arnoldia* **4**, 1-5.
- TRIPP, H. R. H. (1971) Reproduction in elephant-shrews (Macroscelididae) with special reference to ovulation and implantation. *J. Reprod. Fertil.* **26**, 149-159.
- TYNDALE-BISCOE, H. (1973) "Life of Marsupials". 254 pp., Edward Arnold, London.
- VON DER BORCH, S. M. (1963) Unilateral hormone effect in the marsupial *Trichosurus vulpecula*. *J. Reprod. Fertil.* **5**, 447-449.
- WEIR, B. J. (1971) The reproductive organs of the female plains viscacha, *Lagostomus maximus*. *J. Reprod. Fertil.* **25**, 365-373.
- WILSON, I. B. (1960) Implantation of tissue transplants in the uteri of pseudopregnant mice. *Nature (London)* **185**, 553.
- WIMSATT, W. A. (1944) An analysis of implantation in the bat, *Myotis lucifugus lucifugus*. *Am. J. Anat.* **74**, 355-411.
- WIMSATT, W. A. (1945) Notes on breeding behavior, pregnancy, and parturition in some vespertilionid bats of the eastern United States. *J. Mammal.* **26**, 23-33.
- WIMSATT, W. A. (1954) The fetal membranes and placentation of the tropical American vampire bat *Desmodus rotundus murinus*. *Acta Anat.* **21**, 285-341.
- WIMSATT, W. A. (1963) Delayed implantation in the Ursidae, with particular reference to the Black bear (*Ursus americanus* Pallas). In "Delayed Implantation," (A. C. Enders, ed.), pp. 49-76, Univ. Chicago Press, Chicago, Ill.
- WIMSATT, W. A. (1969) Some interrelations of reproduction and hibernation in mammals. In "Dormancy and Survival," (H. W. Woolhouse, ed.), S.E.B. Sympos. no. 23, pp. 511-549, Academic Press, New York.
- WIMSATT, W. A. (1974) Morphogenesis of the fetal membranes and placenta of the black bear, *Ursus americanus* (Pallas). *Am. J. Anat.* **140**, 471-495.
- WIMSATT, W. A. AND TRAPIDO, H. (1952) Reproduction and the female reproductive cycle in the tropical American vampire bat, *Desmodus rotundus murinus*. *Am. J. Anat.* **91**, 415-445.
- WIMSATT, W. A. AND WISLOCKI, G. B. (1947) The placentation of the American Shrews, *Blarina brevicauda* and *Sorex fumeus*. *Am. J. Anat.* **80**, 361-436.
- WRIGHT, P. L. (1966) Observations on the reproductive cycle of the American badger (*Taxidea taxus*). In "Comparative Biology of Reproduction in Mammals," (I. W. Rowlands, ed.), Sympos. Zool. Soc. London, no. 15, pp. 27-45, Academic Press, London.
- ZIEGLER, L. (1843) Beobachtungen über die Brunst und den Embryo der Rehe. Hannover.