

The Evolution of Viviparity

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To begin I should like to remind you of a fact of which you are already quite well aware – unless you are an anti-Darwinian, which is unlikely these days. It is rather like reminding you that you have to breathe in order to obtain enough oxygen, for, common as the fact may be, we do not think of it each time we take a breath. Nor is it likely that each time you look at a biological structure or activity you say to yourself that it has arisen by natural selection. But the fact is that selection is a supreme and all important principle which has channelled and governed every aspect of life that exists today.

I do not propose to say anything new or original today. But I am a great believer in saying familiar, well-known things backwards and inside out, hoping that from some new vantage point the old facts will take on a deeper significance. It is like holding an abstract painting upside down; I do not say that the meaning of the picture will suddenly be clear, but some of the structure of the composition that was hidden may show itself.

The abstraction I hold before you is the development of viviparity. The novel view that we shall take of this well-worn and battered subject is its evolution (Amoroso 1955c).

The many differences in anatomical structure, living habits, and genetic constitution in animals as different as fishes and birds, or reptiles and mammals, often tend to obscure the fact that, irrespective of their phylogenetic rank, all living creatures face much the same problems in their efforts to survive as individuals and as species. They must adjust in some way or the other to the same physical influences, such as those caused by seasonal and environmental changes, and they have at their disposal the same choice of chemical elements from which to synthesize the humoral agencies that regulate their reproductive activities. It must be emphasized, however, that the hormones secreted by the reproductive organs are not essential for the well-being of the individual. Indeed, Hisaw (1959) has reminded us that 'any part or all of the structures directly concerned with reproduction may fail to function or be surgically removed without affecting the general health or life expectancy of the individual. Hence it may be presumed that reproduction is essential only for the propagation of the species, whereas to the individual it is merely a privilege which may or may not be indulged'.

It will be obvious that at present we have too little information to permit easy generalization

regarding the evolutionary history of the sex hormones of vertebrates. Nevertheless, the relative independence of the reproductive processes has made it possible for widely different, adaptive mechanisms to occur without marked interference with the general economy of the body. This seems particularly true in the light of the diverse developments that have occurred in the course of the evolution of viviparity.

The Evolution of Pituitary Control

We can only guess at the answer, of course, but it is possible that the situation as it applies to mammals and other vertebrates is one involving the interaction of the pituitary gonadotrophins and the gonadal secretions. Studies in reproductive endocrinology of cyclostomes and elasmobranchs go far towards establishing the antiquity of endocrine-controlling mechanisms in reproduction, and appear to show that gonad-pituitary relationships are fundamentally the same throughout the vertebrates. But however this may be, it must be remembered that reproductive processes were carried out successfully in co-ordinated fashion long before the advent of a pituitary and its associated gonadotrophins, and that so common a phenomenon as ovulation, which is very generally thought of in terms of gonadotrophic function, occurs in all animals and was, likewise, the rule for millions of years before the advent of a pituitary. Clearly, therefore, it would be of the greatest interest to discover the point in the evolutionary series at which viviparity came under the influence of the pituitary remembering, of course, that internal gestation was not a new discovery by the vertebrates, examples of it being known in insects, as well as in the primitive worm-like arthropod, *Peripatus*, and in some Tunicates (Amoroso 1959).

The fact that the essential features of pituitary organization are already fully outlined in the larva of the lamprey, the most primitive of living vertebrates, emphasizes its central importance in the economy of the vertebrate endocrine system. Thus for clues on its early evolutionary history resort must be made to the protochordates, in many of which specialized cephalic sensory organs have been described. One such is the neural gland of Tunicata which has, from time to time, been held to be homologous with the vertebrate pituitary and may play some part in the co-ordination of reproductive activity by virtue of its sensitivity to the sex products of other individuals. Barrington (1964) suggests that 'these may stimulate the gland, and that this in turn could evoke, in some way, the release of germ cells from its own body. If these substances then proved to be of advantage to the species concerned, even though they are initially available only in trace



Fig 1 Caudal view of amplexus in *Gastrotheca marsupiata*, showing how the pouch on the dorsum of the female is distended and held open by the pelvic limbs of the male at the time of oviposition and insemination. (Reproduced from Amoroso 1959, by kind permission)

quantities, it would then be possible for natural selection to promote their more efficient production'.

Such a view may be going rather farther than the facts justify, but from the standpoint of evolutionary theory there is the further interesting suggestion of Barrington that a cephalic sensory organ, such as the neural gland, sensitive to environmental change or to the products of other individuals (pheromones) and capable of releasing signals, might have become sensitive to the products of its own body, foreshadowing not only the far-reaching internal regulatory power that the adeno-hypophysis now possesses, but also the reciprocal feed-back relationships with other endocrine glands. Accordingly, the situation as it applies to mammals and other vertebrates must represent the culmination of a long series of adaptive mechanisms in which the pituitary gonadotrophins may be regarded as a link in the informational chain between the central nervous system and the gonads, and the pituitary-gonadal interactions as representing a device for regulating gonadal activities, which in their inception were capable of going on in direct response to environmental stimuli (Hisaw 1964).

Oviparity, Oviviviparity and Viviparity

Retention of offspring on or within the body of the parent as a means of providing protection and nutrition, and to ventilate them, is widespread among animals, many variants having arisen independently, many times, in widely separated taxonomic groups (Amoroso 1960*b*). It is clear, moreover, that while there has been a certain degree of parallelism in the evolution of the adaptations, there is also a surprising amount of diversity. Hence adaptations found in the various viviparous states in nature may hold only a

general utilitarian value in common, and cannot be arranged in a progressive evolutionary series. Furthermore, the morphological structures involved may not be homologous and often can be compared only with respect to function. Hence the present discussion will be confined to those aspects of the evolution of viviparity which will serve to illustrate the nature of the problem and to build a picture which may be filled in or erased by the reader.

It is now very generally agreed that the primitive vertebrates were marine aquatic forms and that fertilization was external. Evolutionary innovations are difficult to prove; nevertheless, the features of sexual reproduction associated with habitat and habit suggest that the most primitive type of development was probably that in which innumerable sperm and innumerable minute naked eggs, poor in yolk, were shed into the surrounding water, where fertilization was effected without association of the parents themselves. Fertilization is ensured by chance, aided by some degree of synchronization of liberation of gametes. This may reach a high degree of precision, as in the palolo worm and other forms with lunar periodicity, but these social gatherings (found at mating time) can scarcely be regarded as mating behaviour.

With the appearance of land forms certain new requirements to ensure survival became necessary. The first of these was for some sort of loose association of the sexes, which may have begun as the result of chemical stimulation due to substances extruded at spawning time and may be combined with continued prehension or amplexus, as in some amphibia (Fig 1). Another was for some sort of protection against desiccation.

As well as the secretion of a protective envelope around the egg, developments for assuring greater

survival have included increased storage of nutritive material in the form of yolk, and a concomitant reduction in the number of eggs produced. Moreover, the production of a large cleidoic egg would, in general, presuppose a parallel development of sexual association, as well as the elaboration of intromittent organs to ensure fertilization before the egg was completely closed off from the environment. Such close contacts led ultimately to the complete retention of the eggs within the parent organism, at some level in the gastrointestinal or reproductive tract, or in some specially prepared marsupium or pouch; even the respiratory system of an unrelated animal has been utilized as a site for embryo retention.

Male pipe fishes and sea horses, for example, have specialized brood pouches on the ventral body wall. In other species, either the males or females take up the fertilized eggs and incubate them in their mouths. Among amphibians too, specially developed pouches have been used as a repository for the developing young, an extreme example being found in *Gastrotheca marsupiata* (Fig 1). Such embryos are provided with ample supplies of yolk and except for oxygen obtain little, if any, additional food from the parent.

Adaptive viviparity has been described in a small toad, *Nectophrynoides occidentalis*, which breeds in arid mountain environments. After a gestation period of nine months the young are born in a fully metamorphosed condition and do not, in contrast to almost all other amphibians, require an aqueous habitat. Parturition is accomplished by inflation of the lung sacs and contraction of thoracic muscles, but delivery cannot occur unless the animal finds suitable mechanical support in the environment.

In some such way ovoviviparity arose, but from this point onwards profound changes have occurred in the nature of the egg and in the structure of the female animal. These evolutionary innovations have involved: perfection of internal fertilization; a decrease in the number of eggs which are shed together with a loss of yolk, concomitant with specialization of the embryonic membranes (yolk sac and chorioallantois) and maternal tissues as an organ of nutrition and internal secretion; the adoption of the corpus luteum in the family of endocrine glands; intra-uterine care of a few young to relatively advanced stages in development; the participation of the endocrine secretions of the placenta in extending gestation, with the object of retaining eggs in the genital tract for periods beyond the limits of the normal sex cycle; and the adaptation of the endocrine control of lactation in preparation for the early nutrition of the newborn (Amoroso 1955b).

A point to be noticed in this connexion is that while the female parent is most often the one with which the young have become associated, the male parent, in some species of teleost fishes, has not escaped entirely. This phenomenon, that male as well as female structures may provide nutrient fluids, which would seem the more remarkable if it were not so familiar to zoologists, suggests that in mammalian evolution, the functioning of the mammæ has only recently become confined to the female, explaining their slight sexual dimorphism.

It must by now be clear that with the evolutionary movement of organisms from aqueous to terrestrial habitats many adaptive changes of structure, behaviour and so on must have occurred. But before going on to describe these various adjustments, it may as well be pointed out here at the very beginning of our argument that we need not suppose that modifications of a sexual, or any other kind, could be purposively accomplished. Any marine animal incapable of evolving the necessary mechanism could never have passed through the estuarine regions to colonize the fresh water, and no amount of purposive struggling could make up for the lack of the requisite potentialities. The colonization of dry land too, required numerous and far-reaching adaptational changes, yet today there are few places so barren that no living creatures whatever can be found on them.

Adaptations for Ovoviviparity and Viviparity in

Elasmobranch and Teleost Fishes

Selachian ovoviviparity and viviparity: The cartilaginous fishes, containing as they do oviparous and ovoviviparous forms showing all grades of relationship between the embryo and the maternal organism, as well as viviparous forms, offer special opportunities for the study of evolutionary innovations that accompany the development of internal gestation. Particularly interesting light has been shed on the physiological significance of the variations in composition of the uterine fluids bathing the egg cases while the embryo develops (Amoroso 1960b). As the dependence of the embryo upon the maternal organism increases, its concentration in organic substances increases and there is an increasing accumulation of fat in particular. In the sting-ray, *Trygon violacea*, for example (Table 1), in which there is the closest approximation of foetal and maternal tissues consistent with ovoviviparity, the uterine milk contains upwards of 13% organic substance, 8% of which is in the form of fat. In addition to this, the negative balance of organic substance caused by combustion is gradually changed over into a positive balance when the embryo comes from

Table 1

Percentage composition of uterine milk in various selachian fishes (Ranzi 1934)

Species	pH	Na/D	Water	Organic substance	Fat	Ash
Ovoviviparous:						
<i>Torpedo ocellata</i>	7.0	1.34105	96.3	1.2	0.1	2.5
<i>Torpedo marmorata</i>	7.0	1.34105	96.4	1.5	0.1	2.1
<i>Scymnus lichia</i>	5.9	1.34179	95.1	1.6	0.1	3.3
<i>Centrophorus granulosus</i>	—	1.34192	95.1	1.8	—	3.1
<i>Acanthias vulgaris</i>	7.0	1.34313	95.1	2.4	0.3	2.5
<i>Acanthias blainvillei</i>	—	1.34233	95.1	2.8	—	2.1
<i>Galeus canis</i>	—	—	93.8	4.9	trace	1.3
<i>Mustelus vulgaris</i>	6.5	1.34551	93.6	5.1	0.2	1.3
<i>Mustelus antarcticus</i>	—	—	90.9	7.1	—	2.0
<i>Trygon violacea</i>	7.0	—	86.5	13.3	8.2	1.2
Viviparous:						
<i>Mustelus laevis</i>	6.2	1.35313	89.4	9.1	0.1	1.5

species in which the foetal tissues are brought into closer and closer contact with the maternal organism (Table 2). It is, however, with the development of the yolk sac placenta that viviparity reaches its highest expression in the selachii.

There is some evidence that oestradiol-17 β , oestrone and progesterone can be extracted from the ovaries of the dog-fish, *Squalus suckleyi*, and the marked ability of implants of oestradiol to stimulate the oviducts of the female *S. cuniculus* seems to indicate that the structures are under steroid control (Wotiz *et al.* 1958, Wotiz *et al.* 1960). On the other hand, although corpora lutea constitute one of the most characteristic features of the gonads of these fishes, there is no evidence that they help to maintain pregnancy. However, the evidence of Hisaw & Abramowitz (1938, 1939)

tends to show that the course of pregnancy in the viviparous *Mustelus canis* is not influenced by pituitary removal, at least in its early stages, though nothing is known concerning the later stages and parturition. This is clear evidence that the corpora lutea do not function as endocrine glands of gestation nor are they, as suggested by Bretschneider & de Wit (1947), a result of pituitary luteinization, since the development of corpora atretica from regressing follicles remains unimpaired after hypophysectomy.

Viviparity in teleost fishes: The habit of viviparity has been developed independently in several families of teleost fishes and to different degrees. At one extreme are those (e.g. *Scorpenidae*) in which the embryos are numerous, are supplied with an ample yolk sac, as in most oviparous fishes, and are born while still in a very immature state. Intermediate are fishes (*Pacilliidae*) in which a large yolk sac is still present in the embryos but the embryos are retained in the ovary until they have reached an active swimming feeding stage; the embryos are quite immature sexually. At the other extreme are those fish (*Embiotocidae*) in which a small number of young are retained within the ovary until the embryos are in an advanced stage of development and the males at least are sexually mature and ready for reproduction when born.

In ovoviviparous and viviparous teleostean fishes, the ovary is usually a single unpaired hollow organ, whose cavity is continuous with that of the gonoduct, the physiological equivalent

Table 2

Intake of organic substance by eggs of oviparous, ovoviviparous and viviparous selachian fishes (Ranzi 1932, 1933)

Species	Gestation (months)	Weight of egg at beginning and end of development				Weight of organic substance			
		Egg (g)	Completed embryo (g)	Difference	Change (%)	Egg (g)	Completed embryo (g)	Difference	Change (%)
Oviparous									
<i>Scylliorhinus canicula</i>	6	1.314	2.69	+ 1.376	105	0.614	0.487	- 0.127	- 21
Ovoviviparous:									
<i>Torpedo ocellata</i>	4	6.78	13.37	+ 6.59	97	3.780	2.91	- 0.87	- 23
<i>Torpedo marmorata</i>	6	14.38	27.28	+ 12.90	90	7.680	5.06	- 2.62	- 34
<i>Scymnus lichia</i>	10	129.53	186.66	+ 57.13	44	62.72	53.50	- 9.22	- 15
<i>Centrophorus granulosus</i> ●	10	308.0	360.0	+ 52.0	17	162.0	74.0	- 88.0	- 54
<i>Acanthias blainvillei</i>	9	19.26	38.83	+ 19.57	102	10.68	10.79	+ 0.11	+ 1
<i>Mustelus vulgaris</i>	10	3.928	60.16	+ 56.23	1,432	1.956	8.93	+ 6.974	+ 356
<i>Trygon violacea</i>	2	1.9	118.0	+ 116.10	6,105	0.93	16.07	+ 15.14	+ 1,628
Viviparous:									
<i>Mustelus laevis</i>	5	5.535	189.6	+ 184.1	3,326	2.811	32.70	+ 29.90	+ 1,064

● Combined data of Lo Bianco (1909) and Ranzi (1932)

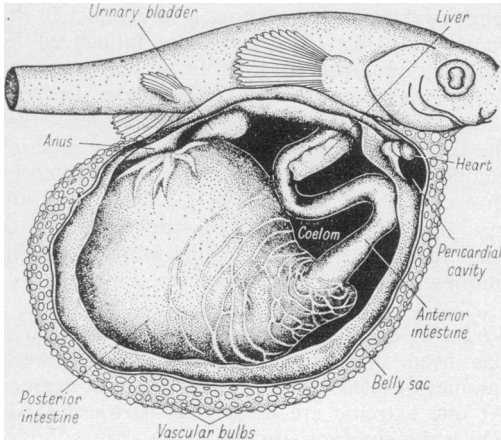


Fig 2A Partially dissected embryo of *Anableps dowei*, showing relations of pericardial cavity, coelomic cavity and expanded posterior intestine. (Redrawn from Turner, and reproduced from Amoroso 1952, by kind permission)

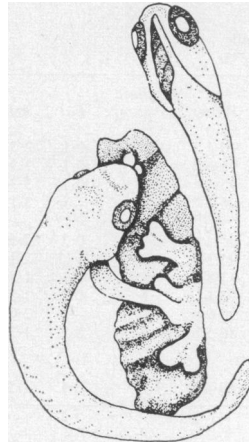


Fig 2B Late stage in the development of *Jenynsia lineata*, showing the attachment of the embryo to ovarian flap. The ovarian tissue extends through the opercula opening into the pharyngeal cavity and a small portion protrudes through the mouth. (Redrawn from Turner C L (1940) *J. Morph.* 67, 297, Figs 2 & 3)

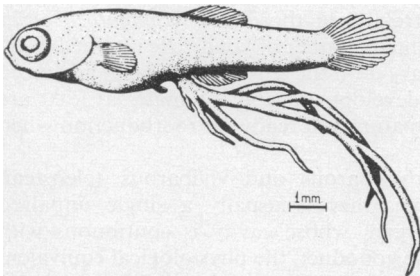


Fig 2c Embryo of *Zoogonecticus cuitzoensis*, showing absorptive trophotenia. (From Turner C L (1940) *J. Morph.* 67, 289, Fig 11)

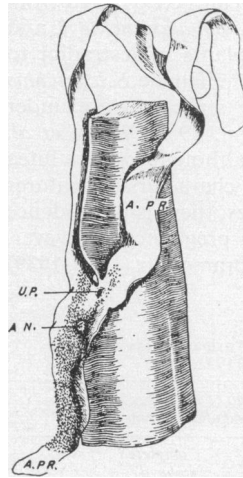


Fig 2D Posterior part of an unborn embryo of *Parabrotula*, a deep sea fish. The ribbon-shaped membranes of the processes absorb materials from the fluids of the ovarian cavity. (Reproduced from Turner 1947, by kind permission)

of the uterus. When internal fertilization occurs it must take place while the egg is still retained within the follicle or after its extrusion into the ovarian cavity or the gonoduct. Hence as an initial step in the evolution of teleostean viviparity it could be assumed that ovulation would have taken place in the ovarian cavity or gonoduct after a shorter or longer interval.

A more advanced step in the development of viviparity in bony fishes is the fertilization of the egg within the ovarian follicle prior to ovulation. Presumably this is dependent upon the ability of the spermatozoa to remain alive within the ovary for long periods of time. This appears to be a function of the ovary itself. In some instances it is the epithelium of the ovarian cavity which assumes a trophic function, whereas in other cases it is the luteal bodies derived from the granulosa and theca cells of the discharged follicles, which provide for the nourishment of

the sperm, and for the young if fertilization takes place. It is clear, moreover, that fertilization within the follicle prevents true ovulation and since ovulation is controlled by a pituitary hormone, it would appear that some endocrine change has been brought about by, or is concurrent with, the new development of intrafollicular fertilization.

The retention of the young embryo within the follicle sets the stage for further steps in evolution, hence we see arising a succession of adaptations

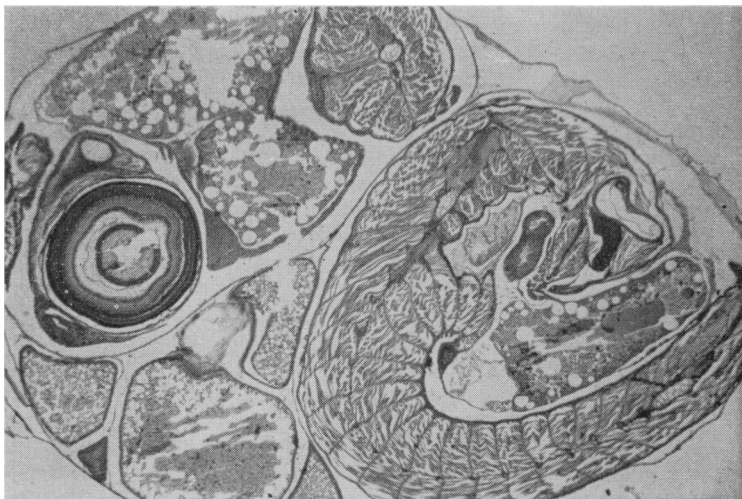


Fig 3 Section of gravid ovary of the guppy (*Lebistes reticulatus*) with embryos in advanced stages of development. $\times 20$. (Reproduced from Amoroso 1959, by kind permission)

(Fig 2) which range from little or no alteration of the oviparous types of structures (guppy, Fig 3) to conditions in which the yolk supply is reduced and a series of temporary but unique structures occur for the nourishment of young (Amoroso 1952, 1959, 1960b).

Superfœtation

Another new direction evolution may take in fishes having follicular gestation is that of superfœtation, the condition in which two or more broods of embryos at different stages of development are harboured within the follicles of the ovary at the same time (Fig 4). The ovary is modified to some extent during gestation to pro-

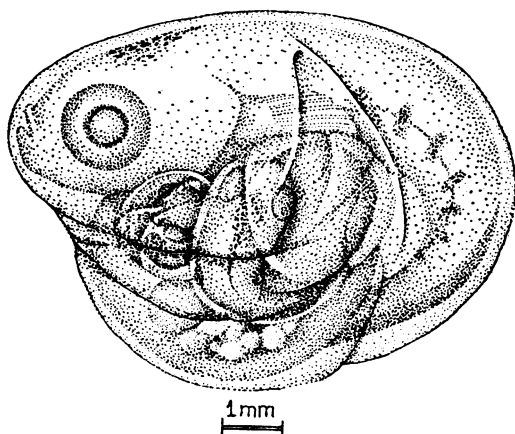


Fig 4 A complete ovary of a pœciliid fish containing six small broods of embryos in different stages of development at the same time, to illustrate superfœtation. The smallest spheres at the base of the ovary are embryos in the first stage of development. (Reproduced from Turner 1947, by kind permission)

vide the embryos with respiratory materials. In these species the yolk is reduced in amount and the ovary becomes more elaborately specialized to provide a nutritive supply.

The structural and functional modification of the ovary incident to the retention and nourishing of the embryos and harbouring the sperm seems to have little effect upon the development of the oocytes and the production of mature eggs proceeds independently. Some of these viviparous fish have short reproductive cycles, in some instances of only one month's duration (*Lebistes*) and new ova are ready for fertilization before the previous brood is born. Many of the mature ova become atretic, but sometimes they become fertilized and a second brood of embryos will be found in the ovary.

Potential superfœtation exists wherever mature ova are formed in an ovary in which a brood of embryos is retained and within which there are viable sperm. As the number of broods within the ovary increases the number of embryos in each brood decreases. Extreme superfœtation, on the other hand, is found in those species in which the yolk sac has decreased in size and special devices have been evolved in the embryos and the follicular capsules for facilitating exchange (Fig 2). This is so, presumably because, with the reduction in the amount of yolk, less time is required for its elaboration and so new groups of ova come to maturity and are ready for fertilization earlier. Secondly, the evolution of a follicular pseudo-placenta makes possible a longer retention of the embryos in the ovary, while the retention of older embryos increases the number of broods within the ovary at any one time.

Effective superfœtation, as far as is known, occurs only in the pœciliid fishes among the

cyprinodonts and does not occur in those vertebrates that have uterine gestation. The reasons for this may be stated as follows:

(1) The oocytes reach maturity seasonally or periodically within the ovarian follicles, after which ovulation occurs. The ova enter the oviducts where fertilization takes place and the developing embryos are then retained in the oviduct or uterus for the period of gestation.

(2) In the mammals, at least, the implantation of the embryos in the uterus brings about changes in the endocrine control of ovulation, with the result that ovulation does not normally occur during gestation; and, the cyclical changes in the uterus which proceed in the absence of fertilization of the ovum are held in abeyance.

(3) In the viviparous reptiles and selachians the time taken for the maturation of the oocytes is extremely protracted. Hence there is a long interval between the birth of one group of embryos and the ovulation and fertilization of the ova which are to form the next brood.

As with selachians, the so-called pre-ovulation corpora lutea form the main mass of endocrine tissue of the teleost ovary and there are reports that steroid compounds similar to those in the spiny dogfish have been isolated from the ovaries of the salmon, *Oncorhynchus nerka*, and the ratfish, *Hydrolagus collicii*. As yet, however, there is no answer to the question whether these luteal bodies have an endocrine function in controlling gestation. Indeed, there is a strong case for it being unanswerable, since extirpation of the corpora lutea is unlikely to be successfully performed on a pregnant teleost and would in any case certainly terminate pregnancy.

Amphibian larviparity and ovoviviparity: The ruptured follicle of the viviparous anuran *Nectophrynooides occidentalis* and the ovoviviparous marsupial frog *Gastrotheca marsupiat*a is quickly transformed into a corpus luteum which persists throughout gestation, but regresses rapidly after the young are born. Lamotte & Rey (1954) on direct evidence credit the corpora lutea with the inhibition of ovulation in *N. occidentalis*, and

conclude that the luteal bodies control gestation in this species. In *G. marsupiat*a, on the other hand, an endocrine function during gestation was not established (Amoroso 1959, 1960b).

Reptilian viviparity: The specific functions of the reptilian corpus luteum are by no means clear, although its presence in both viviparous and ovoviviparous species seems to indicate that here too it is not primarily related to viviparity. There appears to be, nevertheless, a definite correlation between the longevity of the corpus luteum and the egg-laying or retaining habit of the species. In oviparous species retrogression is rapid and is completed shortly after oviposition (Cunningham & Smart 1934). In vivipara, on the other hand, the corpora lutea remain well developed for most, if not the whole, of gestation and have regressed completely within two weeks *post partum* (Cunningham & Smart 1934, Bragdon 1952).

The observations of Bragdon (1951, 1952) on the ovoviviparous garter snake *Thamnophis* and the watersnake *Natrix* and those of Panigel (1956) on the ovoviviparous lizard *Zootoca* are much like those of Hisaw & Abramowitz (1938, 1939) on selachians and indicate that ovariectomy and hypophysectomy during gestation will not usually lead to abortion, but will interfere with parturition and cause the retention of young past term. This interference was more complete subsequent to hypophysectomy than after ovariectomy, thus implying placental participation. These observations are in agreement with the instances cited previously in which corpora lutea do not function as endocrine glands of gestation, a condition probably true of all viviparous anamnia and all but a few doubtful exceptions among live-bearing reptiles (Rahn 1938, 1939, Clausen 1940, Fraenkel *et al.* 1940), in which, it is to be emphasized, an allantoic placenta functions as the organ of exchange.

If this be so, then it is clear, as Hisaw has suggested (Hisaw 1959, 1964), that the next stage in the evolution of viviparity leading to conditions found in mammals was the adoption of the corpus luteum into the family of endocrine

Table 3
Tissues separating fetal and maternal blood

Classification (Grosser)	Maternal tissue (uterine mucous membrane)			Fetal tissue			Gross form of placenta	Typical examples
	Endothelium	Connective tissue	Epithelium	Trophoblast	Connective tissue	Endothelium		
Epitheliochorial	+	+	+	+	+	+	Diffuse	Pig, horse
Syndesmochorial	+	+	—	+	+	+	Multiplex	Sheep, goat, cow
Endotheliochorial	+	—	—	+	+	+	Zonary	Cat, dog, ferret
Hæmochorial	—	—	—	+	+	+	Discoid	Man, monkey, bats, mice, insectivores

Based on Table 1, Amoroso (1952)

glands. He suggests that synthesis of progesterone, probably on a small scale, was the first step in the specialization of the corpus luteum, and that this process was later augmented by the acquisition of competence to respond to the pituitary luteotrophic hormone (LTH).

Evolution of Placentation in Mammals

The evolution of placentation in mammals, that is, the establishment of a respiratory and trophic (nutritional) connexion between mother and foetus, has not been accomplished by the evolution of new foetal structures beyond those encountered in reptiles. Typically in mammals there exist two essentially different main types of placenta.

In the first type, the connexion is established between the uterine wall and the yolk sac with its network of vitelline vessels. In some mammals (e.g. marsupials), the yolk-sac placenta is the only instrument of placentation, whereas in others, like the rabbit, where it has undergone its highest specialization, it functions as an organ of exchange whose importance is not secondary to that of the chorioallantoic placenta. In the second type of mammalian placenta certain portions of the allantoic sac fuse with the adjacent chorion and enter into varying relationships with the uterine wall, the allantoic vessels taking over the transport of substances from the mother to the embryo and vice versa. This is the chorioallantoic placenta, and in all higher mammals is the principal, and in some the only, instrument of placentation.

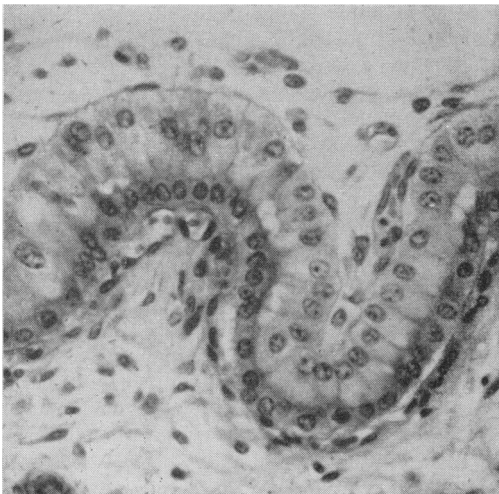


Fig 5 *The placental membrane of the sow at 32 days after insemination. The chorion consists of low folds which are matched by corresponding folds of the endometrium. The fetal epithelium is uniformly columnar and light staining, whereas the uterine epithelium is cuboidal and intensely basophilic. (Placenta epithelio-chorialis). × 275*

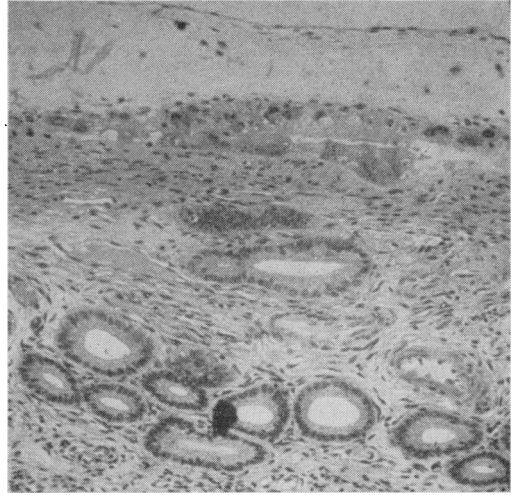


Fig 6 *Section through the intercotyledonary region of the cow's placenta at the fourth week of pregnancy, showing the partial denudation of the uterine epithelium with the creation of a syndesmochorial relationship. × 80*

Traditionally, the chorioallantoic placentas of eutherian mammals have been classified into four principal types according to Grosser's concept of the degree of erosion of the maternal tissues present (Grosser 1909, 1927), the name given to each type indicating the two tissues – one maternal, the other foetal – which are in immediate contact (Table 3). Thus, simple apposition of uterine epithelium and chorion was classed as *epitheliochorial* (Fig 5); erosion of uterine epithelium but not of maternal connective tissue was called *syndesmochorial* (Fig 6); erosion of both the epithelium and the connective tissue was designated *endotheliochorial* (Fig 7); and complete destruction of the uterine tissues so that the maternal blood is directly in contact with the foetal chorion, as in the primates and hyena, was called *haemochorial* (Fig 8). Subsequently Mossman (1926, 1937, 1959) suggested adding a *haemoendothelial* placenta to the types proposed by Grosser, to accommodate the rabbit and the guinea-pig, because he then believed that the tissues of the separating membrane were reduced to the absolute minimum of foetal vascular endothelium. More recently it has been shown (Amoroso 1952) that the allantoic placenta of the marsupial *Perameles*, as seen with the light microscope, is *endothelio-endothelial* (Fig 9) in type, necessitating yet another category.

There can be little doubt that Grosser's grouping of chorioallantoic placentas of mammals into the number of tissue layers between the maternal and foetal circulations, in so far as it provides a ready means for their histological characteriza-

tion, retains its hold upon present-day thinking more effectively than does his judgment of the relative efficiency of epitheliochorial versus hæmochorial placentas. Grosser's basic assumption – that the fewer the layers the greater the speed of diffusion – has not been entirely substantiated. Indeed, from what is known at present, it seems certain that intrauterine foetal welfare is dependent upon a certain preciseness in both the qualitative and quantitative selectivity of placental transfer (Brambell *et al.* 1951, Page & Glendenning 1955). It is obvious, moreover, that the rate of diffusion across a number of semi-permeable layers will be limited by the least permeable layer, and not by their total number. Hence, it is necessary to question Grosser's ascription of increased functional efficiency to the

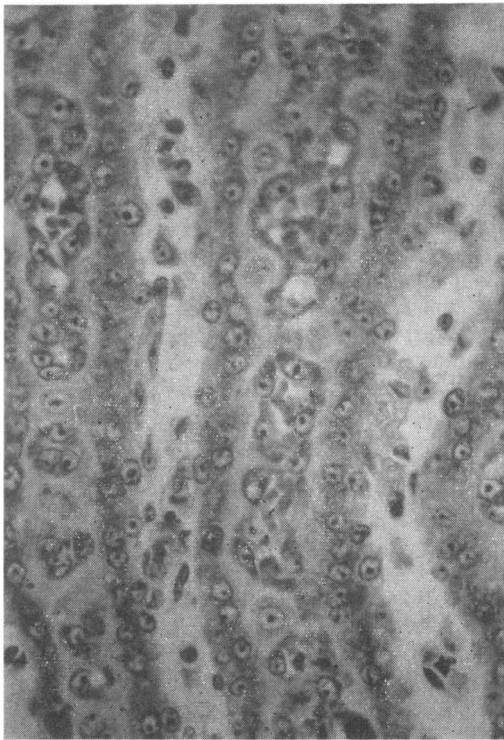


Fig 7 Details of the lamella from the placental labyrinth of a cat at about the 30th day of pregnancy, as seen with the light microscope. Observe the dark staining syncytial trophoblast which encloses decidual giant cells and maternal capillaries. Notice the pale staining matrix which accompanies the maternal blood vessels and giant cells and intervenes between them and the syncytial trophoblast. Observe the hypertrophied endothelial cells lining the maternal capillaries and the thin walled fetal blood vessels lying in the fetal mesenchyme adjacent to light staining cytotrophoblast. This represents the endotheliochorial type of membrane in Grosser's scheme but is better classified as vasochoial. $\times 225$



Fig 8 Section through the ripe placenta of the hyena (*Crocuta crocuta*), showing the fetal villi and the extensive intra-epithelial capillary nets in the syncytial trophoblast. The fetal tissues are in direct contact with circulating maternal blood creating a hæmochorial membrane. $\times 210$

hæmochorial ones while regarding the epitheliochorial ones as relatively inefficient, and to demand experimental proof before accepting the hypothesis that the efficiency of a placenta derives directly from his concept of four morphological types of chorioallantoic placentas.

Placental Hormones and Evolution of Viviparity

The monotremes are the only mammals that lay eggs. The eggs are relatively small but contain enough nutrition to support development up to an advanced stage, though not to the level of self-sufficiency. In all other mammals placental associations of increasing complexity are established between parent and offspring, with the aim of bringing the two circulations into the closest apposition without actual admixture (Amoroso 1952, 1955a, 1961).

The endocrine control of the follicular phase of the mammalian oestrous cycle, up to and including ovulation, is comparable in most respects to the oviparous cycle of nonmammalian vertebrates, differing from this only by the addition of a luteal phase. In higher mammals a corpus luteum is formed by the action of the pituitary luteinizing

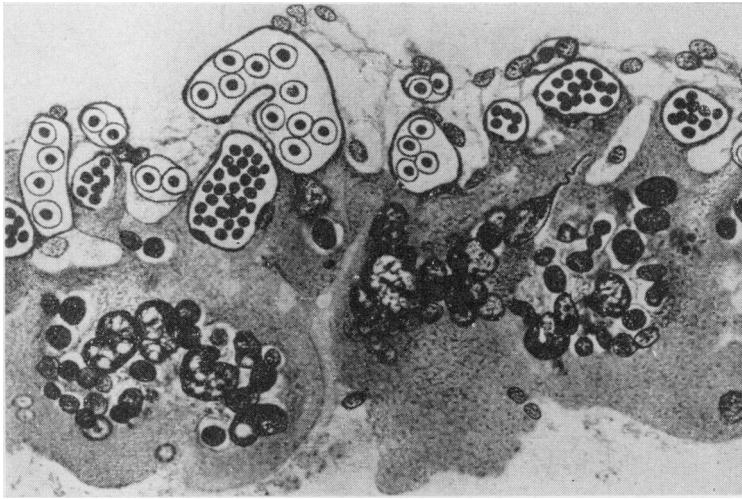


Fig 9 The fetal-maternal junction in the ripe allantoic placenta of the bandicoot (*Perameles*), showing the interlocking of the fetal and maternal tissues, the blood vessels of which are in intimate apposition. (*Placenta endothelio-endothelialis.*) $\times 300$

hormone (LH), and it secretes progesterone in response to pituitary luteotrophic hormone (LTH or prolactin). Therefore, the height of specialization of the mammalian corpus luteum has involved acquiring the capacity to respond to two pituitary hormones as well as to secrete progesterone.

This innovation of a luteal phase in the oestrous cycle of mammals would thus clearly set them apart from other vertebrates, for although luteal bodies have been described in representatives of every other vertebrate class and certain protochordates, there is, as we have noted above, no certain evidence that they are endocrine glands specialized for secreting progesterone. This function, being peculiar to mammals, must have been established late in vertebrate evolution. When and how this took place is, of course, a matter of conjecture, but a good guess might be that it first occurred in the reptilian stock that gave rise to mammals, and that this process (synthesis of progesterone) was later augmented by the acquisition of competence to respond to the pituitary luteotrophic hormone (LTH).

The physiology of the first few days of pregnancy is apparently the same in all mammals as it is fundamentally that of the oestrous cycle. Hence one of the problems confronting mammals in the evolution of viviparity was that of extending gestation with the object of retaining eggs in the genital tract for periods outlasting the duration of a normal cycle. In the monotreme mammals the situation is similar to that of other viviparous vertebrates in which internal fertilization occurs and merely involves the retention of the eggs in the reproductive tract for some 8 days until the shell is formed, and when laid they contain embryos comparable in many respects to those of the hen after about 40 hours of incubation. This

modest gesture towards viviparity in monotremes is in some respects reflected in marsupials.

In the American opossum in which pregnancy lasts for only 12 days, and in which the luteal phase of the oestrous cycle is so prolonged as to be comparable with true pregnancy in duration, parturition is correlated with the involution of the corpora lutea. As the ovaries are necessary in these species throughout gestation, it is assumed that the maintenance of pregnancy does not involve hormonal mechanisms other than those that participate in the regulation of the oestrous cycle. However, as gestation is prolonged other and rather diverse controlling mechanisms appear. The evidence points to the placenta being implicated in these changes, the direction of which is towards the endocrinological specialization of this organ as an adjunct to the pituitary and to the ovary (Amoroso 1955*d*).

The secretion of gonadotrophins, oestrogens and progesterone by the placenta has been described for several species (Amoroso 1955*d*, 1960*a*). When the adoption of pituitary gonadal functions by the placenta is considered from an evolutionary point of view, it seems reasonable to suppose that the process might have followed divergent paths in different mammalian groups. The available evidence points in this direction (Amoroso 1955*b*, 1960*a*).

Placental gonadotrophins differing widely in their physiological functions have been described in several species including the rat, mare, monkey, chimpanzee and the human female. In the rat and probably in all animals in which oophorectomy terminates pregnancy (e.g. mice, rabbits) the stimulation of luteal secretion is the only effect known to be produced by this hormone. In the mare placental gonadotrophins appear in the blood about the time of implantation of the

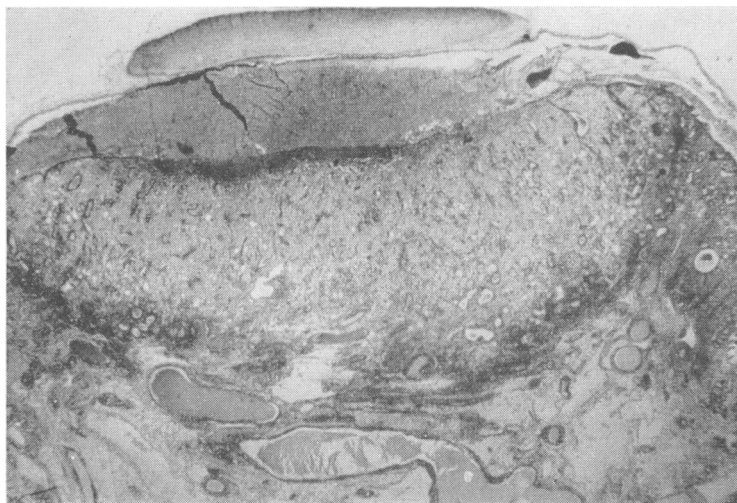


Fig 10 *Endometrial cup from a mare killed on the 56th day of pregnancy. At this stage the cups project from the surface of the uterus. Note also that the uterine epithelium over the surface of the cup is entirely absent, and that there is extensive autolysis of the tissues. The detritic coagulum which has accumulated in the uterine lumen is rich in gonadotrophic hormone and is the product of degenerating interglandular cells (decidua) as well as glandular secretions. × 10. (Reproduced from Amoroso 1959, by kind permission)*

blastocyst and the onset of its production coincides with the appearance of endometrial cups (Fig 10) which secrete it (Amoroso 1955*d*). The part it plays in gestation is not clear but it may be significant that its presence is correlated with the development and growth of numerous accessory corpora lutea.

In man, as well as in the chimpanzee and monkey, the principal property of the hormone is one of luteinization, and there is some evidence which indicates that it can stimulate the secretion of both oestrogen and progesterone by the corpus luteum (Hisaw & Astwood 1942). The fact that the secretion of these substances, from the three groups of mammals represented, is initiated at the time of implantation or attachment of the blastocyst seems to imply that all of them may be instrumental in furthering luteal function. On the other hand, the extent to which the placenta has adopted ovarian functions varies considerably from species to species (Amoroso & Finn 1962). After removal of the ovaries in some of these animals (mouse and rabbit), pregnancy may be maintained by administering progesterone alone, but in the hamster and rat it is necessary to provide oestrogen as well, whereas in man, the monkey and the mare, substitution therapy is unnecessary after a certain stage of pregnancy has been reached. The implication is that while oestrogen and progesterone are necessary for the maintenance of pregnancy in all animals, in the last group (mares and primates) these steroids may be supplied from elsewhere than the ovaries, and it is generally held that the placenta represents the new source. In another group, which includes the rabbit and some murine rodents, oestrogen, but not progesterone, may be supplied from the extra-ovarian source. Finally, in the hamster and rat, which represent yet another

group, neither of these substances can be supplied in sufficient quantities in the absence of the ovaries. That this represents a phylogenetic series may be questioned but, taken in conjunction with the postulated hypophyseal functions, it gives the placenta an important claim to be considered as having decisive effects in extending gestation and in determining the endocrine framework within which viviparity has evolved. Indeed, the probability that the placenta in all eutherian mammals secretes a luteotrophic principle at the time of implantation seems to imply that this was the initial adaptation which made it possible to prolong the life of the corpus luteum, thus extending the gestation period beyond the limits of the oestrous cycle; while the apparent influence of the placenta in augmenting gonadal function through secretion of oestrogen and progesterone, or in forming accessory corpora lutea through a secondary effect on the ovaries, might be regarded as a still further placental specialization allowing a still longer gestation period. Whatever the true explanation it seems clear that the problem of the role of the placenta in the evolution of viviparity remains an intriguing problem which still clamours for solution.

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DISCUSSION

Professor C A Clarke (*University of Liverpool*) asked whether Professor Amoroso could give his views on how the evolution of viviparity (where the foetus was not rejected) could be equated with the evolution of the immune rejection response whereby a homograft was rejected.

Professor Amoroso felt that the question arose because of a misconception. From time to time attempts had been made to solve both the question of the function of the decidua reaction and the problem of why the antigenically foreign foetus was not rejected by the mother as were other

homografts. Thus, it had been postulated that the decidua protected either the mother from sensitization, or the foetus from immune attack. But there was no need to elaborate on the unsatisfactory features of such a hypothesis, since recent work by Simmons & Russell (1962) indicated that it was the trophoblast, rather than the decidua, which gave the foetus its unique dispensation against homograft reaction. It would appear that the trophoblast was in a sense antigenically immature, and the thin layer which, in the human female, separated foetal from maternal tissue throughout most, if not all, of a normal pregnancy was effective in preventing antigenic sensitization of mother by foetus.

A speaker asked whether Professor Amoroso could say a few words about the histological character of the placenta. He asked which cells were responsible for the formation of the many hormones secreted by the placenta and whether they were the same ones which presented an immunological barrier. The fact that there was such a barrier in species below the mammals might not be admitted but, nevertheless, there appeared to be some kind of cellular differentiation or compartmentalization between the mother and the foetus.

Professor Amoroso replied that traditionally, the chorio-allantoic placentas of eutherian mammals had been classified into four principal types according to Grosser's (1909) concept of the degree of erosion of the maternal tissues present; the names given to each type indicated the two tissues – one maternal, the other foetal – which were in immediate contact. Thus, simple apposition of uterine epithelium and chorion was classed as *epitheliochorial*; erosion of uterine epithelium but not of maternal connective tissue was called *syndesmochorial*; erosion of both the epithelium and the connective tissue was designated *endotheliochorial*; and complete destruction of the uterine tissues so that maternal blood is directly in contact with the foetal chorion, as in the human female, was called *haemochorial*. With the advent of the electron microscope, it became apparent that the separating membranes which mediate the metabolic exchange between the foetal and maternal blood streams in man and other mammals with haemochorial placentas, were much more complex than was indicated in Grosser's scheme and that the chorion, which was assumed to be composed of a single layer of trophoblast, might in fact be represented by two or even three layers in the placenta of some animals, the three resulting types being then called haemomonochorial, haemobichorial and haemotrichorial respectively.

Professor Amoroso felt, however, that these refinements were of academic interest only and that there was little doubt that Grosser's grouping of chorio-allantoic placentas of mammals by the number of tissue layers between the maternal and fetal circulations, in so far as it provided a ready means for their histological characterization, retained its hold upon present-day thinking more effectively than did his judgment of the relative efficiency of epitheliochorial *versus* hæmochorial placentas. Grosser's basic assumption – that the fewer the layers the greater the speed of diffusion – had not been entirely substantiated. Indeed, from what was known at present, it seemed certain that intrauterine foetal welfare was dependent upon a certain preciseness in both the qualitative and quantitative selectivity of placental transfer (Brambell *et al.* 1951). It was obvious, moreover, that the rate of diffusion across a number of semipermeable layers would be limited by the least permeable layer, and not by their total number. Hence, it was necessary to question Grosser's ascription of increased functional efficiency to the hæmochorial types while regarding the epitheliochorial ones as relatively inefficient and to demand experimental proof before accepting the hypothesis that the efficiency of a placenta derived directly from his concept of four morphological types of chorio-allantoic placentas.

Concerning the origins of the steroid and gonadotrophic hormones associated with human pregnancy, the work of Midgley & Pierce (1962) strongly suggested that human chorionic gonadotrophin, like the oestrogens and progesterone, was normally formed in the syncytial-trophoblast, rather than in the cytotrophoblastic cells, as tentatively suggested, more than twenty years ago, by Wislocki & Bennett (1943). The relevant point in the present context was that the same layer of trophoblast which was apparently effective in preventing antigenic sensitization of the mother by the foetus was also the tissue responsible for hormone production, so that a hormone-graft relationship could not be entirely excluded.

But Professor Amoroso added that, though not protecting the foetus against immunological attack by the mother, the decidual reaction might, nevertheless be involved in hormone production. For example, in horses and donkeys a potent gonadotrophin, pregnant mare serum gonadotrophin (PMSG) had been demonstrated in the blood and lymph of pregnant females. Its appearance in the body fluids corresponded with the time of attachment of the blastocyst and although its source had not, as yet, been definitely established, the evidence pointed to certain specialized areas of decidual transformation – the endometrial cups (Fig 10) – being implicated in its production.

The evidence was: (1) The appearance of the hormone in the blood and lymph which coincided with the period of development of the cups. (2) When first formed (45th day) the cups contained higher concentrations of the hormone than were found in the blood and lymph. (3) The hormone could be extracted from the endometrium only in the cup area. (4) The jelly-like coagulum, which resulted from the degradation of the tissues of the cup, re-inforced by the secretions of the uterine glands in the vicinity of the cups, was the richest known source of the hormone. (5) The lymph draining the cups contained high concentrations of the hormone and was richer, per gram, than the blood. (6) One could account for the coincident high concentrations of the hormone in the endometrial-cup secretions and in the lymph most easily by assuming an endometrial source – the decidual cells themselves being especially rich in the hormone. But, howsoever this was accomplished, the endometrial transformation in the cups was progressive and profound and was associated with a decidual response and with the presence of demonstrable quantities of gonadotrophic hormones, two phenomena which played essential roles in embryonal implantation.

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The Epidemiology of Human Pregnancy

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The Concise Oxford English Dictionary defines epidemiology as the study of epidemics and although the subject did start in this way it has now become a branch of human biology, being concerned with the study of groups rather than of the individual.

As a species we are social animals and the patterns of human reproduction are much influenced by social as well as biological trends. The papers which follow will be concerned with the detailed relationship of mother and foetus, and I hope they will be better appreciated against a more general appraisal of human pregnancy.