

The Sect.

TS

THE PLURIOVULAR FOLLICLE, WITH SPECIAL REFERENCE TO ITS

OCCURRENCE IN THE FERRET

by D. Mainland, M.B., Ch.B.

Report on Research conducted under the supervision  
of the Professor of Anatomy; Presented for the  
Monro Prize, March 31st., 1927.

*Dear Sir:*

*I am delighted that  
this essay is deserving  
of the Prize  
E. Sharpey Schaffer*

*26.V.27*

*for Gunning Victoria Jubilee Prize*

THE PLURIOVULAR FOLLICLE, WITH SPECIAL REFERENCE TO ITS  
OCCURRENCE IN THE FERRET

by D. Mainland, M.B., Ch.B.

Report on Research conducted under the supervision  
of the Professor of Anatomy; Presented for the  
Monro Prize, March 31st., 1927.

for *Gunning Victoria Jubilee Prize*

---

Prof. Sir E. Sharpey Schaper.

The University New Buildings,

Edinburgh 26 May 1927.

COMMUNICATIONS TO BE  
ADDRESSED TO THE DEAN  
OF THE FACULTY OF MEDICINE.

The enclosed Essay by  
Donald Mainland, M.B. which has  
been lodged for the Lunning  
Victoria Jubilee Prize in Anatomy is  
sent to you herewith for  
examination. Professor Robinson  
has already read the Essay.  
Report

I am satisfied that  
this Essay is deserving

of the Prize  
E. Sharpey Schaper

26.V.27

J. Lorrain Smith  
Dean P.A.S.

*Th. Sect.*

THE PLURIOVULAR FOLLICLE, WITH SPECIAL REFERENCE TO ITS

OCCURRENCE IN THE FERRET

by D. Mainland, M.B., Ch.B.

( 17 Figures )

*Gunning Victoria Jubilee Prize  
in Anatomy 1927.*



# CONTENTS

	page
INTRODUCTION . . . . .	1
HISTORICAL SUMMARY . . . . .	3
MATERIAL AND METHODS . . . . .	6
RESULTS OF THE SYSTEMATIC EXAMINATIONS . . . . .	9
COMPARISON OF PLURIOVULAR FOLLICLES . . . . .	13
THE SPECIES-DISTRIBUTION OF PLURIOVULAR FOLLICLES. . . . .	15
CONCOMITANT PECULIARITIES OF OVARIAN STRUCTURE. . . . .	20
(1) Plurinuclated Ova. . . . .	20
(2) Abundance of Small Follicles . . . . .	24
(3) Epithelial Ingrowths . . . . .	25
THE RELATIONSHIP OF THE OVARIAN CONNECTIVE TISSUE TO THE FOLLICLES. . . . .	27
(1) Secondary Union of Follicles . . . . .	27
(2) Primary Disproportionate Development. . . . .	32
THE DEGREE OF THE ABNORMALITY . . . . .	37
(1) Number of Ova in a Follicle. . . . .	37
(2) Relation of the Epithelium to the Ova within the Follicle. . . . .	39
RELATIONSHIP OF THE AGE OF THE ANIMAL TO THE OCCURRENCE OF PLURIOVULAR FOLLICLES . . . . .	43
GROWTH AND DESTINY OF THE PLURIOVULAR FOLLICLE. . . . .	48
(1) Conversion to Uniovular Follicles. . . . .	48
(2) Degeneration. . . . .	49
(3) Growth. . . . .	50
(i) Growth of the Ova . . . . .	50
(ii) Growth of the Follicle and Maturation. . . . .	53
THE RELATIONSHIP OF PLURIOVULAR FOLLICLES TO FERTILITY . . . . .	56

	page
(1) Coexistence with Diminished Fecundity. . . . .	56
(2) Coexistence with Increased Fecundity . . . . .	56
(i) Direct Influence:	
Monochorionic Twinning. . . . .	56
Biovular Twinning . . . . .	57
(ii) Indirect Influence:	
(a) Concomitance of Small Follicles . . . . .	59
(b) Ovulation-rate or Litter-size . . . . .	60
VALUE OF THE PLURIOVULAR FOLLICLE IN REPRODUCTION . . . . .	68
SUMMARY . . . . .	69
LITERATURE . . . . .	73

## INTRODUCTION

One hundred years ago von Baer (1827) referred to his observation of pluriovular follicles in the ovaries of the dog and the pig. Last year appeared Hartman's paper (1926), partly devoted to these follicles as they occur in the opossum. A glance at the Historical Summary below reveals the frequency with which observations were made on this subject during the intervening century, and gives rise to the suggestion that investigation of it must be nearly complete. Analysis of the previous publications, however, demonstrates in many instances a lack of standardisation. We have, for example, often no evidence of the number of normal ovaries reviewed, and we are left to form a judgment from our general knowledge of the experience of the observer. The widespread distribution of the condition is obvious, but data of the frequency are comparatively meagre, and the possible relationship to twinning or to fertility in general is, in regard to many species, quite obscure. After the example set by the extensive and close observations made by Hartman (1926), it appeared desirable to make a similar survey of another Mammal, - a

member of the Carnivora, the common ferret.

Even in recent years opposing views have been held concerning the origin of the abnormality, and it seemed possible that further light might be thrown on this question, and therefore upon normal ovarian structure, by the investigation of an animal, concerning which, apparently, no record of the abnormality has previously been published.

In the first instance, I have arranged a brief Historical Summary of the findings of previous authors, which is, I believe, almost complete. The notes are grouped, as nearly as possible, under the headings: Year of Publication; Author; Species of Animal; Extent of Observations.

I have next recorded ~~the recorded~~ the numerical results of my attempts to ascertain, roughly, the frequency of the abnormality in the ferret. In the discussion which follows, the study of the pluriovular follicle in different species, so far as our evidence will permit, is made the basis for the study of the condition in the individual. (Attention has been confined to the condition in Mammalian ovaries.)

## HISTORICAL SUMMARY

(The unqualified word "follicle" here refers to the pluriovular variety)

- 1827 von Baer: dog and pig; once in each.
- 1840 Hausmann: dog.
- 1842 Bidder: calf; one follicle.
- " Bischoff: rabbits; two follicles.
- 1845 Bischoff: adult dog; one follicle.
- 1861 & 1863 Klebs: newly-born human; frequent follicles. One 7-years old child; follicles rare.
- 1863 Grohe: human children; repeatedly.
- " Pflüger: cows and dogs, both young and adult; young cats: - in all of these, "tubes" with several ova.
- " Quincke: human children; young cats and rabbits; cow embryos; adult rabbits.
- " Schrön: 400 ovarian "Präparate" of cat: - two follicles (one from a 1-year old animal). 80 "Präparate" of dog ovaries: one follicle.
- 1867 Kölliker: human; two follicles.
- 1869 Plihal: calves and young children: - "tubes" with several ova. Only once seen in an older ovary (18-years old human).
- 1870 van Beneden: human foetus at term; young kangaroo.
- " Slavjansky: human children and adults; rare in both.
- " Waldeyer: human foetuses. Most frequent in dogs.
- 1874 Foulis: general. (chiefly studied were: calf, kitten, young human.)
- 1875 de Sinety: dogs and cats; frequent.
- 1878 Balfour: rabbits; follicles not very uncommon; one shown from a 6-weeks old animal.

- 1879 Balbiani: adult dogs;follicles more frequent than in any other animal.
- " Wagener:rabbits;dogs(including an adult).
- 1880 van Beneden: adult bats.
- 1881 Schulin: a sheep;occasionally in pig and cow;bat; follicles frequent in a human 3 years old.
- 1887 Janosik: cat;rabbit;dog;human.
- 1888 Nagel: human;only once such a follicle,and that in a newly born.
- 1891 Alexenko: human adults.
- 1893 Klien: one human child;nine follicles.
- " Schottländer: human;chiefly foetuses and newly born.
- 1895 Hellin: human;about a year old. (Refers to the findings of Hensen,Kiwisch and Scanzoni,without detail.)
- 1898 von Franqué: human adult;one follicle.
- 1899 Stöckel: one human,aged 29;numerous follicles.Also in a newly born child.
- " Rabl: one human,aged 25;numerous follicles.
- 1900 Bouin(P. & M.): one adult dog ovary;numerous follicles.
- " Schumacher & Schwarz: 2 human adults;numerous follicles.
- 1901 Honoré: adult rabbit.
- " Loeb(L.): young guinea-pigs;occasional follicles.
- " Rosner: nine-banded armadillo;ovaries of one pregnant female;numerous follicles.(Enumeration.) Also foetal armadillo ovaries.
- 1903 Ancel: dogs of various ages;frequent follicles.
- " Cuénot: nine-banded armadillo;four ovaries(one young animal,one adult);a few follicles.(Enumeration.)
- 1905 Schottländer: further discussion of the material of 1893,with addition of adult ovaries.

- 1907 Welch: adult human; one follicle.
- 1908 Smyth: dog; ovaries from 2 pups of one litter; numerous follicles.
- 1908-9 von Winiwarter & Sainmont: young cats; follicles frequent.
- 1909 Chappelier: 4 white mice of same litter; 8 to 32 days old; a few follicles.
- 1911 Schmaltz: adult dogs; follicles quite usual. Pigs; follicles frequent. Sheep; not uncommon.
- 1912 Arnold: human; 18 years old; many follicles. (Enumeration.)
- " Hansemann: human; very frequent.
- " O'Donoghue: Dasyurus; 45 animals: - Two ovaries from one; one from another showed follicles. Both adults. Proportion to normal follicles: - about 10 and 20 per cent., respectively.
- " Tur: cat and rabbit; several instances.
- 1913 Kingsbury: young cats.
- 1915 Corner: pigs; many ovaries; only three follicles.
- 1917 Loeb (L.): 34 guinea-pigs used in underfeeding experiments. One animal; numerous follicles.
- 1921 Häggström: one human; 22 years; both ovaries. In one, 5 follicles; in the other none. (Enumeration.)
- " Woerdeman: one human ovary, probably adult; numerous follicles. (Refers to Strassman's case: human adult; nearly every follicle biovular.)
- 1924 Allen, Francis, &c.: adult mouse; one follicle appears in fig. 31 (Plate 7).
- " Kennedy: 2 dogs; 2 cats; 4 rabbits; 1 guinea-pig; ages various, young and adult. (1 fowl)
- 1926 Hartman: ovaries of about 150 opossums; all ages; two thirds of the animals possess follicles. Adult Macacus rhesus; 2 animals (3 ovaries); follicles numerous. Cat; 1 ovary. Dogs; 2 animals. Follicles numerous in the ovaries or parts examined of these two species.

## MATERIAL AND METHODS

In the present survey 496 ovaries of adult ferrets were used, and 39 and 39 ovaries of young, i.e. non-mature ferrets. In a few instances only one ovary from an individual had been preserved, but in most cases both could be examined. The animals had in most instances been killed by chloroform vapour; a few by coal-gas. The ovaries had been fixed immediately after death by one or other of the following fluids: Strong Flemming (with 2 or 3 per cent. acetic acid), Lenhossek's fluid, Mann's picrorosive sublimate (with and without formol), Maximow's fluid, Perenyi's fluid, Zenker's fluid. Each ovary was cut as a complete series of paraffin sections at 10 or at 7 microns. The stains were chiefly Mayer's Haemalum and Eosin, Heidenhain's Iron-Haematoxylin, Mallory's connective-tissue stain. On some of the specimens I have prepared recently I have used Heidenhain's "Azan" stain, and on others the Picro-Ponceau S stain of Curtis.

As the adult animals were purchased from dealers for breeding, no further information concerning age could be obtained. In the subsequent records, unless it is specially indicated that a statement refers to the ovaries of non-mature animals, the implication is that adults only are referred to. All the various stages of the sexual cycles are represented in the collection of ovaries examined.

Before the results of the present observations are studied, it appears desirable to indicate the limits of the term "pluriovular", as here employed. I do not apply the term to follicles which differ from the normal merely by the presence of other follicles in their thecae. I have observed such follicles and considered them in connection with the theory <sup>of origin</sup> of the true pluriovular condition. This restriction of the term appears from the literature to be traditional. Hartman, however, (1926) objected to the inclusion of primordial follicles in the group, for these might or might not become pluriovular. In the present numerical survey I have ex-

cluded primordial follicles in which the ova are in contact  
or are separated by only a single layer of epithelium, <sup>unless it</sup> passes  
from the non-adjacent side of the one ovum to that of the  
other without leaving a gap or groove externally. This excep-  
tion permits only a few follicles to be included, and they are  
strikingly different from many of the "primordial pluriovular  
follicles" of some writers.

The volumes of the follicles and of the ova, where recorded,  
have been estimated from the formula for the capacity of an  
ellipsoid:  $\frac{4}{3} \pi abc$ , where a, b, c are the semi-axes. The measure-  
ments of follicles are for comparison within the series, and  
were taken (as was the most convenient) so as just to exclude  
the thecae, which were not always distinguishable from the  
surrounding stroma.

9

In order to obtain a series of pluriovular follicles for systematic comparison, a complete survey of the collection of ovaries was first made. During this process, an attempt was made to acquire some slight indication of the frequency of the condition. Different groups of ovaries were formed by random choice, and each ovary in a group was subjected to the same examination. Although I cannot claim that any final determination of the frequency has been obtained, the results of the different methods are of interest.

#### RESULTS OF THE SYSTEMATIC EXAMINATIONS

A. 39 ovaries from 21 different animals. From each ovary two or three slides were examined, situated at equal intervals in the series. The numbers of sections examined averaged approximately one tenth of the total of each ovary.

Ovaries found to contain pluriovular follicles: 13.

Number of pluriovular follicles found: 25.

Number of animals in which the follicles were found:8.

Maximum in any one ovary : 4 (two instances).

Maximum in any one animal : 6 (two instances).

B. 58 ovaries, probably from rather over half that number of animals. \*

Two methods were employed on the same ovaries.

I. From each ovary 10 follicles of volume about .00016 cubic millimetres or over were traced from end to end. This examination included, therefore, follicles which on account of their volume might only reveal themselves as pluriovular when traced through the series containing them. The follicles chosen were those first met when examination of the ovary started from one end of the series, usually on one pole of the organ.

From the 580 follicles, 9 were pluriovular, from 8 different ovaries, i.e., about 1.5 per cent of the follicles examined.

II. From each ovary 10 sections were examined minutely. The intervals between the sections were chosen so as to be as nearly equal as was practicable. The sections chosen amounted on the average to about one from every forty.

\* The unfortunate accidental destruction, some years ago, of certain records prevents the grouping of a number of these ovaries according to animals.

11

In the 580 sections, 33 pluriovular follicles were found, from 19 different ovaries, i. e., very roughly, 0.1 per cent. of the follicles examined. The follicles examined (all those in the sections chosen) included many primordial follicles, and to this fact is, <sup>partly</sup> due the low percentage as compared with that of B I, in which the smallest follicles were excluded. Another factor is the serial nature of the examination in B I.

Maximum number of pluriovular follicles found in any one ovary by these two methods last described: 4 (2 instances). In only one instance was a follicle recorded under both headings. In both Examination A and B II, conducted on different ovaries, the ovaries found to contain pluriovular follicles were about one third of those examined.

C. In 399 ovaries not used in the examinations described above, inspection for other purposes had shown the presence, in some cases, of pluriovular follicles. I re-examined these ovaries, less minutely than those preceding. Two slides, each with about twenty sections, were taken, occasionally more. When the follicles now found were added to those previously found in these ovaries, the results were:

69 pluriovular follicles, from 46 different ovaries.

Total number of pluriovular follicles noted in these examinations of 496 ovaries ( including some follicles observed incidentally in the ovaries used for Examinations A and B): 154, from 82 different ovaries.

...the specimens, but no other conscious attempt was made to form a 'representative' series. The following table was compiled:

- Stage of the animal in the sexual cycle.
- Number of corpora lutea in the animal was examined.
- Position of the follicle in the ovary.
- Shape of the follicle.
- Size of the follicle in cubic millimeters.
- Presence or absence of liquor folliculi.
- Follicular epithelium - the number of layers and the shape of the cells, or an indication of the extent to which the follicle had developed.
- Characteristics in the follicular epithelium.

## COMPARISON OF PLURIOVULAR FOLLICLES

From the 154 follicles obtained, 100 were chosen for examination, so that impressions gained in the previous survey should be verified or corrected. Complete follicles and those best preserved were selected, and the choice was spread widely among the ovaries containing the specimens, but no other conscious attempt was made to form a "representative" series. The following facts were tabulated:

Stage of the animal in the sexual cycle.

Number of corpora lutea if the animal was pregnant.

Position of the follicle in the ovary.

Shape of the follicle.

Size of the follicle in cubic millimetres.

Presence or absence of liquor folliculi.

Follicular epithelium - the number of layers and the

shape of the cells, as an indication of the extent to

to which the follicle had developed.

Degeneration in the follicular epithelium.

Number of ova in the follicle.

Degeneration of ovular nucleus or cytoplasm.

Relative sizes of the ova.

Position of the ova in the follicle, and relationship to each other.

The information so obtained is incorporated, where pertinent, in the subsequent discussion.

Owing to the lack of standardisation and systematic study of the condition within the various species of animals, a comparison of one species with another in this respect yields few data. So far as ascertained, however, they are interesting.

MARSUPIALS. Hartman's extensive researches (1926) on the opossum indicate that the pluriovular follicle is very common in this animal. Approximately two thirds of the preparations examined (ovaries from about 150 animals) showed the condition, and negative results were in some instances due probably to incompleteness of the series of sections. On the other hand, O'Donoghue (1912) examined minutely serial sections of the ovaries of 45 individuals of another species of Polyprotodont Marsupials - Dasyurus viverrinus, and found only two animals in which such follicles were present.

EUTHERIANSMAMMALS. Among the Edentate group, the Texas armadillo was carefully investigated by Patterson (1913), who in over 50 pairs of ovaries did not find a single pluriovular follicle. This forms an instructive contrast to Rosner's results (1901),

based on limited evidence.

Ungulates. Two factors require to be borne in mind as influencing the frequency with which a condition like that of the pluriovular follicle is reported: the extent to which the animal is used for research upon the Reproductive System; and the size of the ovary, determining frequently the chances of its being examined in whole or in part. Probably the pig is the Ungulate most thoroughly investigated at present in this respect. Corner's wide experience led him to conclude that in this animal the pluriovular follicle is uncommon (1915 & 1923), - a conclusion somewhat different from that of Schmaltz (1911)\*. The possibility of differences in different breeds occurs to one's mind, but so also does the difference in the extent of the two sets of observations.

Rodents. The extent to which some of the genera and species of this group are used for ovarian research, together with the occasional appearance of reports upon the pluriovular follicle, might be thought to indicate the infrequency of the phenomenon. On the other hand, the common attitude is perhaps indicated by the illustration of one

\*See note on page 17

\*. Two publications by R. Schmaltz appeared in 1911. One was the section of Ellenberger's "Handbuch der vergleichenden mikroskopischen Anatomie der Haustiere", devoted to the sexual organs; the other was entitled "Die Struktur der Geschlechtsorgane der Haus-säugetiere", which might be presumed to be a "Sonderabdruck" of the section of the Handbook. I have had access only to the latter. This states that in the pig pluriovular follicles contain up to 3 oocytes, and are "häufig" (Band II., S. 538) or "nicht selten" (Ibid., S. 560). Corner's quotation from the other work indicates that Schmaltz found such follicles in almost <sup>every</sup> preparation of the sow's ovary, with up to 6 oocytes; but commonly 4. This is the statement made in the Handbook, regarding the dog (Ibid., S. 563).

such follicle in the mouse ovary, shown by Allen, Francis, &c. 18  
(1924) during discussion of another subject.

Carnivores. Schrön's results from the examination of the dog  
ovary (1863) are by no means in agreement with the belief held by  
Waldeyer (1870), Balbiani (1879), Ancel (1903), and Schmaltz (1911).

The concensus of opinion appears to be that the pluriovular  
condition is common in this animal. Apart from Schrön's (1863)  
observations, no very systematic work on the cat's ovary for the  
present purpose seems to have been carried out. In the present  
examination of the ferret ovary, I lay most stress on the results  
of the observations A and B described above. From these we may  
conclude that the condition is moderately common in the ferret.

Cheiroptera. van Beneden (1880) stated that in the horse-shoe bat  
pluriovular follicles were more abundant than in Vespertilio  
murinus.

Primates. The size of the human ovary tends to lessen the chance  
of an abnormal follicle being found, but this factor is probably  
over-neutralised by the enormous numbers of ovaries examined.

Welch (1907) instituted inquiries among many histologists and patho-

logists, and the verdicts agreed with that of Waldeyer (mentioned by von Franqué, 1898), indicating the rarity of the pluriovular follicle in the human species.

The results of this comparative study, such as it is, appear to indicate: the frequency of the pluriovular follicle in the opossum; its infrequency in Dasyurus; its frequency in the dog; its infrequency in the Texas armadillo, in man, and, at least in certain varieties of pig; its moderate frequency in the ferret.

The paucity of data prevents any extensive correlation between the species-distribution of this condition and that of other phenomena, but such comparisons as are possible are of some value as introductory to the more fruitful study of the individual instance.

## CONCOMITANT PECULIARITIES OF OVARIAN STRUCTURE

### (1) Plurinuclated Ova.

It has been contended by some authors, working with young ovaries, that the multiple nucleus is a precursor of the pluriovular follicle, (Quincke, 1863; Klebs, 1861 & 1863; v. Beneden, 1870. Balfour, 1878, and Eismond, 1898, inclined to a similar view). In adult human ovaries, apparent stages of transformation from the one condition to the other have been observed by Stöckel (1899), Rabl (1899), and Schumacher and Schwarz (1900). Unfortunately, our knowledge of the frequency of plurinuclated ova is as unsystematised as that of the frequency of pluriovular follicles. An investigation of the species-distribution of the former condition reveals its great frequency in the opossum (Hartman, 1926). van Beneden (1880) stated that in the bat plurinuclated primordial ova were met here and there, and, like pluriovular follicles, were more common in the horse-shoe bat. There seems to be no evidence of frequency of the multiple nucleus in the ova of the adult dog. The concomitance of the two conditions in an individual, or in a species (opossum), or in ovaries of young animals (e.g. the cat;

von Winiwarter and Sainmont, 1908-9), does not prove any direct causal relationship. Criticism of this suggested mode of origin of the abnormal follicles has been advanced on various grounds. Bischoff (1863) did not believe in the existence of plurinucleated ova, but considered pluriovular follicles not uncommon. Waldeyer (1870) opposed Klębs' contention that egg-division took place in the follicles after their separation as such. Observation of individual ovaries from various species of animals, and containing numbers of pluriovular follicles, has shown no evidence of association with plurinucleated ova (P. & M. Bouin, 1900; Honoré, 1901; Arnold, 1912; O'Donoghue, 1912; Hartman, 1926 - a cat specimen).

Criticism on more theoretical grounds has been brought forward by Honoré (1901) and O'Donoghue (1912), who have found it difficult to believe that a fairly large number of ova of different sizes in one follicle should have been developed from one precursor. There appears to be little difficulty in supposing that a repeated division could take place, but there is lack of evidence that it does. The argument from difference in size seems to rest on two assumptions: (i) that the growth-potentialities of

the offspring of an originally single oocyte should be equal. One calls to mind as an analogy the similarity of uniovular twins.

(ii) that the situation of each ovum in the follicle is equally favourable for nutrition. We may suppose that contact with the epithelium forming the outer wall of the follicle will be one of the chief factors in the securing of adequate nutrition. In the opossum, where very large numbers of ova are found in the same follicle (Hartman, 1926), this favourable situation cannot be secured by every ovum. In the ferret the numbers of ova are not nearly so great, a single follicle with probably fourteen ova being the greatest deviation from the normal that I have found. It appears to be the rule in this animal that all the ova in a follicle are partly in contact with the outer wall, and yet considerable difference of development is sometimes present in the different ova.

I commenced these investigations with the object of ascertaining the frequency of the plurinucleated ovum in the ferret, as well as studying the pluriovular follicle; but careful scrutiny of thousands of ova in the adult has failed to reveal an instance of this nuclear abnormality. The series of ovaries from young

animals is much more limited; but I have obtained from it evidence of the method of formation of the follicle, and have observed in it numerous pluriovular follicles, and here also I find no evidence that multiplied nuclein in the ovum are connected with these follicles. I am not able to offer any positive evidence concerning the contrary view - that plurinucleated ova arise by fusion of separate ova (Goette, 1875; Rabl, 1899; Hartman, 1926; and others),

*the production of*

(2) Abundance of Small Follicles.

In the horse-shoe bat, as has been noted, van Beneden found pluriovular follicles more common than in Vespertilio murinus. In the latter, also, the same author (1880) stated that primordial follicles were much less numerous than in the former (but individual variation was great). Even in the anoestrous period, the opossum ovary contains a very large number of small follicles (Hartman, 1923). Individual cases in which pluriovular follicles were accompanied by an abnormal abundance of primordial follicles have been reported by v. Franqué (1898; human), Schumacher and Schwarz (1900; human), and Hartman (1926; cat). In the ferret, follicles of various sizes are to be found in the ovaries at all periods of the year, but are not typically quite so abundant as is indicated for the opossum by Hartman's figure 10 (1923), representing the typical anoestrous condition of the ovary of that animal. I am not prepared to correlate any abnormality in this respect with the presence or absence of pluriovular follicles in the individuals I have examined.

(3) Epithelial Ingrowths.

Tube-like ingrowths of the germinal epithelium- the "schlauchförmige Einsenkungen" of Waldeyer - form, as this author pointed out (1870), a marked feature of the ovary of the dog. Balbiani (1879) and Schmaltz (1911) also referred to these in the adult dog. Hartman (1926) compared with these ingrowths the "primordial egg masses" found in the opossum. Balbiani (1879) stated that in the dog they often contained no ova. A further comparison is made by Hartman (1926) with the structure found in the ovary of a 18-year old girl by Plihal (1869), and diagnosed by him as a Pflüger's tube. Individual instances of the coexistence of pluriovular follicles and epithelial ingrowths have been noted by Schumacher and Schwarz (1900: adult human) and by Woerdeman (1921: human, probably adult).

The ferret ovary contrasts with that of the dog in possessing a tunica albuginea that forms a definite narrow band lying close beneath the germinal epithelium. Where strands of ova and epithelium extend inwards from near the free surface, the admixture with spindle-cells and fibrils is astarule intimate. Tube-like downgrowths like those of the dog ovary are very rare. In the adult ferret ovary I have not found that pluriovular follicles are associated with abnormally numerous epithelial ingrowths or follicular strands, but, as will be indicated later, it appears that the production of the abnormal follicle is dependent on the relationship of follicle and connective tissue at this very early stage of follicular development. This question of epithelial ingrowths is, in fact, merely a part of the larger question now to be treated.

THE RELATIONSHIP OF THE OVARIAN CONNECTIVE TISSUE TO THE FOLLICLES

The relationship of the supporting connective tissue to the gland substance supported, is a matter of interest in all glands both to the histologist and to the pathologist, but in the ovary is of special interest. Here the processes of development and decay are always detectable, and here, if anywhere, we should expect to discover failure in the balance between the glandular and the connective tissue.

Two chief types of opinion have been held concerning the mode of action of the connective tissue in the production of pluriovular follicles:

- (1) Secondary union of follicles owing to weakness of the intervening tissue;
- (2) Primary disproportionate development of the follicles and the connective tissue.

(1) Secondary Union. Three suggestions have been made concerning the sizes of the follicles concerned:

- (i) Union during enlargement of primordial follicles, ment.

suggested by Grohe (1863). Observations to test this view are rendered difficult by the similarity between connective tissue cells and flat follicular epithelium, but careful staining minimises this difficulty. Two follicles of primordial size are frequently seen close together, only partly separated by connective tissue, and it is difficult in any single instance to decide whether this tissue is penetrating or being expelled. The former alternative is, of course, favoured by our knowledge of the general tendency of the cells of this tissue to penetrate, and especially the demonstration of this tendency kinetically by tissue-culture experiments ( see, for example, Fischer, 1925, p. 117). The actual frequency with which the partial separation is seen, compared with the frequency of pluriovular follicles, indicates that invasion is proceeding in the vast majority of cases.

(ii) Union of medium-sized or large follicles. Rosner (1901) in armadillo ovaries found that all primordial follicles were uniovular, and published figures to represent stages in a supposed union of ~~non~~<sup>uni</sup>ovular follicles of larger size, even containing liquor

folliculi. Lobulation of the follicles that contained more than one ovum was cited in support of this contention. In the ferret I have on only two occasions seen two follicles (of about medium size), between which the connective tissue cells were not quite so evenly disposed as is usual at that stage of development. It frequently happens that two follicles are brought close together by pressure either from without or from within, and then the follicular epithelium and the thecal interstitial cells become much flattened; the connective tissue remains quite unbroken (fig. 17). In the two instances mentioned above, the follicles were not pressed so closely as to cause much flattening of the epithelium, and the connective tissue cells near were numerous and apparently healthy. An adequate explanation would be that they were, later than usual, entering the colloid-filled interval between the follicles, and not being expelled or pushed aside. Lobulation of pluriovular follicles occurs when a follicle without liquor contains a larger and a smaller ovum; the smaller forms a projection. This appears explicable in the same manner as is the thinning of the epithelium over the smaller

ovum(see below). In follicles with liquor folliculi present, lobulation is not a special feature of the pluriovular variety.

(iii) Union of a small with a large follicle. L.Loeb(1917)discussed the "hypotypical" ovary of a guinea-pig ,which had been underfed, had had its thyreoid gland removed and the lobe of another thyreoid engrafted subcutaneously. Pluriovular follicles here,the author claimed,were formed mostly by "concrecence", - a process by which small follicles,especially primordial,push~~d~~ their way into larger ones,perhaps even into degenerating follicles. The thickness of the granulosa surrounding the different eggs in a follicle was noted to accord with the character of the egg,and this was thought to be compatible only with this view of secondary union.

Ovaries containing pluriovular follicles are met apparently fortuitously in the guinea-pig(Loeb,1901),and it seems at least possible that in the one in question these follicles did not owe their origin to the induced "hypotypical"condition. In the ferret I have noted several instances of the presence of a small follicle in the theca of a larger one(fig.11),but I find no evidence that the former ever lose<sup>s</sup> its integrity. As regards the character of the granulosa,and<sup>1</sup> of the epithelium in general,in contact with the different ova,

~~the granulosa~~, I have observed that, where there is ~~an~~ dispro-  
 portion in size between two ova in <sup>a</sup> follicle without liquor, the  
 epithelium over the external surface of the smaller ovum is of-  
 ten thinner than over the corresponding surface of the other. <sup>(fig. 4)</sup> It  
 appears quite possible to account for this by supposing that the  
 two ova were originally of the same size, but one started to grow,  
 and so tended to arrange about it a ring of neighbouring struc-  
 tures, as occurs around any growing object in the ovary (follicle  
 or corpus luteum). The other <sup>ovum</sup> had ~~in virtue~~, in virtue of its in-  
 ternal tension, resisted flattening, and had projected, stretching  
 the layer of cells outside it. In any case, we may suppose that  
 there is a certain amount of control of the epithelium by the  
 ovum lying closest to it. ~~it.~~

In concluding this section devoted mainly to negative evidence,  
 I may remark that in no ovary showing pluriovular follicles have  
 I found evidence of deficient power of the connective tissue,  
 such as was manifested, according to Loeb (1917) ~~and~~ by the atypical  
 atresia in the ovary of the guinea-pig already mentioned. Moreover,  
 so far as the eye can judge, I have seen no quantitative deficiency  
 of the connective tissue.

(2) Primary Disproportionate Development of the follicles and the connective tissue. Suggested by Schrön(1863), considered possible by Balfour(1878), this view was advanced most satisfactorily by Schottländer(1893),<sup>§ 1905</sup> who stated that pluriovular follicles resulted from the irregular growth of connective tissue in the formation of follicles from egg balls. The Bouins(1900), Honoré(1901), O'Donoghue(1912) and Hartman(1926) adopted a similar explanation. In a number of cases this view has been assumed without proof or without a consideration of the various possible factors at work. Mere exclusion of the other two possible modes of origin of the abnormality (the origin from plurinucleated ova or from secondary failure of connective tissue) hardly justifies one in adopting this third possibility without an attempt to find <sup>apparent</sup> possible stages in the process. This fact, and the value of comparing in detail the abnormal with the normal, have induced me to suggest what appear to be the important factors in the production of the pluriovular follicle.

Even in an ovary with fairly numerous pluriovular follicles, it is not easy to find material for the proof or disproof of the suggestion under consideration. Even in these most favourable specimens the proportion of follicles that are destined to become pluriovular must, in the ferret at least, be fairly small.

In the ferret ovary, as Robinson(1918) pointed out, primordial ova are usually surrounded by a single layer of flattened epithelial cells. Examination of numerous specimens at that stage has indicated the possibility of five factors being present in the abnormality of balance between the follicular and the connective tissues, probably to different degrees in different instances:

(i) Rate of growth of oocytes. I have seen instances of two ova lying with their adjacent sides parallel and separated by epithelium that was very much flattened. It appears that in such

cases growth of the oocytes has been more rapid than can be accommodated by displacement of neighbouring structures, and the follicles instead of forming two separate spheres, tend to form a single one, which may then be surrounded by a common layer of connective tissue

(ii) Distribution of the follicular epithelium. In some instances only a single layer of flat epithelium lies between two primordial ova. This condition is more frequently met than is the small pluriovular follicle, and it therefore appears probable that some other factor must be necessary for the production of the latter, even if the single epithelial layer plays a part. Small pluriovular follicles, indeed, frequently show only one layer of flat epithelium between two ova.

(iii) Growth of follicular epithelium. The sulcus around the area of contact of two primordial follicles is in some instances filled with epithelium (figs. 12 & 13), which may even form a projection outwards instead of the usual depression. Connective tissue cells lie alongside. The next stage to this appears to be suggested by rather more developed follicles (fig. 14), possessing epithelium which is broader than before on the non-

adjacent surfaces of the ova. In the area between the ova the epithelium is flat; at the margins of this area, i.e. in the sulcus between the ova, it is pyramidal. The edge of the follicle near the sulcus is uninterrupted; connective tissue cells are parallel to the edge.

(iv) Slowness of growth or lack of penetrative power of the connective tissue. As mentioned above, I have never seen any generalised deficiency of this kind. The last factor (iii) implies a local defect of the connective tissue, at least relative to the follicular epithelium.

(v) Distribution of the connective tissue. In some regions of an ovary the connective tissue cells are not so evenly distributed as in other regions. They may be arranged in strands along the sides of a row of two or <sup>primordial</sup> three follicles, and none at right angles to this. They appear less favourably placed than usual for the surrounding of each single follicle before the epithelium of two or more primordial follicles <sup>to form a common syncytium.</sup> has united. In some instances the factor responsible for this distribution of the connective tissue seems to be the rate of ingrowth of strands from near the germinal epithelium. Comparison with the ingrowths of the dog ovary is suggested.

Whichever of the suggested factors may be responsible, the essential attainment appears to be an unbroken epithelial surface. There is thus no capillary interval left between the follicles. Instead, there is a capillary interval between the follicular surface and the surface of neighbouring structures, and we may suppose that, under the capillary and surface-forces here acting, the young connective tissue cells are compelled to migrate and thus form the rudiments of a theca common to the epithelium which surrounds the several ova. (Compare Fischer, 1925, page 39, for the importance of capillary forces in the migration of cells).

## THE DEGREE OF THE ABNORMALITY

(1) Number of ova in a follicle. From the literature, it appears that the minimal deviation from the normal number of ova is the commonest - the biovular follicle. The tables given by Rosner (1901) and Arnold (1912) indicate that this holds within individual ovaries that contain a number of pluriovular follicles. The importance of serial sections in an estimation of this kind need hardly be emphasised. From the hundred pluriovular follicles which I have completely examined, the following figures were obtained :

74 follicles each contained 2 ova.

18 " " " " 3 "

2 " " " " 4 "

3 " " " " 5 "

2 " " " " 6 "

1 follicle contained, as nearly as could be determined, 14 ova, all of them small.

Records of follicles containing a considerable number of ova have been made : about 10 ova (the Bouins, 1900:dog); 12 (Hartman,

1926: Macacus rhesus); 13 (Arnold, 1912: human); over 100 (Hartman, 1926: opossum; this is exclusive of "egg-masses"). The last figure appears to indicate that frequency of the condition is accompanied by greatness of degree.

(2) Relation of the epithelium to the ova within the follicle.

van Beneden (1880) described in the horse-shoe bat two types of compound follicle : one in which two or three eggs were in direct contact; the other in which follicular epithelium separated the ova. Both types have been recorded in subsequent publications. The results of the examination of the 100 follicles in the ferret may be summarised as follows:

Ova in contact: 37 follicles. Of these -

22 follicles showed no liquor folliculi; 9 showed a trace;

5 showed liquor; and in 1 the liquor had apparently been absorbed .

Ova separated by epithelium: 63 follicles. Of these -

24 follicles showed no liquor folliculi; 10 showed a trace;

28 showed liquor; and in 1 the liquor had apparently been absorbed.

Elongated follicles containing several ova in a row, mentioned by Schumacher and Schwarz (1900) are apparently common in the opossum (Hartman, 1926). They occur, but are <sup>very</sup> uncommon in the ferret, (fig. 10)

Although the distinction is not of great intrinsic value, the presence or absence of epithelium between two growing ova is important in regard to the theory of origin of the zona pellucida. Foulis(1874) and van Beneden(1880) showed that two ova in contact developed the zonae pellucidae just as did ova surrounded by epithelium - an indication that the ovum, and not the epithelium, was the responsible factor. The observations of O'Donoghue (1912) and Hartman (1926) support this view, as do some of my own. The other possibilities (emigration or degeneration of the epithelium after it had formed the zonae) were not considered likely by Hartman (loc.cit). In most cases degeneration can easily be dismissed. To prove that emigration does not occur in any given instance would be difficult but the numbers given above suggest that invasion of the interval between the ova occurs as the follicle enlarges, after the zona is at least partly formed. (The index of size in the list above is the presence or absence of liquor folliculi.) In the majority of the small pluriovular follicles that I have examined, the adjacent sides of the growing ova were in some degree parallel. Where epithelium was present between such ova, it was very much

thinned and yet in many instances unbroken. It appears that it can suffer great pressure without losing its continuity. From a surface-view that I have obtained of such epithelium, I consider it probable that the pressure merely thins the epithelial syncytium, separating the nuclei somewhat; and that, after the pressure is reduced, the epithelium becomes thicker again.

In the larger pluriovular follicles that have been described, various degrees of separation of the ova have been noted, from those follicles in which a common cumulus contained two or even more closely apposed ova (O'Donoghue, 1912) to those described by Smyth (1908) and by Welch (1907), in which the two ova lay at wide separate parts of a large follicle. Of the 100 follicles that I have examined specially, there were 30 in which the cumulus was observable, either completely or partly formed. They may be grouped as follows:

Follicles in which the ova possessed separate cumuli: 20.

Follicles in which the ova lay in cumuli which were in the process of separation from each other: 8.

Follicles in which the ova possessed a single cumulus: 2.

The first appearance of the liquor folliculi in the ferret pluriovular follicle is commonly among the epithelium in the sulcus between the ova (fig.7), and not so commonly near the pole or poles of the follicle, as is usual in the uniovular variety. Subsequently the cumuli containing the different ova become, in the majority of cases, widely separated (figs 1, 2 and 9). We may probably account for this wide separation by supposing that the parts of the follicular wall to which the ova are attached, being thicker, are less elastic; and that the other portions will be more stretched by the increasing liquor folliculi.

fully follicles in the process of formation. The ovaries were examined from 4 weeks (1 specimen); 7 weeks (5 specimens); and about three months after birth. The ovaries were examined in the same group, but not all born of one mother. The ovaries of this group were fully formed follicles and in all of these ovaries some of the follicles were pluriovular.

I examined the ovaries of three animals about three months old, and found the following:

RELATIONSHIP OF THE AGE OF THE ANIMAL TO THE OCCURRENCE OF  
PLURIOVULAR FOLLICLES

The historical summary given above shows that some of the past observations on these follicles have been concerned only with young animals. Klebs (1861) and Grohe (1863) emphasised the comparatively great frequency of such follicles in the ovaries of young children. They are common in the ovaries of the recently born cat (v. Winiwarter and Sainmont, 1908-09).

Of the 39 ovaries of young ferrets that I have examined, three-quarters were from the earliest days after birth, and showed principally follicles in the process of formation. The remainder dated from 4 weeks (1 specimen); 7 weeks (2 specimens); and about three months after birth. The animals contributing to this second group were not all born of one mother. The ovaries of this group showed fully formed follicles and in all of these ovaries some of the follicles were pluriovular.

I examined the ovaries of three animals about three months old, and noted the following:

Animal killed at 11 weeks old:

Right ovary (about 1/16th of the ovary examined): 22 pluriovular follicles.

Left ovary (about 1/13th of the ovary examined): 11 " "

Animal killed at 12 weeks old:

Right ovary ( about 1/15th of the ovary examined): 12 " "

Left ovary ( " " " " " " " " ): 10 " "

Animal killed at about 3 months old:

Right ovary ( about 1/15th of the ovary examined): 18 " "

Left ovary (about 1/20th of the ovary examined) : 23 " "

Other slides of these ovaries were similar to those giving the record  
At the age of three months the follicles in the ferret ovary can

be arranged in three groups:

- (1) Deeply placed, cord-like follicles with little or, sometimes, no cavity.
- (2) More peripheral, large follicles, often piriform or lobulated.
- (3) Numerous small spheroidal follicles, close to the surface of the ovary.

Pluriovular follicles are mostly found in classes (1) and (2).

The relationship of these structures to the pluriovular follicles

of adult ovaries is raised by the article of Woerdeman(1921), who described a human ovary, probably adult/ in which were two types of pluriovular follicles:

(1) Some which had apparently arisen from medullary cords.

(2) Some which had apparently arisen from egg-balls.

The author emphasised this distinction because of the supposed homology between medullary cords and seminiferous tubules. This homology was <sup>accepted</sup> ~~suggested~~ by v.Winiwarter for the non-mature rabbit (1900) and applied by him and Sainmont(1908-09) to the non-mature cat; but criticised by Kingsbury(1913), after investigation of the cat. In the adult ferret ovary, as I have stated, I have occasionally seen follicles with several ova in a row. They are small follicles lying in the cortex, and do not differ except in shape from the other pluriovular follicles. I have found, in the adult ferret, no evidence that pluriovular follicles arise from medullary cords. v.Winiwarter and Sainmont, indeed, (1908-09) considered it questionable whether the medullary structures of the adult were the same as those of the young animal.

The abundance of degeneration in the pluriovular and other follicles in the young ferret ovary (as in that of the young cat, (v. Winiwarter and Sainmont, loc. cit.), indicates that we must account for pluriovular follicles in the adult by factors present there, as I have endeavoured to do. Nevertheless, the ovary of the young animal is of value in this study, because, as Kingsbury (1913) pointed out, it illustrates the effects of varying relationships of follicular tissue and stroma. The young ferret ovaries indicate that the lack of balance between these two <sup>tissues</sup> groups, incident to the growth and development of the ovary, is responsible for the greater frequency of the pluriovular follicle and also for the greater variety of shape. (fig. 15). One of the most striking facts in the three-months ovary is the comparative infrequency of pluriovular follicles among the smaller follicles near the periphery - the follicles that have been produced when the relation of connective tissue to follicular tissue is more like that of the adult.

I have, therefore, been led to the conclusion that the factors suggested above as the cause of the pluriovular follicle in the

adult, are those responsible in the young also. They may be summarised as the relationship, in regard to distribution and growth-rate, of the ovum and follicular epithelium on the one hand, to the connective tissue on the other.

## GROWTH AND DESTINY OF THE PLURIOVULAR FOLLICLE

(1) Conversion to uniovular follicles by ingrowth of stroma - a view entertained, as regards small, or, especially primordial follicles by a number of authors, some of whom, however, regarded other fates as also possible. It has been held by Quincke (1863); Schottländer (1893); Hellin (1895); Stöckel (1899); Rabl (1899); Hansemann (1912). Hartman (1926) criticised the application of the term "pluriovular" to primordial follicles, which might, or might not become definitive pluriovular follicles. In the adult ferret, I do not consider it at all common for ~~the~~ even the smallest pluriovular follicle, as defined earlier, to be converted into uniovular follicles. One of the rarely seen long follicles, containing several ova in a row, appears to be undergoing the process at one (fig. 10) part. This process of separation seems more likely in some of the follicles of young ovaries, but here it is probably accompanied by degeneration (fig. 16).

(2) Degeneration. Instances of this were described and discussed by Honoré(1900) and Hansemann(1912).The latter considered that a uniovular follicle could arise by degeneration of all ova in a follicle except one. Atresia of the pluriovular follicle has been accepted as the common fate by Janoik(1887), Schmaltz(1911), Hartman(1926)

The evidence of degeneration in ovum, follicular epithelium or both, noted during my examination of the 100 ferret pluriovular follicles, may be summarised as follows:

- (i) Follicles with no liquor folliculi (total - 46):  
9 degenerate; 3 commencing to degenerate; 4 doubtful.
- (ii) Follicles with a trace of liquor folliculi (total - 19):  
9 degenerate; 2 commencing to degenerate; 4 doubtful.
- (iii) Follicles with liquor folliculi in moderate or large quantities (total - 33):  
28 degenerate; 3 commencing to degenerate.
- (iv) Follicles from which the liquor folliculi had probably been absorbed (total - 2): Both degenerate.

(3) Growth.

## (i) Growth of the Ova.

The ova in a pluriovular follicle may be all about equally developed, whether they are two or more in number (Schrön, 1863; O'Donoghue, 1912; Arnold, 1912). Non-degenerate biovular follicles may contain ova unequally developed (Loeb, 1901). Primordial ova may be contained in advanced follicles, along with larger ova (Schottländer, 1893; Loeb, 1917; Hartman, 1926). It was as small ova that Staziewitch (see Alexenko, 1891) and also Schottländer (1893) interpreted the "Nährzellen" of Nagel (1888). (Hartman, 1926, suggested that Schottländer, Nagel and others had before them different structures - true ova and swollen granulosa cells. The latter are common in or adjoining the cavity of atretic follicles in the ferret (fig. 6), but the resemblance to ova is usually superficial.)

In the examination of the 100 pluriovular follicles in the ferret, it was noted whether the ova in a given follicle were equal in size, and in thickness of zona pellucida. The estimate in most cases was made by the eye; but even so

inexact a method revealed that 37 out of the 100 follicles contained (figs. 3 & 4) ova that were obviously unequal. Various degrees of disparity in size were met. Thus, Follicle No. 33 contained one large ovum and three just above primordial size. (fig. 5) Follicle No. 66 contained two ova, of which the volumes, as determined by measurement, were in the ratio of 7 to 3. In Follicle No. 71 were 6 ova, of volumes, expressed in cubic millimetres:

.00015	.00017	.000012
.000029	.00049	.000024



This disparity might be accounted for in various ways:

(a) Inclusion of a small follicle in a larger one (Loeb, 1917).

This theory of the formation of these follicles has already been discussed.

(b) Arrested development of the smaller ovum, suggested by Arnold (1912), who further suggested that the smaller ova would very probably degenerate further. The series I have examined hardly proves this. Neglecting the instances in which an ovum is smaller than its fellow because it is degenerate and crumpled, one still sees

follicles in which one small ovum is degenerate, while another, larger, is not. But the reverse may also be the case; and, further, great differences in degree of degeneration may be seen in ova of similar stages of development in the same follicle. In any given follicle, therefore, one cannot assert that the smaller ovum is necessarily nearer degeneration.

(c) Different thresholds of response to growth stimuli (Hartman, 1926). Outside pluriovular follicles it is quite common to see differences in size in ova which, from their position, we may presume to have arisen at the same time, and are now apparently in the same environment. The factors causing the one to grow are insufficient to move the other from its quiescent state. The first may, therefore, soon reach the almost inevitable stage at which the balance of metabolism is no longer in favour of life. The second may now either have modified its power of response or been stimulated by other influences, and proceed to grow. This applies equally within the follicle.

## (ii) Growth of the Follicle and Maturation.

Many of the pluriovular follicles described in the literature are small, even primordial (e.g. those of Stöckel, 1899; Rabl, 1899; <sup>Smith,</sup> Hansemann, 1912). Large follicles have, however, been recorded (e.g. those of Welch, 1907; Schulin, 1881; V. Franque, 1898; Smyth, 1908; O'Donoghue, 1912).

The volumes of the 100 follicles I have examined were estimated, for purposes of comparison within the series, and a note of the presence or absence of liquor folliculi was taken. The results may be grouped as follows (volumes expressed in cubic millimetres):

Follicles of .000 01 or over; under .000 1 : 4 (no liquor).

Follicles of .000 1 or over; under .001 : 27 (2 with a trace of liquor; rest without).

Follicles of .001 or over; under .01 : 43 (2 with liquor probably absorbed; 7 with liquor; 17 with a trace; 17 without).

Follicles of .01 or over; under .1 : 23 (all with liquor).

Follicles of .1 or over; under 1.0 : 3 (all with liquor).

The largest follicle had a volume of 0.74 cubic mm.

An attempt might be made to extract evidence of the cause of the condition from the numbers of follicles of different sizes. This would be unjustified. A fair number of the follicles had been detected in general surveys of the ovaries, and the examples most likely to be detected were those somewhat larger than the smallest, and yet small enough to present a part of both ova in at least one of the sections of the follicle.

The following ripe pluriovular follicles are on record:

One in a dog (Bischoff, 1845); two in Dasyurus (O'Donoghue, 1912); one in a pig (Corner, 1915). I have never seen a ripe pluriovular follicle in the ferret.

THE RELATIONSHIP OF PLURIOVULAR FOLLICLES TO FERTILITY

"Fecundity" was the term used by Pearl (1912) "to designate the innate reproductive capacity of the individual organism, as denoted by its ability to form and separate from the body mature germ cells." It is with this, rather than with general fertility, that we are concerned here. The relationship of pluriovular follicles to fecundity may be one of two chief types:

(1) Coexistence of these follicles with diminished fecundity or sterility (Loeb, 1917). The evidence from ovulation-rate in the ferret (see below) inclines in this direction; but is quite inadequate.

(2) Coexistence of the follicles with increased fecundity. This may imply either direct influence upon the fecundity, or an indirect relationship - the presence of a common cause.

(i) Direct: - Monochorionic twinning was held by Rosner (1901) and Ancel (1903) to be due to the liberation of more than one ovum from one follicle. This contention was criticised by Cuénot (1903), and, as regards the armadillo (for which Rosner thought it established) it has not survived before the constructive work of Newman and

Patterson on specific polyembryony. (See Newman and Patterson, 1910; Patterson, 1913; Newman, 1917.) The probable rarity with which two ova in a follicle mature and are liberated, <sup>(see below)</sup> renders this suggestion ~~very~~ <sup>the ferret</sup> improbable for ~~the ferret~~. Moreover, close apposition of ova, necessary for the fusion of chorions, easily results from the simultaneous liberation of ova from separate follicles.

Biovular twinning. von Baer (1827) suggested that rupture of a pluriovar follicle would account for cases in which the (total) number of ova liberated was greater than that of the corpora lutea. Cases of this nature have been recorded (Bischoff, 1842; Corner, 1915; Lillie, 1923). In none of these cases, however, <sup>it appears</sup> does that monozygotic twinning or embryonic budding can be excluded. Adequate proof of the liberation of two ova from one follicle can only emerge from a complete examination of ova and ovaries soon after rupture of the follicles. Two animals in the series at present under consideration furnish useful evidence in this respect. From each animal both ovaries with oviducts were preserved as a complete

series of sections with the recently liberated ova still within.

Animal No.1. Killed 94 hours after insemination.

Ovary and oviduct No.(i). (Series I.,No. 59.):

Corpora lutea: 5 ; Ova in oviduct: 5.

Ovary and oviduct No.(ii). (Series II.,No.391.):

Corpora lutea: 3; Ova in oviduct: 4.

Both ovaries contained fairly numerous pluriovular follicles.

Three of the four ova in No.(ii) were at the 7- or 8-cell stage of segmentation;but the other ovum was unsegmented,was of volume only about a third of each of the others. It presented a zona pellucida well developed, but not quite so thick as on the others, and its nucleus, clearly defined, was peripherally placed; but showed no signs of maturation.

Animal No.2. Killed 41 hours after insemination.

Ovary and oviduct No.(i) - left side. (Series II.,No.194.):

Follicles just ruptured: 7 ; Ova in middle of oviduct: 7.

Ovary and oviduct No.(ii) - right side. (Series II.,No.195):

Follicles just ruptured: 3 ; Ova: 4 (3 in middle of oviduct,

1 in periovarian space), at pronuclear stage. No pluriovular follicles were seen in a thorough examination of both of these ovaries. The ovum in the periovarian space was probably degenerating.

(ii) Indirect:- Hellin (1895), von Franqué (1898) and Hartman (1926) have held that the tendency to fertility, manifest in twinning or large size of litters, conduces to the production of numerous ova, and to the formation of pluriovular follicles. Various types of evidence may be adduced in support of this view.

{a) Concomitance of numerous small follicles and pluriovular follicles has already been discussed. Even if this concomitance be accepted as a common rule, which is not supported by evidence from the adult ferret, it is not clear that a tendency to fertility is manifested by simple abundance of small follicles. It is equally possible that the latter merely indicates a retention

of a non-mature condition by the ovary; and in the non-mature state pluriovular follicles are fairly frequent. Hellin(1895), indeed, admitted this; but looked upon multiparity itself as a less highly evolved condition than uniparity, the ovaries of the multiparous being more embryonic in structure. It should be urged against this, that the fertility of younger animals is as a general rule less than that of somewhat older ones (Marshall, 1922); and it appears that this lower fertility is actually due to lower fecundity (Hammond, 1914).

The work of Pearl (1912) on birds affords proof that there is little relationship between abundance of oocytes and actual fecundity.

(b) Ovulation rate or Litter-size. Hellin (1895) laid

stress on the frequency of records of pluriovular follicles in the multiparous animals, as compared with the uniparous. The uniparous bat casts some doubt on this generalisation; and the mere absence of records is poor evidence of the absence of the abnormality.

The litter-size is not a very satisfactory means of determining the fecundity, on account of the various influences which tend to produce prenatal death in various amounts in different species (Robinson, 1921; Corner, 1923). It is most desirable to ascertain either the number of ova soon after ovulation, or the number of recent corpora lutea during or just after pregnancy.

Different species of animals may be first considered; and subsequently individual animals. Hartman (1926) gave the average number <sup>of ova</sup> shed by the opossum at one ovulation as 22 per animal. (Further detail is contained in an earlier article by this author, 1919.) There is, undeniably, co-existence of the follicular abnormality and fecundity in this species.

extremely fertile animal, and it is improbable that the actual fecundity is very great.

A comparison may be made between these animals, in which pluriovular follicles are apparently numerous, and other species, in which such follicles appear rare. Wallace (1923) held that a sow ought to rear 10 or more pigs in each litter after the first. The collection of pig ovaries concerning which we have the most definite pronouncement upon the rarity of pluriovular

The greater fertility (and probably also greater fecundity) is possessed by the species in which the pluriovular follicle is the least common.

Hill (1910) considered Diaparsus viviparus an animal with a great tendency to fertility, and mentioned several cases of 10 to 12 ova extracted at ovulation, and others even more numerous. In this species, O'Donoghue (1911) is probably referring to the ovaries of the species of Diaparsus mentioned only as Diaparsus, which would be pluriovular condition. It appears that the fecundity in this species

follicles, is that of Corner. ( The ovulation-rate of these animals (Corner, 1923) suggests that they belong to breeds of under the average fertility of British animals as given by Wallace. The lowest ovulation-rate in Corner's animals is that of a group of 156 sows, among which the rate was  $8.109 \pm .118$  per animal. In view of the size of the animal, however, the fecundity is <sup>still</sup> to be looked upon as high. It therefore appears justifiable to hold that, in regard to dog and pig, the greater fertility (and probably also greater fecundity) is possessed by the species in which the pluriovular follicle is the less common.

Hill (1910) considered Dasyurus viverrinus an animal with a great tendency to fertility, and mentioned several cases of 20 to 25 ova extruded at ovulation, and others even more remarkable. In this species, O'Donoghue (1912), in repeatedly examining serial sections of the ovaries of 45 animals, discovered only 3 <sup>ovaries</sup> (2 animals), which showed the pluriovular condition. It appears that the abnormality in this <sup>species</sup> animal is

occasional; and its relationship to <sup>the</sup> fecundity of the species is very doubtful.

In the ferret, the number of ova shed was given by Robinson (1921) as 9.95 per animal; and breeders consider 6 the average birth-rate per animal. Compared with the dog, the ferret has by no means a low fertility, and yet shows no abundance of pluriovular follicles.

Individual instances of abnormally high fertility accompanied by pluriovular follicles in the ovary, have seldom been reported. Ten children had been borne by a woman of 41 whose ovary showed the follicular abnormality (Schumacher and Schwarz, 1900). Smyth's case (1908) was that of a rather small Gordon setter, with 14 pups in her first litter. The ovaries of two of the pups were examined, and found to contain pluriovular follicles. The first litter of another daughter was 9. Hartman's criticism (1926) of these observations seems perfectly just: pluriovular follicles are probably frequent in dogs; the ovaries of the mother were not examined, and therefore the suggestion that the tendency to plurio

ovular follicles is hereditary, receives no support. The ovaries of young animals, moreover, appear frequently to contain follicles of this type. Further, I note that Heape (1906-08) gave 8.68 as the average litter-size for setters (19 different litters). Even admitting that the litter of 9 was a first litter, we hardly appear justified in granting that it was abnormally high.

Woerdeman (1921) gave an indirect reference to Strassman's case, in which it was alleged that the ovary of a woman who died while giving birth to twins was found to contain two ova in nearly every follicle. I have not been able to trace this further. Coincidence must always be borne in mind when a few cases of this kind are under discussion.

In the ferret series that I have examined, I have not, unfortunately, always been able to group the ovaries in pairs according to the animals to which they belonged, but the number of recent corpora lutea in the various ovaries has been noted, in regard to those containing pluriovulat<sup>t</sup> follicles. The average number of ova shed by each <sup>ferret</sup> ovary at one ovulation is just under 5. With

this may be compared the following figures, obtained from the ovaries which showed most abundant pluriovular follicles:

Two ovaries from one animal contained respectively 5 and 3 corpora lutea.

Two ovaries from another animal contained respectively 4 and 3 corpora lutea.

Single ovaries, all from different animals, contained corpora lutea as follows: 4 (3 ovaries); 3 (2 ovaries); 1 (1 ovary).

Admittedly, in none of these ovaries were the pluriovular follicles very numerous in proportion to the follicles of uniovular type, but the evidence indicates that the tendency to the abnormality of follicles may exist in an ovary which does not, by its ovulation-rate, show increased fecundity.

The foregoing evidence appears to justify the conclusions:

(i) that, ~~occasionally~~ occasionally, rupture of a pluriovular follicle occurs, and in such cases only one of the ova may be mature and healthy;

(ii) that there is no adequate ground for supposing a connection to exist between pluriovular follicles and fecundity.

## THE VALUE OF THE PLURIOVULAR FOLLICLE IN REPRODUCTION

The presence of any abnormality in an individual or a species prompts the question whether this abnormality is an advantageous modification on the way to be established, or a disadvantageous one, which may tend to be eliminated.

The most obvious disadvantage of the pluriovular follicle as a mode of reproduction is exemplified by the instance given above, in which, of the two ova from one follicle, one was by no means ready for liberation. This is probably the factor that would prevent the uniovular type of follicle being displaced by the pluriovular. The question may also be raised, whether the ova in the multiple follicle are not at a disadvantage as regards nutrition. One may, indeed, speculate upon this at great length, but an answer would only be obtained by further investigation, particularly of the area of the follicular surface and the volume of the liquor folliculi at corresponding stages of development in the normal and the abnormal follicle.

## SUMMARY

A Historical Summary of the previous records of the pluriovular follicle is given, and it is pointed out that analysis reveals the lack of standardisation of these, and the consequent uncertainty of the actual frequency of the condition.

Various systematic examinations of 496 adult ferret ovaries have been made. The numerical results are set forth. Two separate groups of the ovaries were chosen at random, and sections were examined. In both groups, about one third of the ovaries showed the pluriovular condition.

The proportion of pluriovular follicles to the total number of follicles examined in a fairly large series was, very roughly, 0.1 per cent. When the smallest follicles were excluded, and the sections were examined serially, the percentage was about 1.5.

100 pluriovular follicles have been specially investigated, and the results are introduced into the discussion.

The species-distribution of the follicles is indicated, as far as possible. The opossum and the dog are noted as frequently exhibiting it. In Dasyurus, in the Texas armadillo, in man, and in at

least certain varieties of pig, the condition appears rare.

The concomitant species-distribution of other characteristics of ovarian structure serves as an introduction to the study of the individual animal in the following respects:

- (1) Plurinuclated ova. The ferret negatives the suggestion that there is a relationship to the pluriovular follicle.
- (2) Abundance of small follicles. No evidence is obtained from the ferret.
- (3) Tubular ingrowths of the germinal epithelium - notable in the dog; rare in the ferret.

The relationship of connective tissue to follicular cells is treated.

- (1) The possibility of secondary failure of the separating connective tissue at various stages of follicular development is discussed, and dismissed as improbable in the ferret ovary .
- (2) The factors involved in the primary failure of the connective tissue to separate primordial follicles, are suggested. The pluriovular follicle in the ferret is attributed to this lack of balance

between follicular and connective tissues.

The degree of the abnormality is considered. Biovular follicles were found to be the most common. The greatest deviation from the normal in this series was a small follicle with probably 14 ova.

The relationship of ova to epithelium in the pluriovular follicle is seen in many instances to lend support to the view of Foulis and Van Beneden, that the zona pellucida is formed by the ovum itself.

Pluriovular follicles are found more abundantly in the non-mature ferret than in the mature. 39 young ferret ovaries have been examined for this purpose. The factors producing the condition are the same, qualitatively, as in the adult.

The growth and destiny of the pluriovular follicle are discussed. Conversion of true pluriovular follicles to uniovular is held to be rare. Degeneration of the ova and variations in the extent of growth of the various ova in a follicle are illustrated.

The relationship of the condition to fertility, in particular to "fecundity" (Pearl) is treated. Direct influence, by rupture of an abnormal follicle, is considered rare. Two instances of rup-

ture are recorded, in the ferret. Indirect relationship is discussed in the light of possible types of evidence:

(1) Abundance of small follicles - doubtful evidence of fecundity.

(2) Ovulation-rate (and litter-size). Pluriovular follicles may occur along with great fecundity (opossum); but they may occur abundantly without great fecundity (dog and bat). Fecundity may be great and the follicular abnormality not common (Dasyurus and pig). In the ferret, fecundity is moderately high, and the follicular abnormality is not very frequent. The evidence from individual instances is inadequate. The ferret ovaries which show most abundant pluriovular follicles do not show a high ovulation-rate.

The most obvious disadvantage of the pluriovular follicle is illustrated by the rupture of a follicle in which one of the ova was not ripe.

LITERATURE

Alexenko, N., (1891), Ann. de Gynécologie, XXXV, p. 417.

Allen, E., Francis, B. F., & Co., (1924), Amer. Jour. Anat., XXXIV, p. 133.

Ancel, M. P., (1903), C. R. Soc. de Biol., Paris, LV, p. 1049.

Arnold, L. I., (1912), Anat. Rec., VI.

Baer, C. E. von, (1827), "de ovi mammalium et hominis genesi..." Lipsiae.

Balbani, G., (1879), "Lecons sur la Génération des Vertébrés", Paris.

Balfour, F. M., (1878), Q. J. Microsc. Sci., XVIII - new series, p. 383.

Beneden, E. van, (1870), Mémoires couronnées et mémoires des savants  
étrang. publ. p. l'Acad. R. de Belgique, Bruxelles,  
XXXIV.

" " " , (1880), Arch. de Biol., I., p. 475.

Bidder, F., (1842), Arch. f. Anat., Physiol., u. wiss. Medicin (Müller).

Bischoff, T. L. W., (1842), "Entwicklungsgeschichte des Kaninchen-Eies",  
Braunschweig.

" " " , (1845), "Entwicklungsgeschichte des Hunde-Eies",  
Braunschweig.

" " " , (1863), "Über die Bildung....", Sitzb. d. königl. bayer.  
Akad. d. Wissensch., München, I.

Bonnet, R., (1897), Anat. Hefte, IX.

Bouin, P. & M., (1900), C. R. Soc. de Biol., Paris, LIII, p. 17.

Chappelier, A., (1909), *Ibid.*, LXVI, p. 543.

Corner, G.W., (1915), *Carneg. Inst. of Wash. Pub. No. 222., Contrib. to Embryol. No. 5.*

" " , (1923), *Amer. Jour. Anat.*, XXXI, p. 523.

Cuénot, M.L., (1903), *C.R. Soc. de Biol, Paris*, LV, p. 1391.

Eismond, J., (1898), *Bibliogr. Anat.*, VI., Fasc. 6.

Fischer, A., (1925), "Tissue Culture", Copenhagen.

Foulis, J., (1874), *Trans. Roy. Soc. Edin.*, XXVII, p. 345.

Franqué, O. von, (1898), *Zeitschr. f. Geburtshülfe u. Gynäkologie*, XXXIX, 326

Goette, A., (1875), "Die Entwicklungsgeschichte der Unke (*Bombinator igneus*)", Leipzig.

Grohe, F., (1863), *Arch. f. path. Anat. u. Physiol. u. f. klin. Med. (Virchow)*, XXVI, S. 271.

Häggeström, P., (1921), *Upsala Läkareförenings Förhandlingar*, XXVI.

Hammond, J., (1914), *Jour. Agric. Sci.* VI, p 263.

Hansemann, V., (1912), *Roux's Archiv*, XXXV, Eier im Ovarium"

Hartman, C.G., (1919), *Jour. of Morph.*, XXXII., p. 1.

" " " , (1923), *Amer. Jour. Anat.*, XXXII, p. 353.

" " " , (1926), *Ibid.*, XXXVII., p. 1.

Hausmann, U.F., (1840), "Ueber die Zeugung und Entstehung des wahren weiblichen Eies bei den Säugethieren und Menschen," Hannover,

- Heape, W., (1906-08), Proc. Camb. Phil. Soc., V., 14.
- Hellin, D., (1895), "Die Ursache der Multiparität der Uniparen Tiere überhaupt und der Zwillingschwangerschaft beim Menschen insbesondere", München.
- Hill, J. P., (1910), Q. J. Microsc. Sci., LVI, p. 1.
- Honoré, Ch., (1901), Arch. de Biol. XVII.
- Janosik, J., (1887), Sitzb. d. Kais. Akad. d. Wiss., Math.-Naturw. Classe, XCVI., Heft. III., S. 172.
- Kennedy, W. P., (1924), Jour. of Anat., LVIII.
- Kingsbury, B. F., (1913), Amer. Jour. Anat., XV, p. 345.
- Klebs, Dr., (1861), Arch. f. path. Anat. u. Physiol. u. f. klin. Med. (Virchow), XXI, S. 362.
- " " , (1863), Ibid., XXVIII, S. 301.
- Klien, R., (1893), Sitzb. Ges. Morph. Phys., München, IX. (Reference from Zoolog. Jahresbericht (Mayer) für 1893, Berlin, 1894.)
- Kölliker, A., (1867), "Handbuch der Gewebelehre des Menschen", Leipzig.
- Lillie, F. R., (1923), Biol. Bull., XLIV., p. 47.
- Loeb, L., (1901), Jour. of Med. Research, VI., p. 39.
- " " , (1917), "The concrescence of follicles...." Biol. Bull., XXXIII. P. 187.
- Marshall, F. H. A., (1922), "The Physiology of Reproduction", London.
- Marshall, F. H. A. & Halnan, E. T., (1917), Proc. Roy. Soc. (B), LXXXIX.

- Nagel, W., (1888), Arch. f. mikrosk. Anat., XXXI., S. 342.
- Newman, H. H., (1917), "The Biology of Twins", Chicago.
- Ne " " & Patterson, J. T., (1910), Jour. of Morph., XXI., p. 359.
- O'Donoghue, C. H., (1912), Anat. Anz., XLI., S. 353.
- Patterson, J. T., (1913), Jour. of Morph., XXIV., p. 559.
- Pearl, R., (1912), Jour. Exp. Zool., XIII., p. 153.
- Pflüger, E. F. W., (1863), "Ueber die Eierstöcke der Säugethiere und des Menschen", Leipzig.
- Plihal, Fr., (1869), Arch. f. mikrosk. Anat., V., S. 445.
- Quincke, H., (1863), Zeitschr. f. wiss. Zool., XII., S. 483.
- Rabl, H., (1899), Arch. f. mikrosk. Anat., LIV., S. 421.
- Robinson, A., (1918), Trans. Roy. Soc. Edin., LII., p. 303.
- " " " , (1921), Edin. Med. Jour., March.
- Rosner, M. A., (1901), Bull. Internat. de l'Acad. des Sciences de Cracovie.  
C. R. des Seances. Classes des Sciences math. et nat.  
p. 443.
- Schmaltz, R., (1911), in Ellenberger's "Handbuch der vergleichenden  
mikroskopischen Anatomie der Haustiere", II,  
Berlin.
- Schottländer, J., (1893), Arch. f. mikrosk. Anat., XLI., S. 219.
- " " " , (1905), Monatssch. f. Geburtsh. u. Gynäk., XXI., S. 622.

Schröbn, O., (1863), Zeitschr. f. wiss. Zool., XII, S. 409.

Schulin, K., (1881), Arch. f. mikrosk. Anat., XIX, S. 442.

Schumacher, S. & Schwarz, C., (1900), Anat. Anz., XVIII, S. 1.

Sinétý, Dr. de, (1875), Arch. de. Physiol. norm. et pathol., (2me serie), II.

Slavjansky, K., (1870), Arch. f. path. Anat. u. Physiol. u. f. klin. Med., LI, 47

Smyth, E. A., (1908-08), Biol. Bull., XIV, 319.

Stöckel, W., (1898-99), Arch. f. mikrosk. Anat., LIII, S. 357.

~~Surface, ., (1908-09), Biometrika, VI.~~

Tur, J., (1912), C. R. Soc. de Biol., Paris, LXXII., (I), p. 336.

Wagener, G. R., (1879), Arch. f. Anat. u. Physiol. (Anat. Abtheilung), S. 175.

Waldeyer, W., (1870), "Eierstock und Ei", Leipzig.

Wallace, R., (1923), "Farm Live Stock of Great Britain", Edinburgh.

Welch, J. E., (1907), Bull. Lying-in Hosp., New York, March.

Winiwarter, H. von, (1900), Arch. de. Biol. XVII.

" " " " & Sainmont, G., (1908-09), Arch. de Biol., XXIV.

(also separately as "Nouvelles Recherches sur l'ovogenese et l'organogenese de l'ovaire des mammiferes (chat) Liège, 1912.)

Woerdeman, M.W., (1921), Koninklijke Akad. van Wetenschappen te  
Amsterdam, Proceedings, XXIII., 1st part.  
p. 448.

THE PLURIOVULAR FOLLICLE, WITH SPECIAL REFERENCE TO ITS  
OCCURRENCE IN THE FERRET, by D. Mainland.

Illustrations

Except where indicated, all specimens illustrated are  
from the ovaries of adult ferrets.

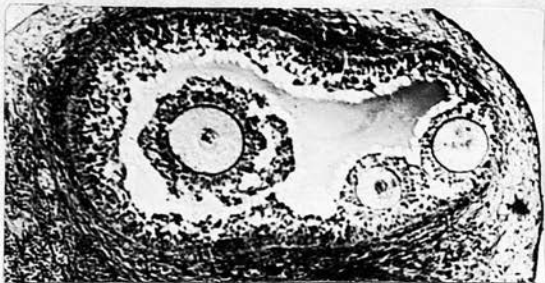


Fig.1. Large Follicle showing Three Ova, unequal in Size, and situated in separate Cumuli, at various Distances from each other.

(III., 51. Fixed in Mann with Formol; Stained by Heidenhain's Iron-Haematoxylin; Magnif'n. 75)

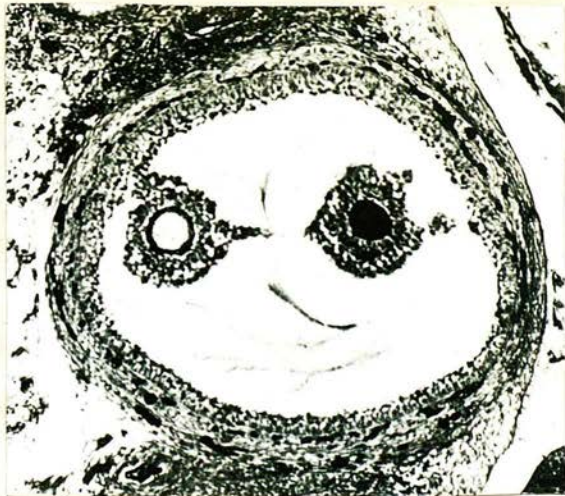


Fig.2. Large Biovular Follicle, showing part of a Strand of the Zona Granulosa stretching between the two Cumuli, which are being separated from each other as the Follicle enlarges.

The dark mass in the Cumulus on the right is Zona Pellucida, deeply stained.

(III., 46. Fixed in Perenyi; Stained by Heidenhain's Iron-Haematoxylin and Orange G; Magnification 75)



Fig.4. A Biovular Follicle. The Larger Ovum is centrally placed. The Epithelium over the Smaller Ovum is thinner than that over the Larger. That the Thickness of the Epithelium is largely dependent on Pressure is illustrated by the Monovular Follicle on the left.

(III., 45. Fixed in Mann with Formol; Stained by Heidenhain's Iron-haematoxylin; Magnification 215)

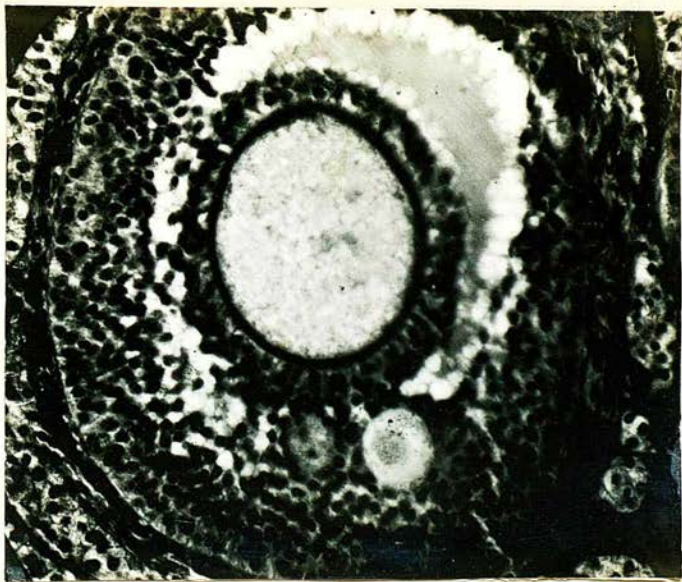
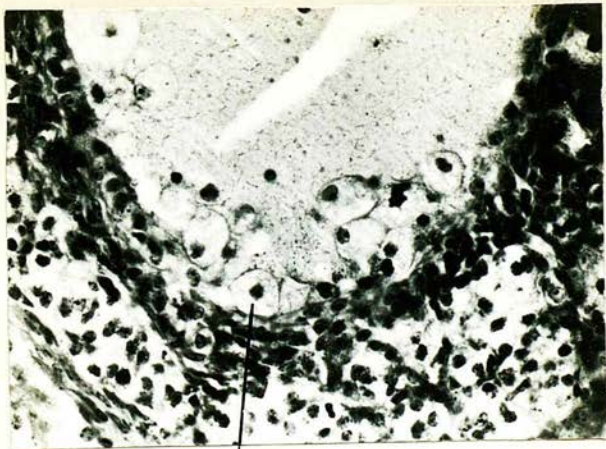


Fig.5. Two Very Small Ova at the base of the Cumulus of a Large one. (The sections show three small ova in this region).

(III., 29. Fixed in Mann; Stained by Mayer's Haemalum and Eosin; Magnification 280)



Swollen granulosa cell

Fig. 16. Swollen Cells of the Zona Granulosa of an Atretic Follicle. The resemblance to small ova is superficial, and seldom causes doubt in determining whether a follicle is plurio- vular or not.

(II., 358. Fixed in Maximow; Stained by Heiden- hain's Iron-Haematoxylin; Magnification 340)

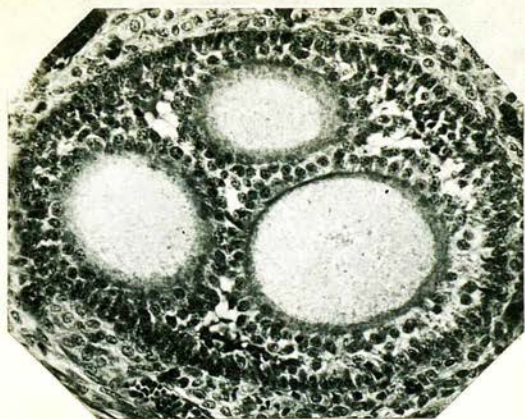
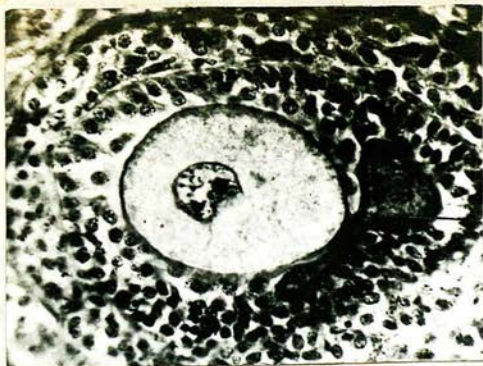


Fig.7. Triovular Follicle, showing commencement of formation of Liquor Folliculi in the sulci between the ova.

(G.31.S1.14. Fixed in Zenker; Stained by Mayer's Haemalum and Eosin; Magnification 225.)



Deeply stained  
degenerate ovum

Fig.8. Bi-ovular Follicle showing great difference  
in degree of Degeneration in the two Ova.  
(III., 73. Fixed in Zenker; Stained by Mayer's  
Haemalum and Eosin; Magnification 300)

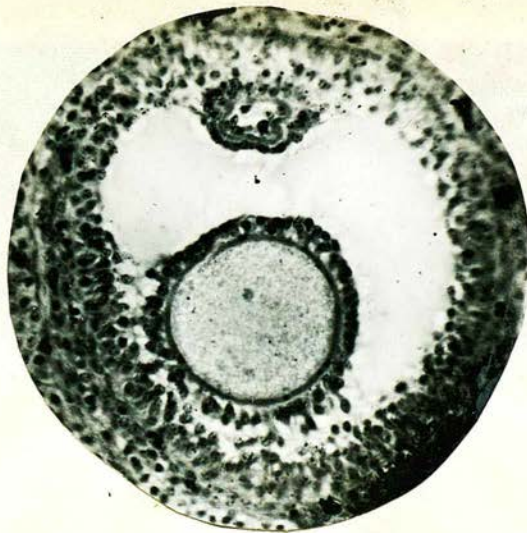


Fig.9. A Large Biovular Follicle, showing Ova in separate Cumuli on opposite walls. One of the Ova, probably developed to about the same extent as the other, is much more degenerate.

(III., 3. Fixed in Zenker; Stained by Mayer's Haemalum and Eosin; Magnification 225)

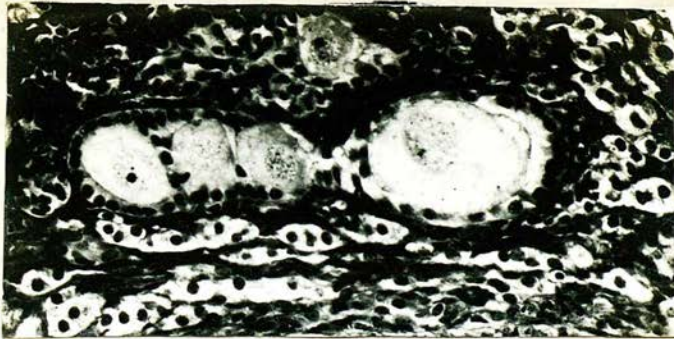
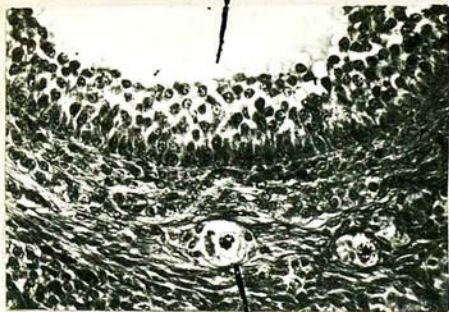


Fig.10. A Long Pluriovular Follicle - a form rarely seen in the Adult Ferret. It is possible that a Monovular Follicle, containing the largest Ovum, is about to be formed.

(II., 66. Fixed in Mann; Stained by Mayer's Haemalum and Eosin, restained by Curtis' Picro-Ponceau S; Magnification 300)

Cavity of large follicle



Primordial follicle

3. 11. A Primordial Follicle in the Theca of a Large one.

This is a fairly frequent occurrence. There appears to be no evidence that it leads to the formation of a true Pluriovular Follicle. (The Epithelium of the small follicle lies at the opposite ends of the ovum more abundantly than alongside it, because of the strains set up by the growth of the large follicle.)

., 45. Fixed in Zenker; Stained by Mayer's Haemalum & Eosin; Magnif'n. 210)

Flat Epithelium  
on Non-adjacent  
Surface of Ovum



Epithelium in  
Interovular  
Sulcus

Connective Tissue  
alongside the  
Epithelium

Fig.12. Suggested Early Stage in the Formation of a Pluriovular Follicle. The Connective Tissue is excluded from the Interovular Sulcus by Follicular Epithelium. (I., 59. Fixed in Mann; Stained by Mayer's Haemalum and Eosin; Camera Lucida Water-colour Drawing; Magnification 530.)

Epithelium in  
Interovular  
Sulcus



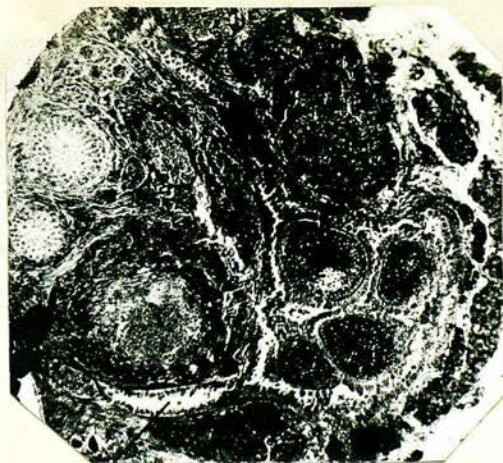
Connective  
Tissue

Epithelium on  
Non-adjacent  
Surface of Ovum

Fig.13. Small Pluriovular Follicle, slightly further developed than that of Fig.12. The Epithelium fills the Interovular Sulci, and forms the uninterrupted edge of the Follicle. The Connective Tissue lies parallel to this. (III., 30. Fixed in Mann; Stained by Mayer's Haemalum and Eosin; Camera Lucida Water-colour Drawing; Magnification 700.)



Fig.14. Biovular Follicle more developed than the Follicles of Figs.12 and 13. The Epithelium over the Non-adjacent Surfaces of the Ova is broader. The Epithelium in the Interovular Sulcus at (a) projects slightly. (I., 23. Fixed in Zenker; Stained by Erythrosin and Light Green; Camera Lucida Water-colour Drawing; Magnification 530.)



Epithelial process

Fig.15. Follicle from a Ferret aged 3 Months.

Four Ova are seen. The irregularity of shape, and especially the presence of long processes, forms a contrast to the Follicles of Adults.

(IV., 10 Fixed in Flemming; Stained by Heidenhain's Iron-Haematoxylin; Mag'n. 100)



Fig.16. Biovular Follicle from a Ferret aged 12 Weeks. Formation of two Monovular Follicles may have been occurring, accompanied by Degeneration. (IV., 8. Fixed in Zenker; Stained by Heidenhain's Iron- Haem-

atoxylin and Orange G; Camera Lucida Water-colour Drawing; Magnif'n 280



Fig.17. Two Follicles pressed closely together,  
without impairment of the layers of Conn-  
ective Tissue between them.

(II., 93. Fixed in Mann; Stained by Mayer's  
Haemalum and Eosin; Magnification 175)