PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Proc. R. Soc. Lond. B 1968 **169**, 167-176
doi: 10.1098/rspb.1968.0004

Proc. Roy. Soc. B. **169**, 167–176 (1968)
Printed in Great Britain

Oogenesis in lorises; *Loris tardigradus lydekkerianus* and *Nycticebus coucang*

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(*Communicated by Sir Solly Zuckerman, F.R.S.—Received* 13 *July* 1967)

[Plates 36 to 39]

A histological study of ovaries in three pairs of foetal and 67 pairs of postnatal slender lorises of different age groups ranging from suckling young to adults was made. Oogonia in interphase and mitosis, as well as non-follicular germ cells in various stages of meiotic prophase, were present in all the ovaries. Similar germ cells were also found in 12 pairs of postnatal slow loris ovaries.

A quantitative estimation of the primordial germ cell population was also made in the 67 pairs of postnatal slender loris ovaries. The total number of germ cells decreases with age from the time of birth until puberty. In the adults the primordial germ cell population varies in relation to the different phases of the reproductive cycle. This suggests that oogenesis in postnatal lorises may be under endocrine control. The number of germ cells increases progressively during pro-oestrus and oestrus and reaches a peak of *ca.* 171 000 cells during early pregnancy. Thereafter the number declines to a level of about 20 000 during lactation and drops to a level of *ca.* 10 000 during anoestrus. The fate of all the freshly formed germ cells during each oestrous cycle is not known. It is likely that most of them perish since the number of atretic cells is also high during phases of increased oogenetic activity. It remains to be shown whether any of the newly formed cells contribute to the definitive germ cell population.

Introduction

The earlier concept that oogenesis or neo-formation of germ cells occurs in the ovary of common laboratory mammals during their adult life has been shown to be incorrect (see Zuckerman 1951, 1956 for reviews). It has been clearly established that oogenesis ceases before the onset of puberty in most mammalian species that have been investigated, e.g. rat, mouse (Franchi, Mandl & Zuckerman 1962), guinea-pig (Ioannou 1964), rabbit (Teplitz & Ohno 1963; Kennelly & Foote 1966), man and rhesus monkey (Baker 1963, 1966).

Exceptions to this mammalian pattern are found in certain species of the family Lemuroidea, where oogenesis has been reported to occur in adults (Gerard 1920, 1932; Rao 1927; Gerard & Herlant 1953; Herlant 1961; Petter–Rousseaux 1962; Petter–Rousseaux & Bourlier 1965; Ramaswami & Anand Kumar 1965; Butler 1964). Ramaswami & Anand Kumar (1965) found primordial germ cells (germ cells without a definitive layer of granulosa cells) in *Loris*, which occurred in discrete nests in the ovarian cortex. These germ cells were present in addition to the definitive oocytes (oocytes in follicles). In their view the number of primordial germ cells not only increased during the pregnancy as Rao (1927) had suggested but also during the period of oestrus. However, neither set of observations was based on any quantitative estimation of the germ cells.

In the present study a quantitative estimation was made of the primordial germ cells in the slender loris to determine whether the germ-cell population in nests varied between animals. This was done by estimating the total number of primordial germ cells in ovaries obtained from animals of different age groups ranging from suckling young to adults, and adults killed at different stages of the reproductive cycle. Secondly, ovaries of slow lorises were studied histologically to determine whether the persistence of oogonia into adult life is a feature common to both the slender and slow lorises.

Materials

(*a*) *Slender loris* (Loris tardigradus lydekkerianus)

Sixty-seven female slender lorises were obtained from forests around Bangalore (South India). These were killed within 48 h of their capture. The reproductive tract and ovaries were dissected out and immediately immersed into Bouin's aqueous fluid. Gravid uteri which had conceptuses in advanced stages of development were opened and the foetuses taken out. The foetuses were also fixed in Bouin's fluid. The reproductive tracts and ovaries of three foetuses (crown-rump lengths 17, 20 and 24 mm respectively) were removed for histological studies. The tissues were transferred to 70% ethanol after 18 to 24 h fixation.

(*b*) *Slow lorises* (Nycticebus coucang)

Twelve female slow lorises were imported from Vietnam. These were killed immediately on arrival and their reproductive tracts fixed as described for the slender loris.

The details of the 67 slender and 12 slow lorises used in this study are shown in table 1.

Methods

Histology

The reproductive tracts in the foetal and suckling young lorises (with the ovaries attached) were embedded in paraffin and sectioned serially at 5 μm thickness. The ovaries from the other animals were separated from the reproductive tracts, processed and sectioned in the same way.

Where early pregnancy was suspected (because the uterus was swollen) the entire uterus was serially sectioned at 10 μm thickness. Otherwise only representative sections of the uterus were cut. Representative sections of the vagina of each animal were also cut. The histological features of the reproductive system were used to identify the stage of the oestrous cycle reached by the non-pregnant, non-lactating lorises at the time of death (table 2).

The sections were stained with Wiegert's haematoxylin-eosin.

Quantitative procedures

(1) *Differential counts*

Chalkley's (1943) technique was used to estimate the proportion of germinal and non-germinal cells in germ cell-nests. For every pair of *Loris* ovaries differential counts of about 1000 to 2000 cells were made at a magnification of

Table 1. The numbers of lorises examined at different stages of sexual maturity and reproductive cycle

species	prepubertal		adults						
	suckling young	vagina not patent	anoestrus	pro-oestrus	oestrus	post-oestrus	early* pregnancy	mid-pregnancy*	lactation
slender loris	5	7	6	11	8	1	14	9	6
slow loris	—	2	2	—	—	—	7	1	—

* Early pregnancy includes blastocyst stage to 17 mm (crown-rump length) embryos. Mid-pregnancy includes embryos measuring 20 to 28 mm crown-rump length. This classification of pregnancies is purely arbitrary.

Table 2. Histological criteria used in determining the stage of the reproductive cycle of the non-pregnant, non-lactating lorises

(Based on Ramaswami & Anand Kumar 1965.)

stage of the oestrous cycle	ovary	uterus			vagina	
		epithelium	stroma	glands	epithelium	fibro-muscular stroma
anoestrus	no corpus luteum	regressed	regressed	regressed	regressed	thick
pro-oestrus	no corpus luteum	hypertrophied, few mitotic figures	slightly oedematous	enlarged but without glandular secretion	columnar	slightly thin
oestrus	corpus luteum when present is newly formed	hypertrophied, few mitotic figures	highly oedematous	enlarged and with glandular secretion	cornified	thin
postoestrus	degenerating corpus luteum present	regressed	regressed	regressed	mucified	thin

×1000. The germ cells were classified as normal or atretic and the normal cells further subdivided as follows: (*a*) oogonia in interphase, mitotic prophase, mitotic metaphase (including anaphase and telophase); (*b*) oocytes at leptotene, zygotene-pachytene and diplotene. Oocytes at zygotene and pachytene were grouped together because of difficulties in telling these two stages apart. Germ cells were classified according to their nuclear configuration as described by Rao (1927). Replicate counts were made on 12 randomly chosen ovaries. The margin of personal error in consistently identifying the different stages of the germ cells was about 10 %.

(2) *The total volume of germ cell-nests in a pair of ovaries* (*Vn*)

Every 20th section of each ovary was projected on to paper at a magnification of ×100 and the outlines of germ cell-nests were drawn. The total volume of the nests per pair of ovaries was calculated by the planimetric method of Dornfeld, Slater & Scheffè (1942).

(3) *Volume of germ cells occupied by different cell stages* (*Vc*)

This was determined by multiplying the total volume of nests of germ cell (*Vn*) in a pair of ovaries by the percentage differential count obtained for each stage in the same pair of ovaries.

(4) *Volume of each cell stage* (*C*)

Fifty germ cells at each stage were chosen at random and their outlines drawn by camera lucida at a magnification of ×1200. Assuming the germ cells to be spherical, their volume was calculated using the mean diameter of each cell stage.

(5) *The total number of germ cells at each stage was determined by dividing Vc by C.*

The following precautions were taken to minimize bias in estimating the germ cell populations:

(i) The ovaries were given code numbers and at the time of estimating the numbers of germ cells neither the age nor the sexual state of the animals from which the ovaries were removed was known.

(ii) The histology of the reproductive tract of the lorises was examined (for assessing the stage of the sexual cycle of the animal) after the germ cell population was estimated.

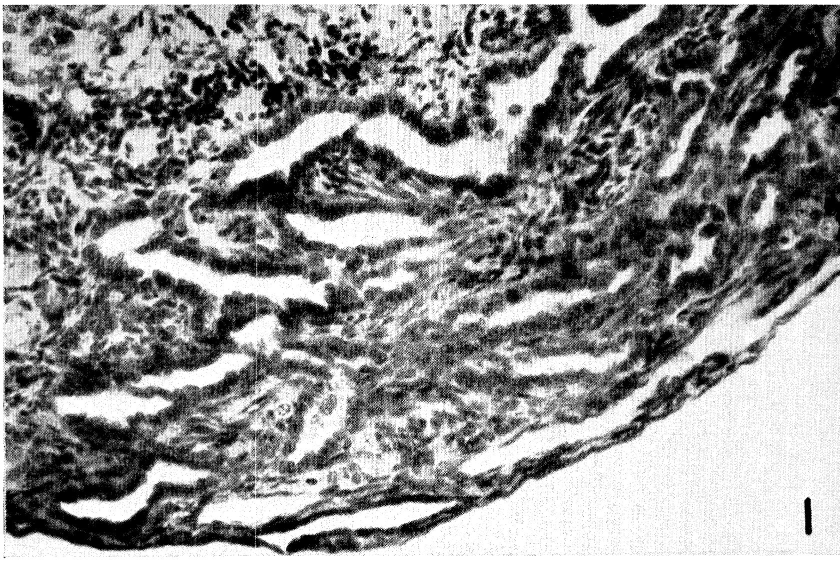
Observations

1. *Slender loris*

Histology

The external surface of the ovary in postnatal lorises has a large number of indentations which appear to be formed by invaginations of the germinal epithelium. These invaginations form a system of subsurface crypts in the ovarian cortex (figure 1, plate 36). The germinal epithelium did not show any histological changes in relation to the reproductive cycle. Mitoses were seen only rarely.

Oogonia in interphase and in various stages of mitosis, as well as non-follicular oocytes in different stages of meiotic prophase, were present in all the ovaries.



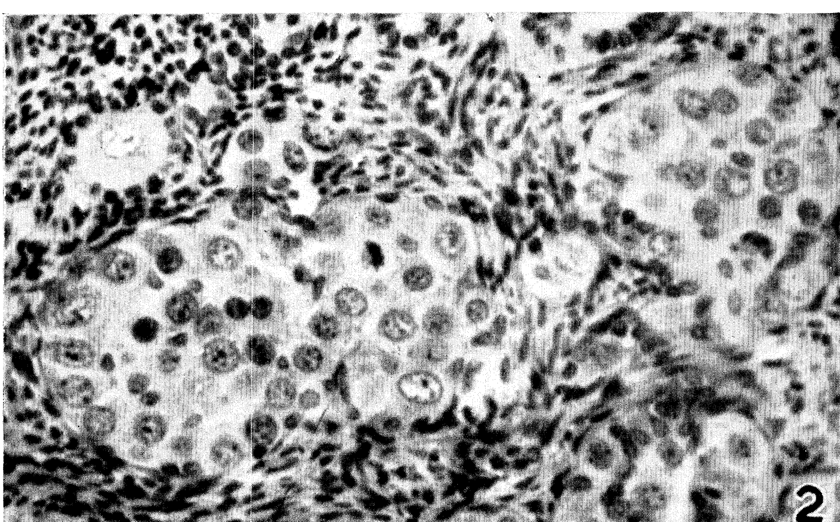


FIGURE 1. Subsurface crypts in the ovarian cortex of *Loris*. (× 500.)

FIGURE 2. Primordial germ cells in the ovary of an adult slow loris. These germ cells occur in nests as shown in this picture. (× 1350.)

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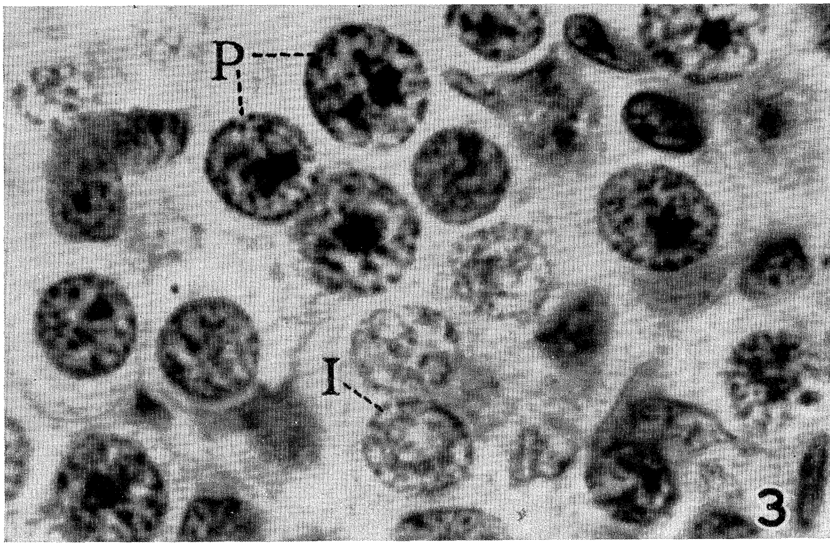


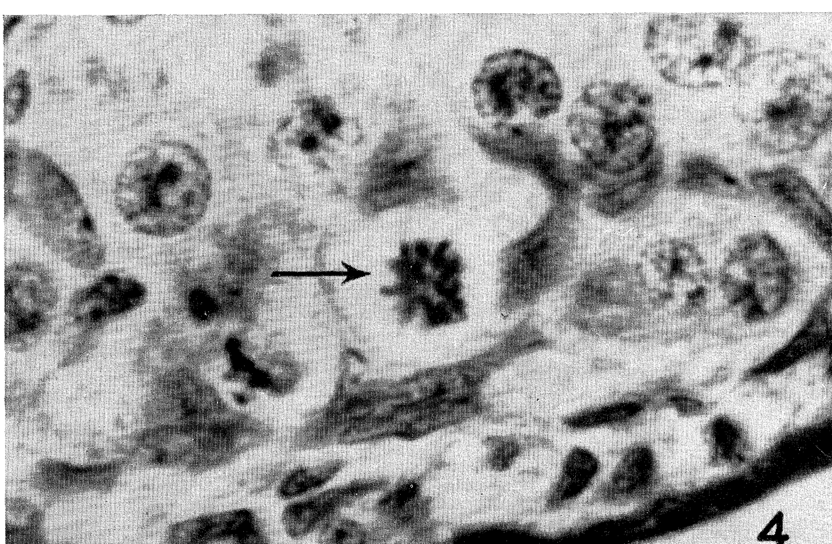
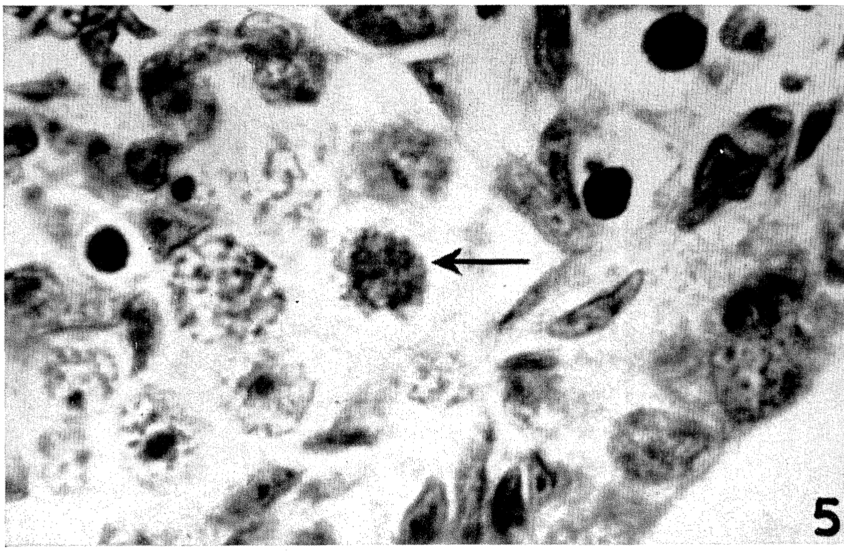


FIGURE 3. Adult *Loris* ovary. Oogonia in interpase (I) and prophase (P). (× 3000.)

FIGURE 4. Adult *Loris* ovary. Oogonium in metaphase. (× 3000.)



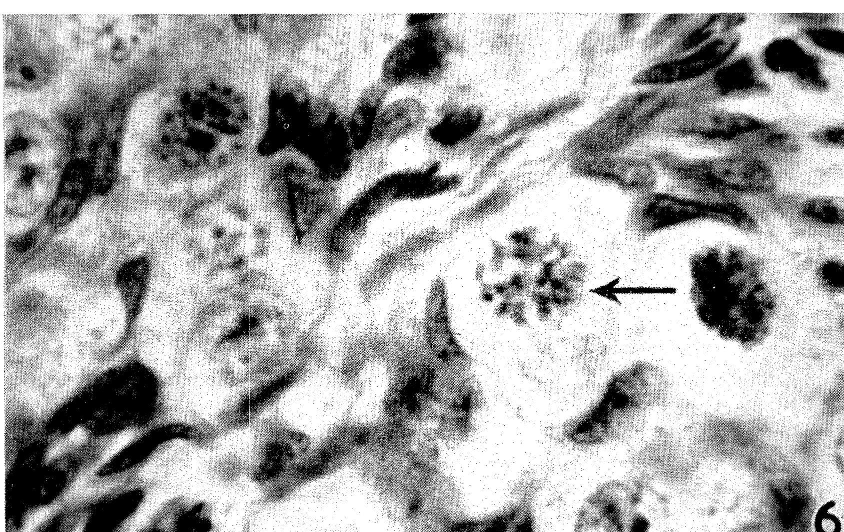
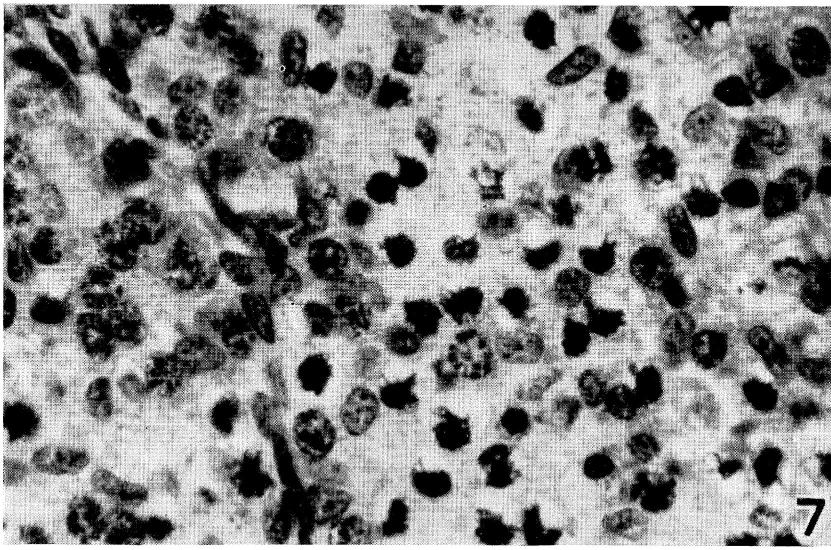


FIGURE 5. Adult *Loris* ovary. Primordial germ cells at leptotene. (× 3000.)

FIGURE 6. Adult *Loris* ovary. Primordial germ cells at pachytene. (× 3000.)



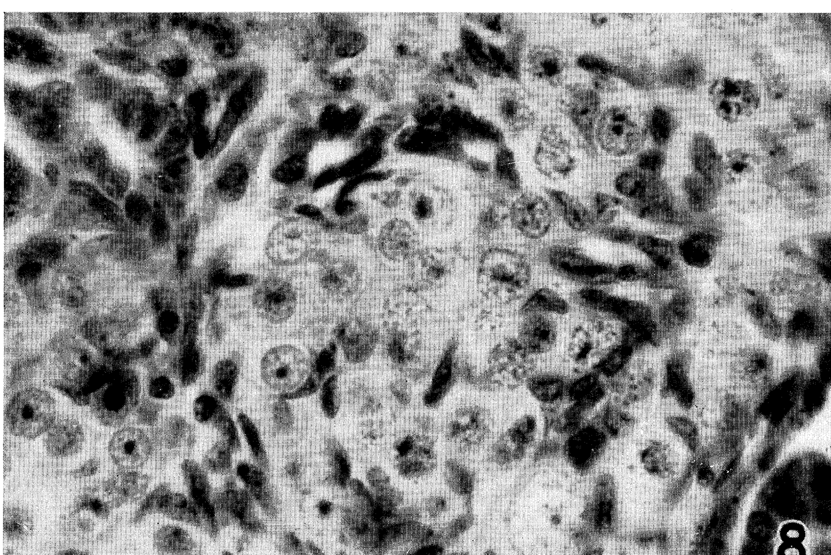


FIGURE 7. Foetal *Loris* ovary. Note the large number of atretic primordial germ cells with dark nuclei. (× 1350.)

FIGURE 8. Adult *Loris* ovary. Primordial germ cells in a nest. (× 1350.)

Table 3. The distribution of the mean number of primordial germ cells in the slender loris

slender lorises	oogonia at: inter-phase	oogonia at: prophase	oogonia at: meta-phase	oocytes at: leptotene	oocytes at: zygotene and pachytene	oocytes at: diplotene	total no. of oogonia	total no. of oocytes	atretic germ cells	total no. of normal germ cells	total no. of germ cells
(1) suckling young (5)	5060 ± 1096	10180 ± 798	1460 ± 417	2020 ± 539	15820 ± 5409	5540 ± 1724	16700 ± 2311	23380 ± 7672	81120 ± 21741	40080 ± 6875	121200 ± 26145
(2) prepubertals (7)	1314 ± 350	6928 ± 1211	670 ± 231	111 ± 85	5971 ± 1213	3420 ± 2103	8922 ± 1792	9502 ± 5108	51128 ± 13735	18414 ± 4554	69542 ± 17897
(3) anoestrus (6)	868 ± 441	1083 ± 286	56 ± 33	86 ± 82	1483 ± 311	506 ± 114	2007 ± 760	2075 ± 1185	7166 ± 990	4082 ± 956	11248 ± 2008
(4) proestrus (11)	2113 ± 453	6495 ± 1441	403 ± 87	287 ± 30	7700 ± 1387	3064 ± 1524	9021 ± 1981	11051 ± 2941	35950 ± 5517	20062 ± 3566	56012 ± 6506
(5) oestrus (9)	7328 ± 1829	19500 ± 4724	1414 ± 473	3328 ± 2312	20885 ± 4826	9571 3186	28242 ± 7026	33784 ± 10324	102680 ± 21825	62026 ± 12066	164706 ± 30337
(6) post-oestrus (1)	2400	6000	200	—	2100	800	8600	2900	30500	11500	42000
(7) early pregnancy (14)	2285 ± 395	19000 ± 3490	1475 ± 334	1062 ± 340	31164 ± 6790	8550 ± 2520	22760 ± 5219	40776 ± 9650	108128 ± 24330	63536 ± 12631	171664 ± 35902
(8) mid-pregnancy (9)	1055 ± 821	3611 ± 927	163 ± 59	33 ± 21	4214 ± 1212	650 ± 218	4829 ± 1807	4897 ± 1451	13855 2924	9726 ± 2358	23785 ± 4801
(9) lactation (6)	450 ± 164	1430 ± 958	126 ± 52	63 ± 29	3021 ± 1007	313 ± 145	2006 ± 1174	3397 ± 1181	14566 ± 3782	5403 ± 1660	19969 ± 5104

(± indicates the standard error of the mean; the figures shown in parentheses indicate the total no. of animals examined.)

These primordial germ cells occurred in nests in the ovarian cortex. In two exceptional cases, germ cells were also present at the hilar region of the ovary.

The nuclear configuration in germ cells at interphase, mitosis and stages of meiotic prophase was similar to that described by Rao (1927), Brambell (1930) and Ioannou (1967) (figure, 3, 4, plate 37; 5 and 6, plate 38), and did not differ between foetal and postnatal loris ovaries.

There was inadequate material to justify a quantitative estimation of the germ-cell population in foetal ovaries. However, far more atretic germ cells were seen in foetuses than in adults (figure 7 and 8, plate 39).

Quantitative

The total number of primordial germ cells in animals belonging to different groups is highly variable (table 3). For example, in one exceptional prepubertal

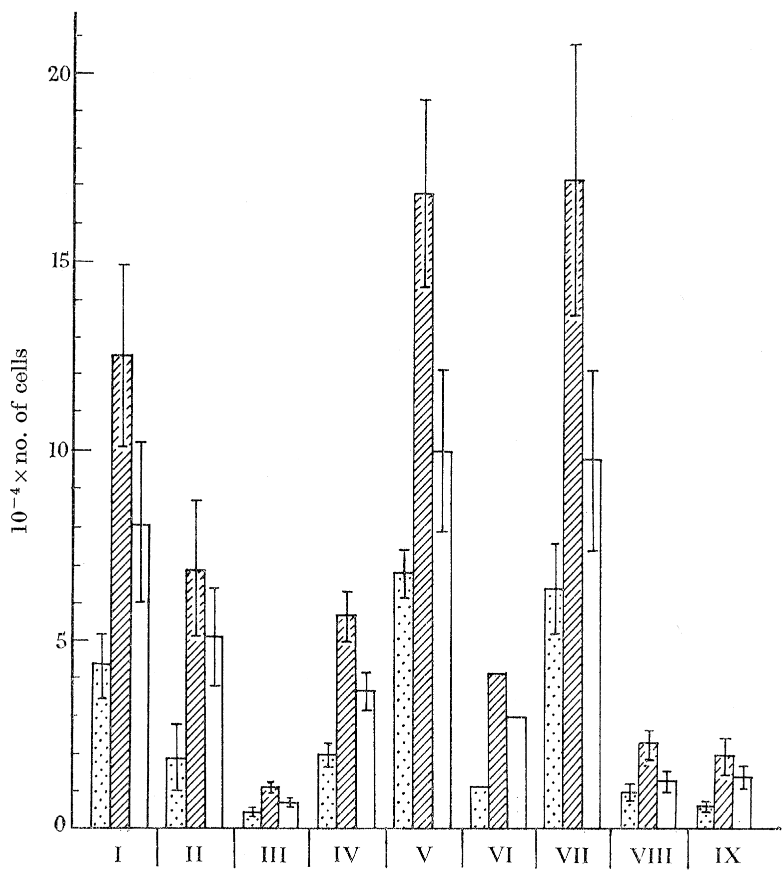


FIGURE 9. Histogram showing mean distributions and standard deviations of the primordial germ cells in *Loris*. 1, Suckling young. II, Prepubertals. III to IX adults at different stages of the reproductive cycle. 3, Anoestrus. 4, Proestrus. 5, Oestrus. 6, Post-oestrus. 7, Early pregnancy. 8, Mid-pregnancy. 9, Lactation. *Key*: ▦, total number of normal primordial germ cells; ▨, total number of normal and atretic primordial germ cells; □, total number of atretic primordial germ cells.

TABLE 4

		mean percentage distribution of:											
		oogonia			oocytes								
slender loris	mean germ cell-nest volume (mm^3)	inter-phase	pro-phase	meta-phase	lepto-tene	zygo-tene and pachy-tene	diplo-tene	atretic germ cells	Σ% of oogonia	Σ% of oocytes	oogonia : oocytes ratio	Σ% of normal germ cells	Σ% of all germ cells
suckling young	0·037	1·73	4·56	0·31	0·71	5·00	2·91	12·46	6·60	8·62	0·76	15·22	27·68
prepubertal	0·026	0·09	5·06	0·19	0·02	3·63	1·54	11·97	5·34	5·19	1·02	10·53	22·50
anoestrus	0·007	1·04	3·99	0·08	0·10	4·13	1·08	9·39	5·11	5·31	0·96	10·42	19·81
pro-oestrus	0·024	0·12	6·11	0·15	0·13	3·80	0·80	9·6	6·38	4·73	1·34	11·11	20·71
oestrus	0·084	1·22	4·71	0·20	0·18	3·08	1·89	9·42	6·13	5·15	1·19	11·28	20·70
post-oestrus	0·019	1·70	5·10	0·10	—	1·40	0·80	9·80	6·90	2·20	3·13	9·10	18·90
early pregnancy	0·070	0·55	4·75	0·34	0·24	5·16	2·01	8·98	5·64	7·41	0·76	13·05	22·03
mid-pregnancy	0·007	0·40	7·40	0·17	0·05	4·70	0·70	12·40	7·97	5·45	1·46	13·42	25·82
lactation	0·008	1·80	5·30	0·10	0·20	3·90	1·20	9·20	7·20	5·30	1·35	12·50	21·70
mean volume of germ cells at different stages (μm^3)		1313	1591	1200	1094	1256	1761	1009	—	—	—	—	—

loris (L 100) the number was as low as 777 compared with a mean of *ca.* 68000 for this group. The figure obtained for this animal was considered abnormal and was not included in the results of the present study.

Comparisons of the numbers of primordial germ cells in suckling young, prepubertal and anoestrous adults indicate that the total number is inversely proportional to age. Furthermore, in adult lorises the number fluctuates in relation to the reproductive cycle. The total number of primordial germ cells in anoestrous loris ovaries is *ca.* 10000. There is a progressive increase in the number of germ cells during pro-oestrus, oestrus and early pregnancy, reaching a maximum of *ca.* 171000 during early pregnancy. At mid-pregnancy the number of germ cells begins to decline and continues to do so until lactation when the mean is *ca.* 20000. The total number of atretic germ cells also increases during oestrus and early pregnancy (table 3 and figure 9). The ovaries of only one loris in the post-oestrous condition were available for study. The differential counts for this animal suggest that the number of primordial germ cells declines if conception does not follow oestrus (table 3). During anoestrus the total percentages of oogonia and oocytes are almost equal. This ratio is altered during the other stages of the reproductive cycle. In animals that are in pro-oestrus, oestrus, early pregnancy and lactation, the total percentage of oogonia is more than the total percentage of oocytes. During mid-pregnancy this ratio is reversed, when the total percentage of oocytes is more than that of oogonia (table 4).

2. *Slow loris*

The germinal epithelium of the slow loris does not form the deep invaginations into the cortex which are seen in the slender loris, and consequently the system of crypts is much less well developed.

The primordial germ cells in *Nycticebus* occur as isolated groups or nests in the cortex (figure 2, plate 37). These nests contain oogonia in different stages of mitosis and oocytes at various stages of meiotic prophase. The nuclear configuration of these germ cells resembles that of the slender loris.

Discussion

The quantitative estimates of the primordial germ-cell populations undertaken in the present study support the observations of Rao (1927) and Ramaswami & Anand Kumar (1965) that the number of primordial germ cells varies in phase with the reproductive cycle. The present work has also shown that oogenesis is heightened during pro-oestrus, oestrus and early pregnancy. The source of the germ cells formed during successive reproductive cycles is uncertain. It is unlikely that the germinal epithelium contributes towards the population of germ cells since the cells of this layer were seen to divide infrequently. It is probable that some oogonia persist into adult life and these contribute to the germ-cell population by mitotic divisions.

The fact that there is considerable individual variation in the total number of primordial germ cells, as well as the number of germ cells at different stages of mitosis and meiosis, indicates that oogenesis may occur continuously during post-natal life with spurts of intense activity at pro-oestrus, oestrus and early pregnancy. The fluctuation in the numbers of primordial germ cells in phase with the reproductive cycle suggests that oogenesis in adult lorises may be under endocrine control. It has been shown that the number of primordial germ cells in the ovaries of anoestrous lorises is increased after the administration of oestrogen (Anand Kumar 1966). This stimulating effect of oestrogen might be a direct one or be mediated through the hypophysis. It still remains to be shown whether oogenesis in untreated lorises is stimulated by the gonadal or hypophyseal hormones.

The inverse correlation between the total percentage of oogonia and non-follicular oocytes in lorises at pro-oestrus, oestrus, and early pregnancy on one hand and those at mid-pregnancy on the other, indicates that at least some of the oogonia formed during the former stages of the reproductive cycle enter into meiotic prophase during mid-pregnancy. Whether the non-follicular oocytes become primordial follicles and ever contribute to the population of the definitive germ cells is not known. Since the number of atretic cells is also high during oestrus and pregnancy, it may be that the majority of the newly formed germ cells perish and do not form primordial follicles. However, the final proof of the fate of the newly formed germ cells may lie with the use of a labelled *DNA* precursor such as triated thymidine. The primordial germ cells in galagos are known to incorporate tritiated thymidine (Butler 1965; Ioannou 1967). What is not known is whether these labelled germ cells would be incorporated into follicular envelopes if allowed to continue their development for a sufficiently long period. Work on these lines in the slender loris is currently in progress.

The expenses for this study were defrayed from a grant made by the Ford Foundation to Professor Sir Solly Zuckerman, K.C.B., F.R.S. I am deeply grateful to Sir Solly for his encouragement in this work.

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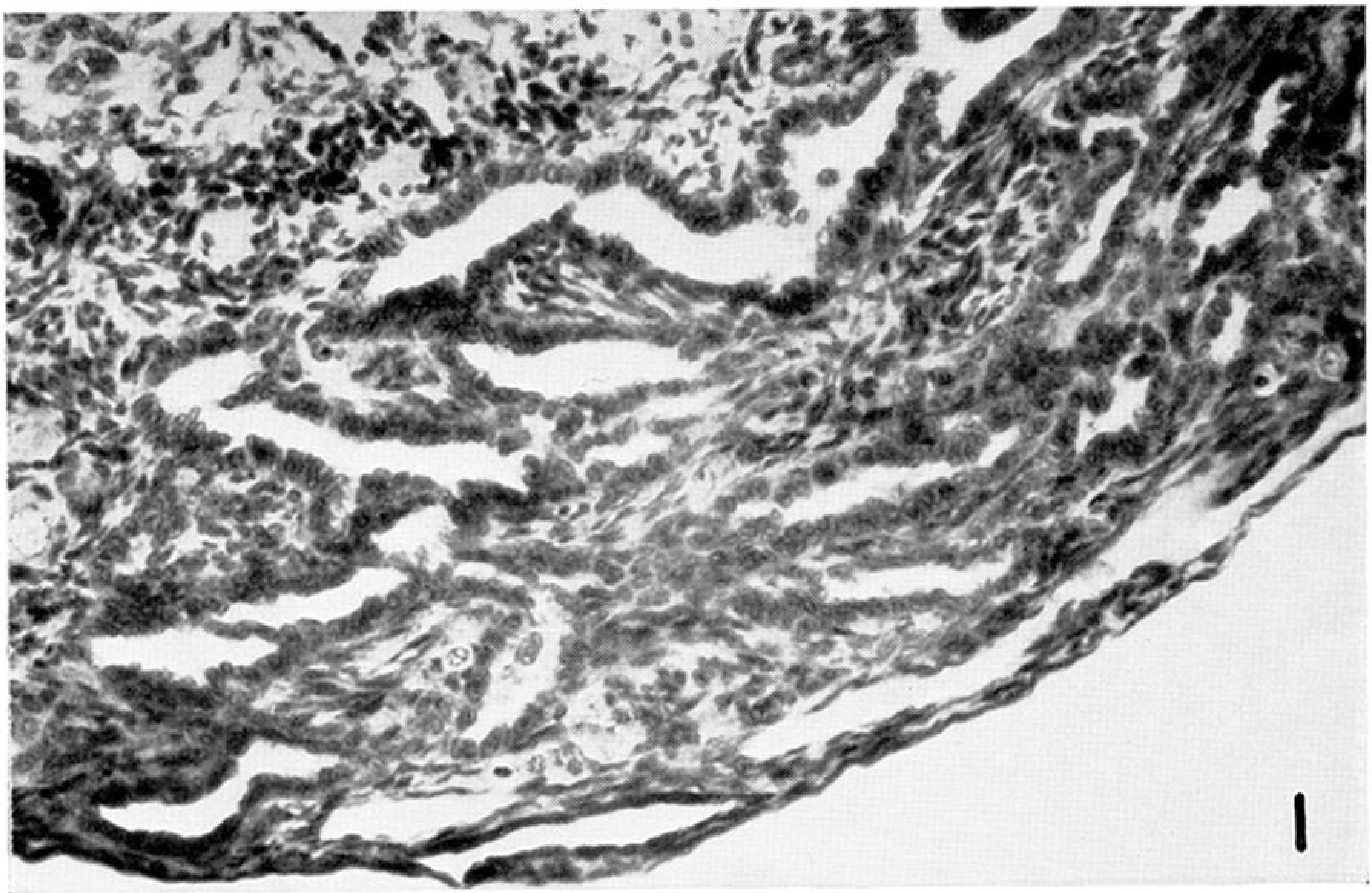


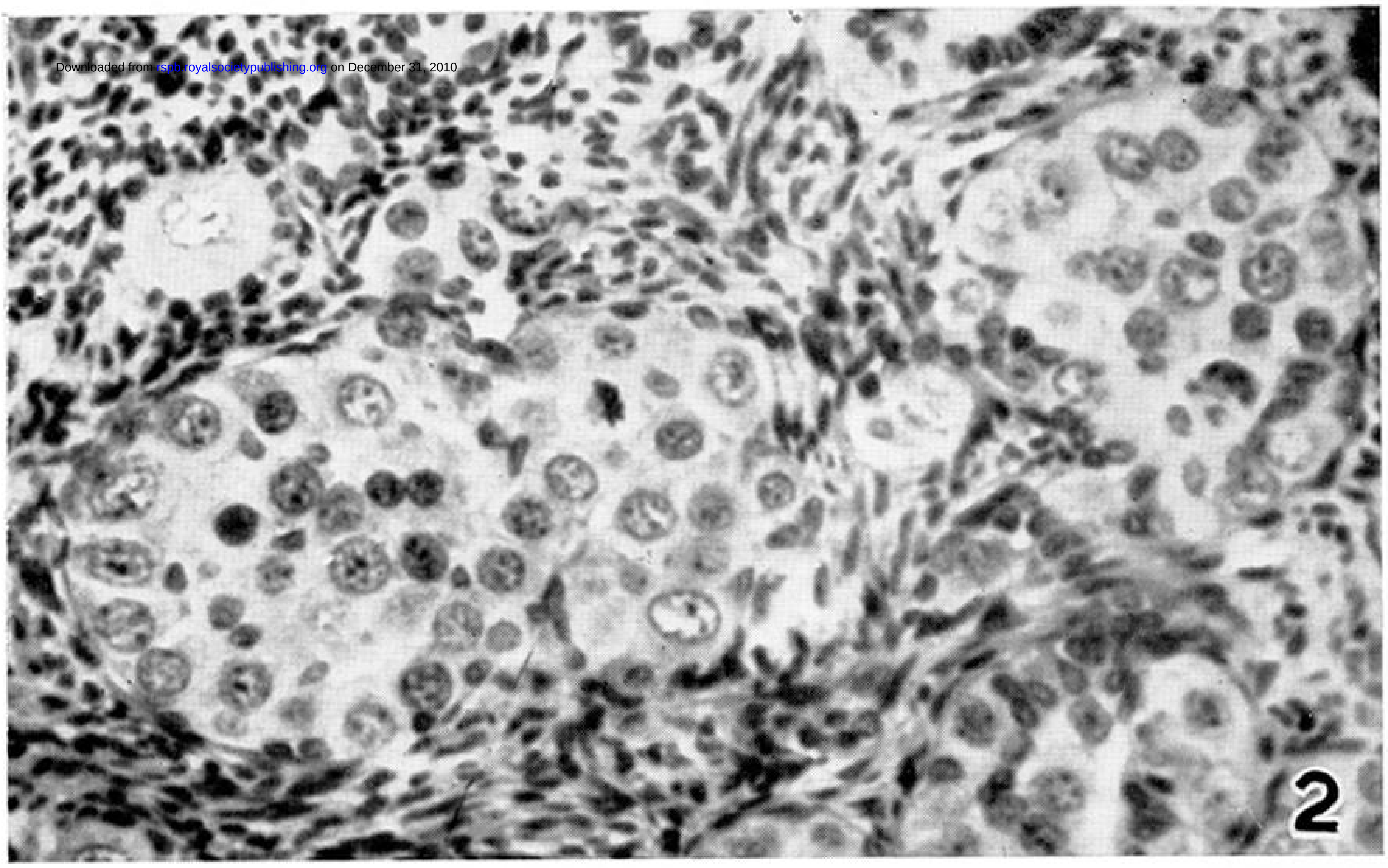
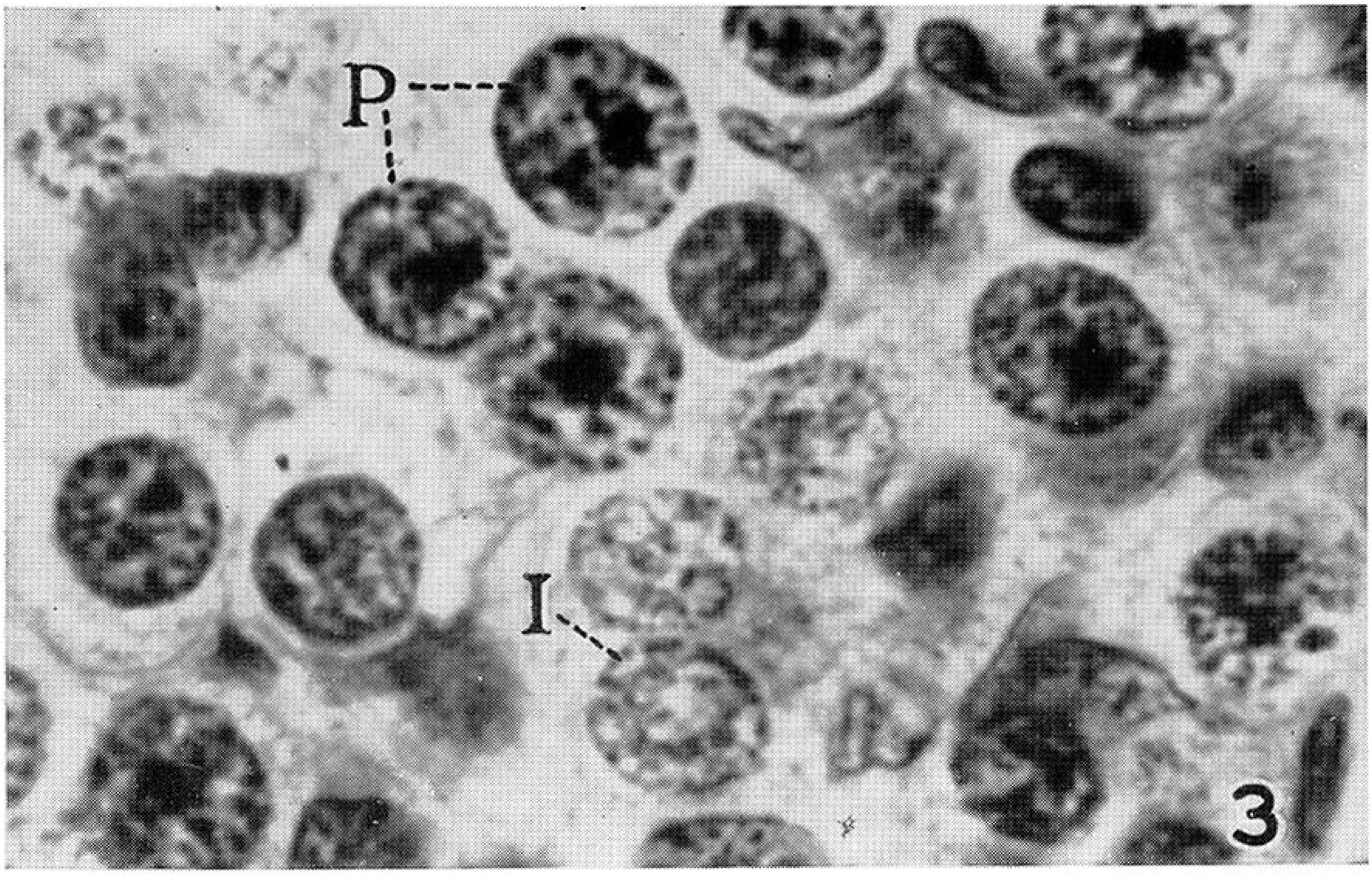


FIGURE 1. Subsurface crypts in the ovarian cortex of *Loris*. (× 500.)

FIGURE 2. Primordial germ cells in the ovary of an adult slow loris. These germ cells occur in nests as shown in this picture. (× 1350.)



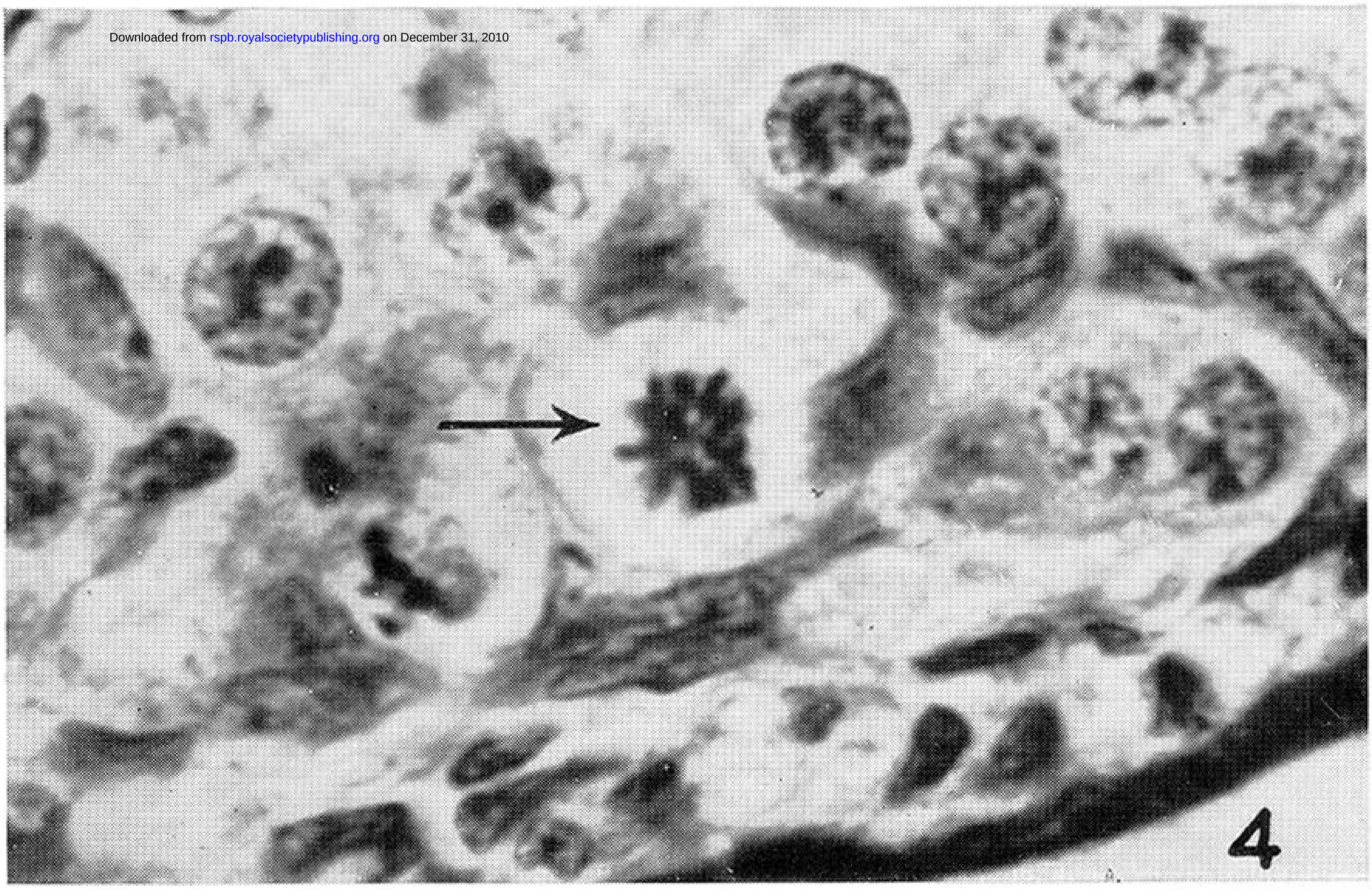
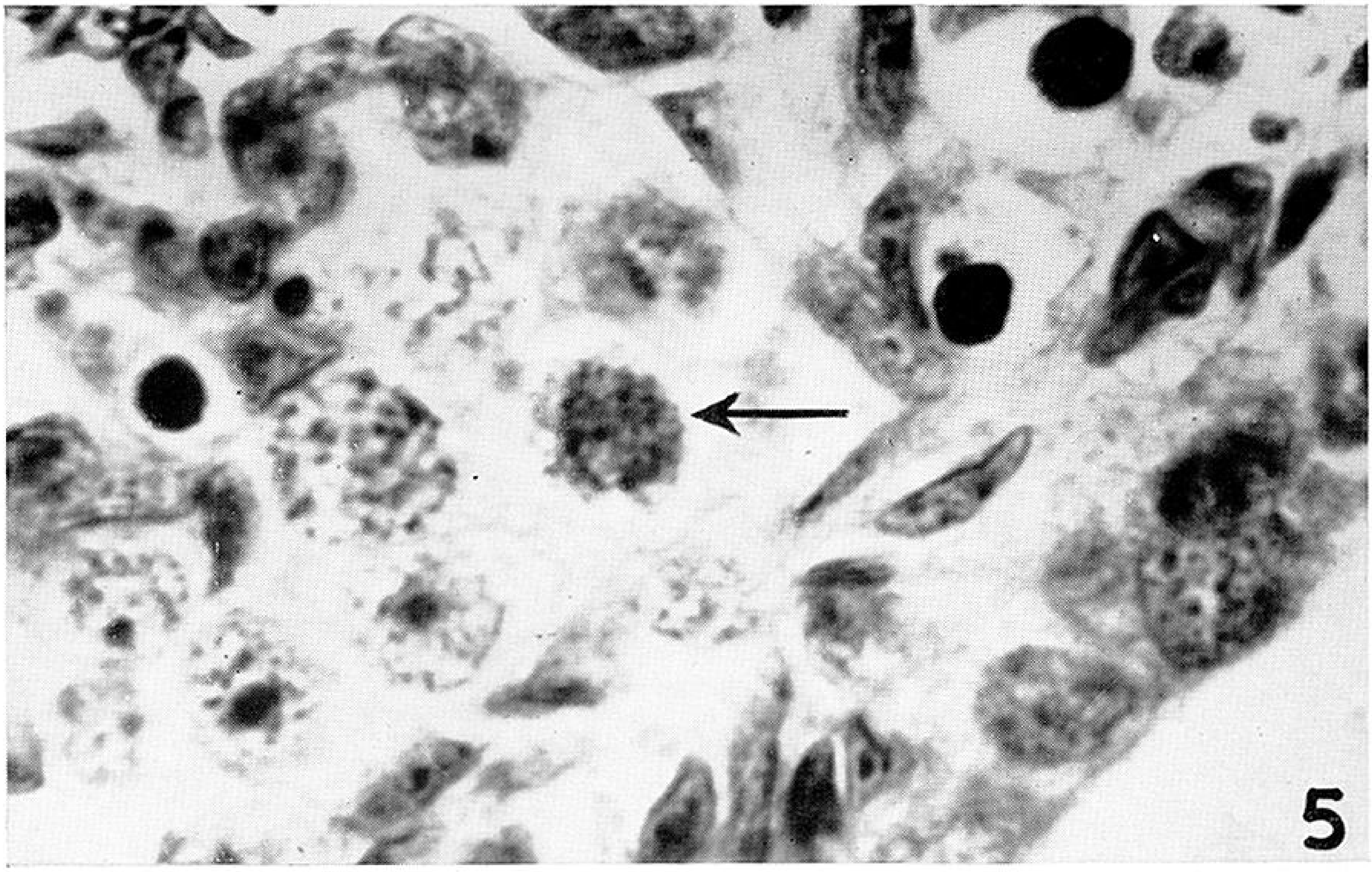


FIGURE 3. Adult *Loris* ovary. Oogonia in interpase (I) and prophase (P). (× 3000.)

FIGURE 4. Adult *Loris* ovary. Oogonium in metaphase. (× 3000.)



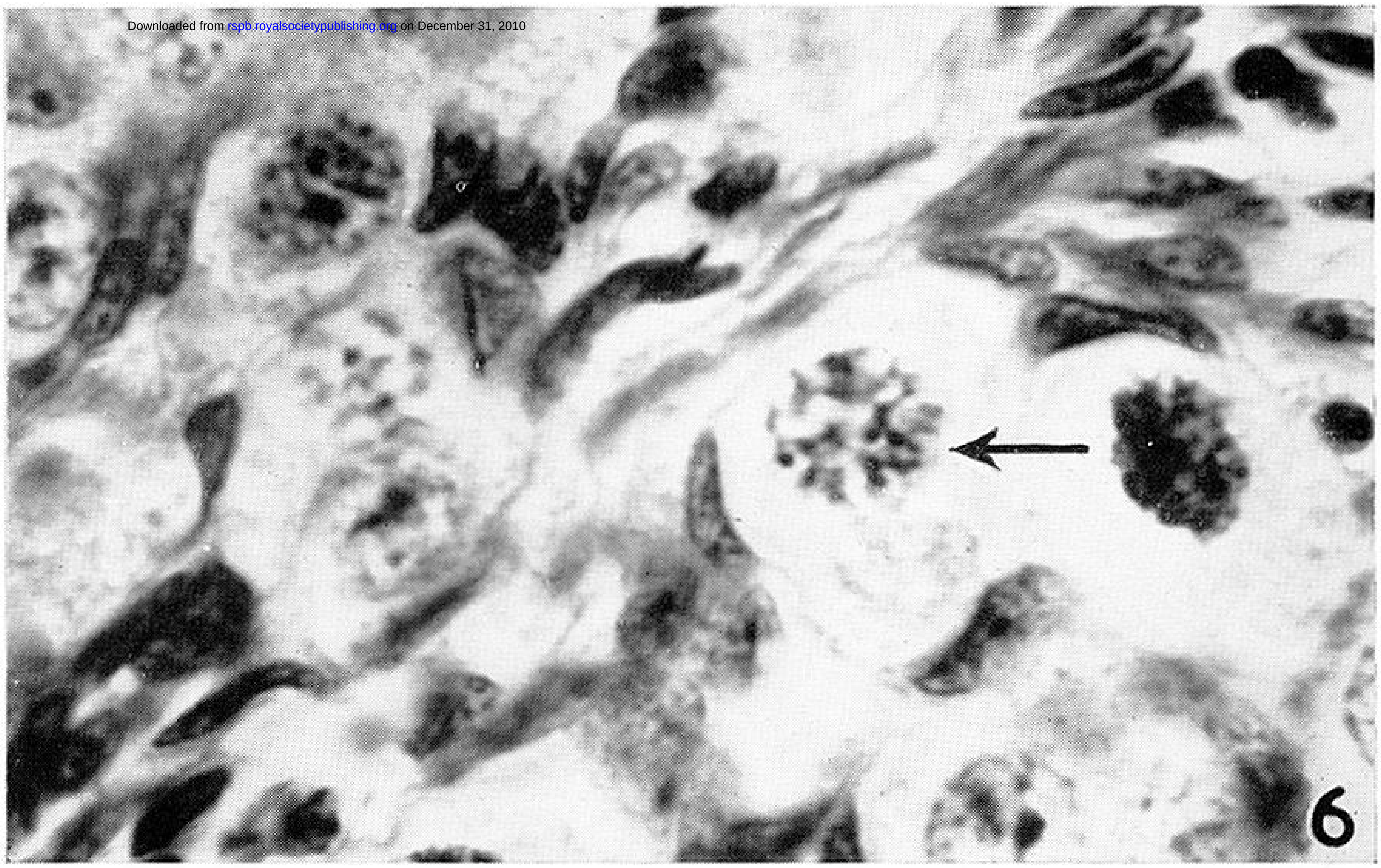
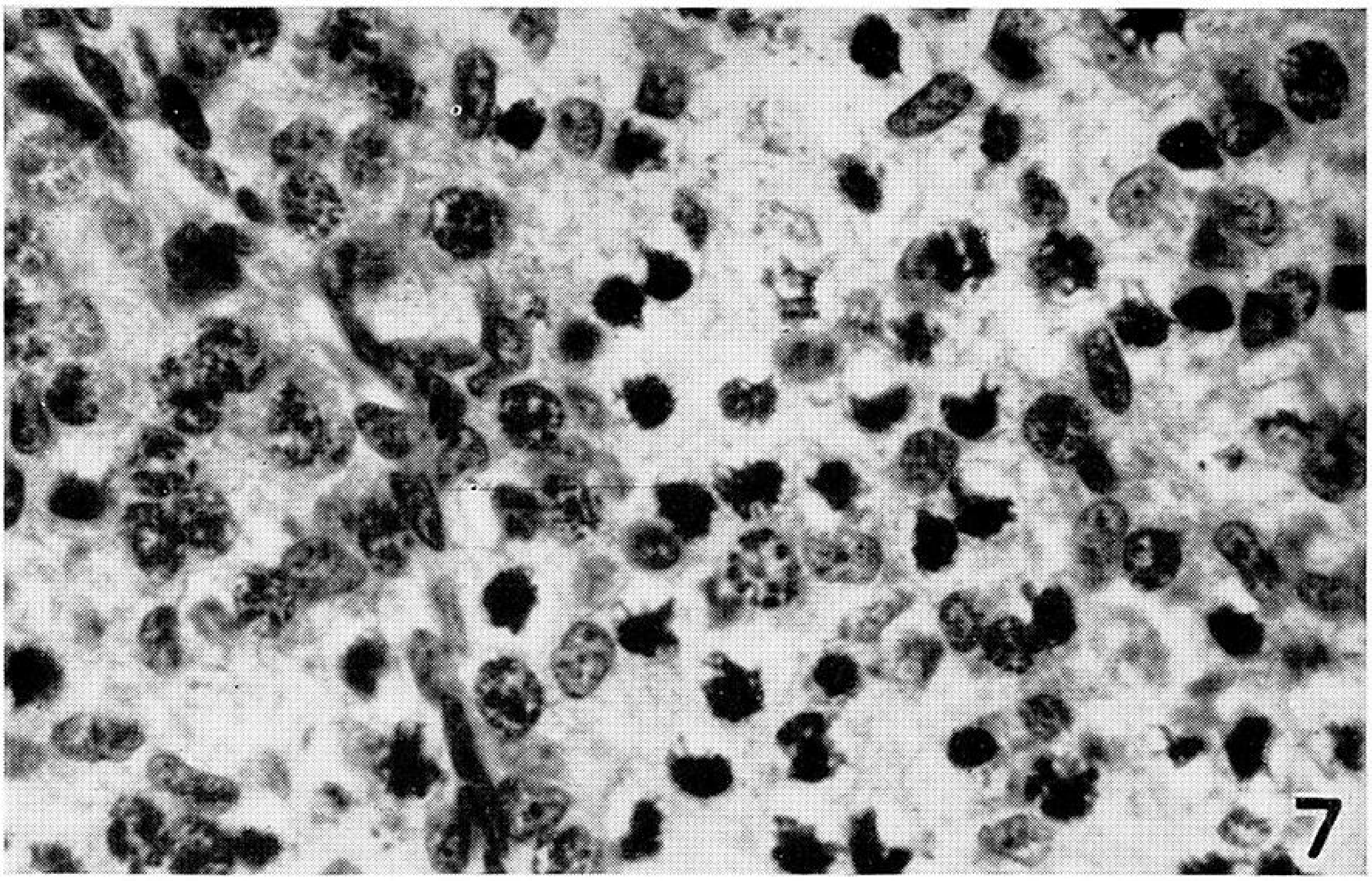


FIGURE 5. Adult *Loris* ovary. Primordial germ cells at leptotene. (× 3000.)

FIGURE 6. Adult *Loris* ovary. Primordial germ cells at pachytene. (× 3000.)



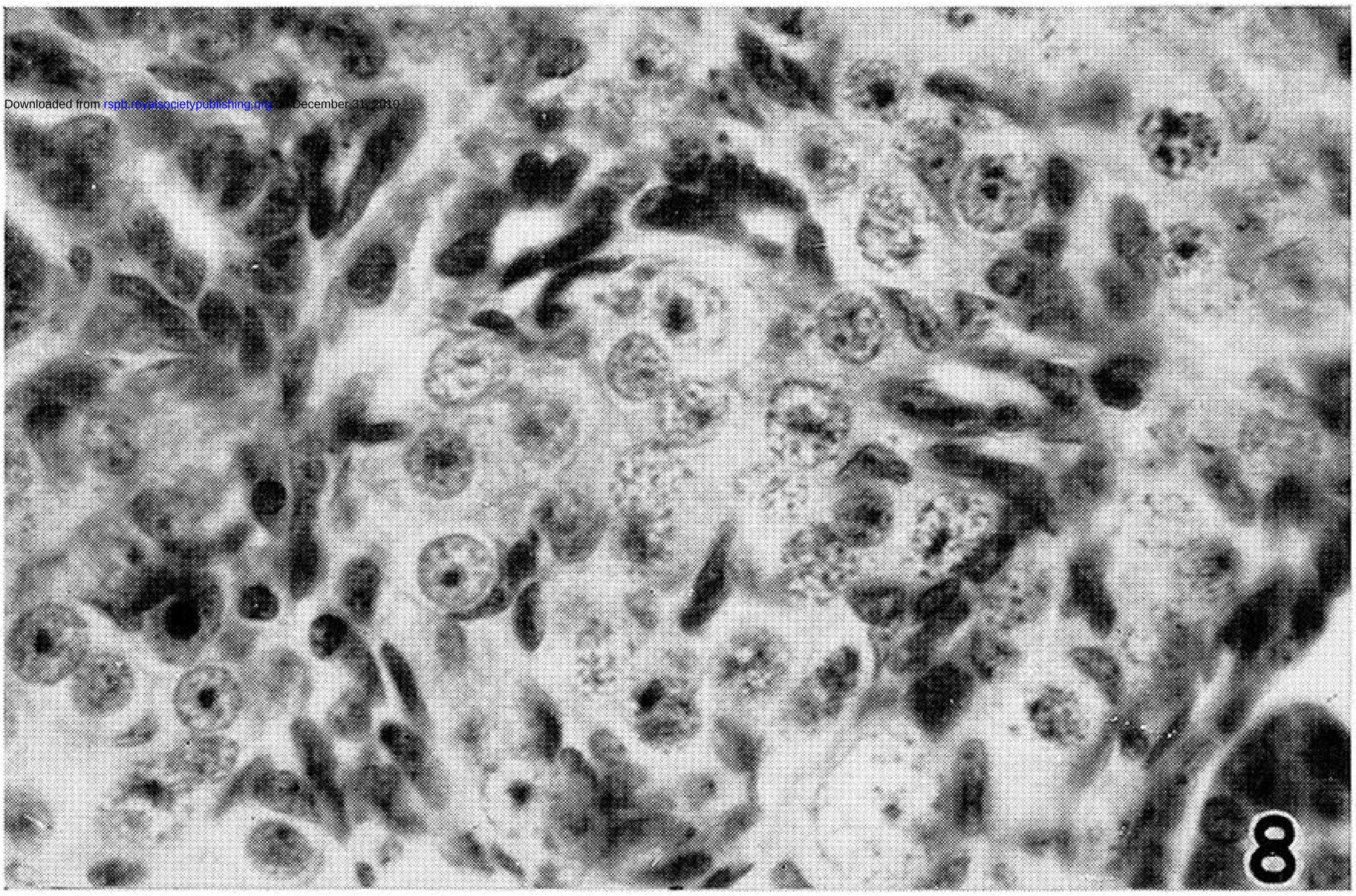


FIGURE 7. Foetal *Loris* ovary. Note the large number of atretic primordial germ cells with dark nuclei. (× 1350.)

FIGURE 8. Adult *Loris* ovary. Primordial germ cells in a nest. (× 1350.)