Reproductive failure due to the entrapment of oocytes in luteinized follicles of the little bulldog bat

(*Noctilio albiventris*)

J. J. Rasweiler IV

*Department of Cell Biology and Anatomy, Cornell University Medical College, 1300 York Avenue, New York, New York 10021, U.S.A.*

**Summary.** The reproductive tracts of 112 female little bulldog bats collected around the onset of a breeding season in the Cauca Valley of Colombia were examined histologically. Of the 88 females with luteinizing/luteinized follicles or new CL, 72 carried tubal ova or uterine blastocysts and 16 were non-pregnant. In 14 of the latter follicle rupture had apparently failed to occur, and the oocyte or a collapsed zona pellucida was found within a luteinizing or luteinized follicle. These structures appeared to be functional because most of the affected bats demonstrated preferential stimulation of the ipsilateral oviduct and/or uterine horn. Two of these animals also had a second, older luteinized follicle which contained the remnants of an oocyte. None of these 14 bats exhibited the uterine modifications thought to be associated with a previous pregnancy or had prominent mammary glands. Such reproductive features were, in contrast, frequently demonstrated by other females in the population. These observations suggest that the luteinization of unruptured follicles may have occurred in prepubertal members of the population and reflect immaturity of the hypothalamo-pituitary-ovarian axis in these individuals.

**Introduction**

In most mammals the determination of whether ovulation has occurred or not often depends upon the observation of secondary events associated with the formation of corpora lutea, such as changes in plasma progesterone or urinary pregnanediol levels, basal body temperature in man, and endometrial histology. Unfortunately, none of these provides evidence that follicles have ruptured and that oocytes have actually been released. During studies on reproduction in a wild population of the little bulldog bat, *Noctilio albiventris*, a surprisingly large number of the non-pregnant females examined around the onset of a breeding season were found to possess apparently functional luteinized follicles with entrapped oocytes or their remnants. These observations serve to emphasize the importance of considering derangement of the ovulatory process as a possible cause of infertility in other mammals. They also raise the question as to why a trait which precludes conception continues to be exhibited by a significant proportion of the bat population.

**Materials and Methods**

All of the bats examined in this study were collected in rural areas of the Cauca Valley of southwestern Colombia (Rasweiler, 1977). Their reproductive tracts were removed within 16 h after
capture, fixed in Zenker's fluid for 10–12 h, washed overnight in tap water and processed through graded ethyl alcohols, cedar wood oil and benzene to paraffin wax. The tracts were then serially sectioned at 6 µm. The sections were stained with haematoxylin and eosin, haematoxylin and buffered Giemsa stain (pH 4.5), Masson's trichrome procedure, or the periodic acid–Schiff (PAS) technique. Some sections from each tract were incubated for 1 h at 37°C in 0.1% α-amylase (α-1, 4-glucan 4-glucanohydrolase; Sigma Chemical Company, St Louis, MO, U.S.A.) dissolved in a 0.02 M-phosphate buffer (pH 6) to remove any glycogen before being stained by the PAS procedure. Parallel sections were incubated under similar conditions in the buffer alone and then stained. The glycogen-hydrolysing ability of the enzyme solution was confirmed by processing pieces of liver and skeletal muscle with sections of the reproductive tracts.

**Observations**

*Reproductive condition of bat population*

In all of the females captured between late February and mid-April (N = 112) a high degree of reproductive synchronization was observed. Pregnant animals (N = 72) carried either tubal ova or uterine blastocysts with the most advanced blastocysts being in the process of amniogenesis. With the exception of one case of twinning, all of the bats carried single embryos. The remaining animals were non-pregnant with no recent CL (N = 21), preovulatory with a mature Graafian follicle (N = 3), or non-pregnant with a luteinizing/luteinized follicle or new CL (N = 16) (Rasweiler, 1977, 1978). Many of the 21 non-pregnant bats lacking CL were captured early in the study interval. All possessed growing ovarian follicles, most exhibited some stimulation of the epithelium in one or both oviducts, and many had been inseminated (Rasweiler, 1978). Only 2 of the females in this group demonstrated uterine modifications thought to be indicative of a previous pregnancy (see below).

*Oocyte entrapment in luteinized follicles*

All of the non-pregnant reproductive tracts associated with a luteinizing follicle or new CL were examined carefully in an effort to account for the fate of the oocyte or ovum. In one bat the latter could not be located, while in another an ovum was present in the oviduct but degenerating. In each of the remaining 14 animals the oocyte or a collapsed zona pellucida was found within the single luteinizing (3 bats) or recently luteinized follicle (11 bats) (Pl. 1, Figs 1, 3 & 5). These luteinized follicles were very similar in appearance, and apparently also in function (see below), to the CL of

**PLATE 1**

**Figs 1 and 2.** Luteinized follicles of different ages in the left and right ovaries of a little bulldog bat. These were the only luteinized follicles or CL present, and both contained the remnants of oocytes (arrows; also see Figs 3 & 4). Masson's trichrome (Fig. 1); PAS-haematoxylin (Fig. 2), × 81.

**Fig. 3.** Higher power view of a parallel section through the degenerating oocyte present in the luteinized follicle shown in Fig. 1. Masson's trichrome, × 547.

**Fig. 4.** Higher power view of the collapsed zona pellucida found in the luteinized follicle shown in Fig. 2. PAS-haematoxylin, × 547.

**Fig. 5.** Luteinizing follicle containing an entrapped oocyte (arrow). The clumping of granulosa cells evident around the oocyte is not typical of mature preovulatory follicles in little bulldog bats. Masson's trichrome, × 81.

**Fig. 6.** Recently ruptured follicle from a bat which carried a pronuclear-stage ovum. The stigma is evident at the ovarian surface. Masson's trichrome, × 187.
early pregnancy in this species. Furthermore, in 2 of the animals another, older luteinized follicle which also contained the remnants of an oocyte was present in the ovary contralateral to the newly luteinized follicle (Pl. 1, Figs 2 & 4). All of these bats were collected at a time when most other females in the population were in early pregnancy (see Rasweiler, 1977: Fig. 1). The presence of uterine spermatoza in 10 of the 14 animals with entrapped oocytes indicated that most had mated.

In 2 of the luteinizing follicles with entrapped oocytes the changes had progressed beyond what might be expected to accompany any preovulatory luteinization: the oocyte appeared degenerate, the cumulus cells were clumped in a fashion not typical of preovulatory follicles (Pl. 1, Fig. 5; for examples of normal preovulatory follicles, see Rasweiler, 1977) or no longer surrounded the oocyte, and blood was present in the membrana granulosa and the antrum. In the 3rd luteinizing follicle some hypertrophy of the granulosa cells was evident and the oocyte had undergone the first meiotic division; however, the follicle appeared small (about 270 × 340 µm) for a preovulatory follicle in this species, and many cumulus cells possessed pycnotic or karyorrhectic nuclei. It is unclear whether this follicle was in the process of atresia or being converted into a luteinized follicle.

None of the luteinizing/luteinized follicles with entrapped oocytes exhibited signs of stigmata, whereas these could be recognized on the surface of CL developing from ruptured follicles (Pl. 1, Fig. 6) until the embryo reached the late morula stage.

Reproductive history of bats with entrapped oocytes

The 14 animals in which ovulatory failures had occurred were examined further for evidence of previous pregnancies. None exhibited uterine modifications thought to be indicative of a prior pregnancy such as increased PAS staining of arterial walls, fibrotic changes in the walls of veins, or the presence of PAS-positive and lipofuscin-rich macrophages in the uterine stroma (Pl. 2, Figs 7 & 8). All of these females also had mammary glands with non-prominent nipples, suggesting that they had not recently suckled young. In contrast, the vascular changes and activated macrophages thought to be indicative of a previous pregnancy were evident in the uteri of 20 of the 29 bats carrying tubal or early uterine embryos, particularly on the non-gravid side of each tract (which was also usually the location of a regressing CL). All 20 of the females with these uterine characteristics had prominent nipples. Of the remaining 9 animals, none exhibited any uterine vascular changes, 3 possessed PAS-positive macrophages (whose presence might be associated with factors other than the involution of a post-partum uterus), and only 1 had prominent nipples.

---

**PLATE 2**

Fig. 7. Section through the uterine horn of a preovulatory bat in which the blood vessels exhibit changes thought to be a result of a previous pregnancy. Relative to normal vessels the artery (A) shown here contains a much greater abundance of amylase-resistant, PAS-positive material in its tunica media, and its companion vein (V) has a thicker, more fibrous wall. PAS-haematoxylin, × 187.

Fig. 8. Macrophages (e.g. at arrow) in the endometrial stroma of a bat which carried a tubal morula and may have also been pregnant previously. These cells contained lipofuscin granules and an abundance of amylase-resistant, PAS-positive material. PAS-haematoxylin, × 547.

Fig. 9. Ovarian structure (delimited by arrowheads) which contained many amylase-resistant, PAS-positive cells. This may represent the remnants of a CL. PAS-haematoxylin, × 187.

Fig. 10. Higher power view of a portion of the ovarian structure shown in Fig. 9. Many of its cells appear darkly stained (e.g. at arrow) because of their content of amylase-resistant, PAS-positive material. PAS-haematoxylin, × 547.

Fig. 11. Regressing CL which contains a cluster of amylase-resistant, PAS-positive cells (arrow). Additional PAS-positive cells were scattered through this CL and around its periphery. PAS-haematoxylin, × 187.
Most of the bats with entrapped oocytes also had a discrete structure in one ovary which contained many cells rich in amylase-resistant, PAS-positive materials (Pl. 2, Figs 9 & 10). Olive-brown lipofuscin pigment was present in some of these cells. Two lines of evidence suggest that these structures may represent the remnants of old CL. Firstly, in all but 2 of the bats they were found in the ovary opposite to that containing the luteinizing/luteinized follicle, and bats of this species exhibit a high incidence (about 92%) of alternation of successive ovulations between the two ovaries (Rasweiler, 1979). The 2 exceptional animals were those which had 2 successive luteinized follicles with entrapped oocytes in opposite ovaries. The structure under discussion was present in the same ovary with the most recent luteinized follicle in one bat but could not be found in either ovary in the other bat. Secondly, regressing CL were the only other ovarian bodies observed to have such an abundance of PAS-positive cells (Pl. 2, Fig. 11). Depending upon the age of the CL the latter may be macrophages and/or involuting lutein cells (see Paavola, 1977, 1979). It seems unlikely that these ovarian bodies represent the remnants of CL formed around the onset of the breeding season, such as during non-fertile cycles. Similar bodies were observed in nearly all (22 of 24) of the non-pregnant females lacking new CL, many of which were captured at that time. Furthermore, the uterine endometrium in most of these animals was atrophic and thus probably had not been stimulated recently by luteal hormones (Rasweiler, 1977, 1978).

Unilateral reactions

During early pregnancy differential stimulation of the oviducts and uterine horns is frequently evident in female bulldog bats (Rasweiler, 1978). Similar observations were made in the 14 bats with entrapped oocytes. In each of the 3 bats with a luteinizing follicle the ipsilateral oviducal ampulla was more dilated with fluid than was the contralateral one. In 12 of the 14 animals the epithelium of the oviduct ipsilateral to the luteinizing or recently luteinized follicle was also more hypertrophied, and the secretory cells in the ampullary and isthmic epithelium on that side contained larger quantities of glycogen. As a result these cells usually appeared much more heavily vacuolated than those on the contralateral side after being stained by the non-PAS procedures. In the 2 exceptional bats the oviduct contralateral to the luteinized follicle appeared preferentially stimulated. This probably represented a prevoluntary reaction, since the luteinized follicles in these animals appeared older histologically and substantial follicular development was evident in the non-luteal ovary.

Unilateral endometrial reactions were also observed in 10 of the 14 bats with entrapped oocytes. Generally these took the form of preferential endometrial development in the horn ipsilateral to the luteinizing or recently luteinized follicle and was most pronounced at its cranial end. The extent of endometrial growth varied between animals and appeared to be related to differences in luteinization of the follicles. Such reactions were evident only in 1/3 bats possessing an early luteinizing follicle but were prominent in 9/11 animals with a more fully luteinized follicle.

In all of the bats with entrapped oocytes the bursa surrounding the ovary containing the luteinizing or recently luteinized follicle was more dilated with fluid than that around the opposite ovary. A similar difference between the luteal and non-luteal sides was generally observed in the pregnant bats. The bursal fluid in all of these animals also contained an abundant, granular precipitate. Usually this was not evident within the oviducts, although very small amounts of a similar material were observed around the newly ovulated ova in the oviducts of 4 bats.

Discussion

Previous studies have provided evidence that the little bulldog bat is monovular (with rare exceptions), breeds seasonally in at least part of its range, and possesses a lengthy gestation period which extends for some months (Anderson & Wimsatt, 1963; Rasweiler, 1977; Hood & Pitocchelli,
Entrapment of oocytes in luteinized follicles

1983). It was therefore surprising to find that more than 15% of the females collected with luteinizing/luteinized follicles or CL during the breeding season in the Cauca Valley of Colombia exhibited reproductive failure presumably due to the entrapment of oocytes. Furthermore, in 2 of these females follicles in successive cycles had apparently luteinized without rupturing. For a species with a low reproductive potential the persistence in the population of a trait which results in such a failure rate clearly seems peculiar. Even if these females could still ovulate and conceive within the same breeding season, they would presumably then give birth later than most of the population and possibly at a less favourable time of the year for healthy development of the young. As well as ovulatory failure the reproductive performance of the bat population is also probably reduced by fertilization failures and prenatal mortalities in the females which do ovulate. Post-ovulatory reproductive wastage has been found to be significant in other mammals (Brambell, 1948; Adams, 1960, 1970; Hanley, 1961; Biggers, 1969; Edey, 1969; Ayalon, 1978; Small, 1982; Hagen & Kephart, 1980).

Further examination of the affected females failed to uncover any conclusive evidence that they had previously carried uterine conceptuses, and all had non-prominent mammary glands. On the other hand, vascular changes and activated macrophages thought to be indicative of previous pregnancies were frequently seen in the uteri of other females in the population, and these individuals also generally had prominent nipples. These observations suggest that the luteinization of unruptured follicles may have occurred in prepubertal bats and reflect immaturity of the hypothalamo–pituitary–ovarian axis. Many other species exhibit non-fertile cycles around the onset of puberty or at the end of anoestrous periods (Grant, 1933; Cole & Miller, 1935; Deanesly, 1934, 1935; Brambell & Rowlands, 1936; Brambell & Hall, 1939; Buechner, Morrison & Leuthold, 1966; Short, 1976; Foster, 1977; Apter, Viinikka & Vihko, 1978; Lemarchand-Béraud, Zufferey, Reynold & Rey, 1982; Metcalf, Skidmore, Lowry & Mackenzie, 1983), but it is generally unclear how frequently this is associated with the luteinization of unruptured follicles. However, the latter phenomenon may be a significant cause of infertility in women (MARIK & HULKA, 1978; Craft, Shelton, Yovich & Smith, 1980; Dmowski, Rao & Scommegna, 1980; Coulam, Hill & Breckle, 1982; Konincx & Brosens, 1982). Accessory CL are known to develop from luteinized unruptured follicles in a variety of mammals (Mossman & Duke, 1973; Amoroso & Perry, 1977; Weir & Rowlands, 1977).

Nearly all of the bats with entrapped oocytes also possessed a single ovarian body which contained many PAS-positive cells. Although the origin of these bodies could not be established with certainty, there is good reason to believe that they may represent the remnants of CL formed before puberty. CL are typically present in the ovaries of fetal and immature giraffes (Kayanja & Blankenship, 1973) and neonatal degu (Weir & Rowlands, 1974), and can also occur, albeit rarely, in the fetal human ovary (Miles & Penney, 1983).

The luteinizing/luteinized follicles containing entrapped oocytes appeared to be functional, since most of the affected bulldog bats exhibited unilateral oviducal and/or uterine reactions similar to those of pregnant animals. The development of the endometrial reaction in particular seems to be closely correlated with formation of the CL and is therefore presumably dependent upon luteal hormones (Rasweiler, 1978).

The fact that typical unilateral effects were evident in these bats also has implications concerning the routes by which ovarian hormones may be delivered preferentially to one side of the tract. This indicates that full development of these reactions does not depend upon follicle rupture and passage of the follicular contents down the ipsilateral ducts. Observation that the unilateral reactions begin to develop before ovulation (Rasweiler, 1978) also points to the involvement of another mechanism for delivering hormones preferentially to one side of the tract. The possibility remains that significant quantities of steroids might diffuse out of the ovary containing the follicle or new CL even in the absence of a rupture point. The bursa surrounding the luteal ovary in early pregnant animals and those with entrapped oocytes usually appeared more dilated with fluid than that around the opposite ovary. Much of this fluid may be an ovarian exudate (Konincx, Renaer &
Brosens, 1980). However, some species of bats exhibiting unilateral oviducal reactions have holes in the ovarian bursa through which ovarian secretions presumably would escape (Rasweiler, 1972; de Bonilla & Rasweiler, 1974). In most bulldog bats, the bursal fluids contained a proteinaceous precipitate which usually was not evident in the oviducts. This raises the question of whether there is sufficient movement of the bursal fluid down the oviducts to account for preferential stimulation of one side of the tract. It has been proposed elsewhere that development of the unilateral reactions may actually depend upon a countercurrent exchange of hormones between the ovarian venous and/or lymphatic drainage and the arterial supply to the ipsilateral ducts (Rasweiler, 1978).

The field work was pursued while I was associated with the Tulane University International Center for Medical Research. The assistance of Dr. M. E. Thomas and the use of facilities at the Cali Station of the Smithsonian Tropical Research Institute are also gratefully acknowledged. The work was supported by a grant from the Johnson Fund of the American Philosophical Society and by NIH Grant AI-10050.

References

Entrapment of oocytes in luteinized follicles


Received 8 August 1983