AMER. ZOOL., 32:264–275 (1992)

Role of the Corpus Luteum and Progesterone in the Evolution of Vertebrate Viviparity[1]

IAN P. CALLARD, LISA A. FILETI, LORELEI E. PEREZ, LISA A. SORBERA, GEORGIA GIANNOUKOS, LORRIE L. KLOSTERMAN, PAUL TSANG, AND JOHN A. MCCRACKEN[2]

Department of Biology, Boston University, 5 Cummington Street, Boston, Massachusetts 02115
Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672
and
[2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

SYNOPSIS. For the past 25 years we have used a comparative strategy designed to identify and describe the endocrine parameters of the oviparous-vivparous transition and subsequent gradual reduction in hepatic yolk protein precursor (vitellogenin) synthesis associated with placental viviparity. Our approach has been to study vertebrate groups in which both oviparous and viviparous modes are common (reptiles, elasmobranchs). We have provided evidence for the control of follicular (granulosa/theca) and luteal steroidogenesis, and the cellular basis of gonadal steroid hormone action on the key target tissues (oviduct, liver). Our results, some of which are summarized below, have led us to suggest that ovarian progesterone (follicular or luteal in origin) has a dual role in the evolution of viviparity: 1. To inhibit myometrial contractions, thus providing a primary condition for egg retention and viviparity. 2. To inhibit estrogen-induced hepatic vitellogenin synthesis as part of both normal oviparous cycles and as a concomitant of placental evolution.

INTRODUCTION

The idea that the corpus luteum and the hormone progesterone were involved in the retention of eggs, and hence viviparity, was first clearly enunciated by Hisaw (1959): ". . . It may not be entirely fanciful to think of the primitive endocrine function of the corpus luteum as being one of facilitating the old reptilian custom of carrying ova in the uterus until a favorable time and place was found to lay the whole clutch at once." This idea has been carefully developed by this laboratory as indicated by publications in the primary literature since 1964 (see, for example, Callard and Leathem, 1964, 1965; Chan *et al.*, 1973; Callard and Hirsch, 1976; Ho and Callard, 1984; Kleis-San Francisco and Callard, 1986*a, b*) and has been most recently restated by Shine and Guillette (1988) in the *Journal of Theoretical Biology.* In addition, we have extended the idea to include a parallel role for progesterone in the down-regulation of hepatic vitellogenin synthesis as a corollary of viviparity and placental development (see, for example, Callard *et al.*, 1972*a*; Ho *et al.*, 1981, 1982; Riley and Callard, 1988*a, b*; Riley *et al.*, 1988; Callard *et al.*, 1990*a, b*).

EVOLUTION OF VIVIPARITY

Taxonomic and embryological evidence from reptiles and other groups indicates that the ancestral mode of reproduction in vertebrates is oviparous (Weekes, 1935; Matthews, 1955; Amoroso, 1959). However, in all vertebrate groups with the exception of birds, viviparity has evolved to varying extents and frequencies. The occurrence of elasmobranch and reptilian genera comprised of both egg-layers and live-bearers shows that viviparity has arisen from egg-laying several times (Tinkle and Gibbons, 1977; Shine and Bull, 1979). Even subspecies within a single genus may be reproductively bimodal (*e.g., Sceloporus aeneus aeneus,* oviparous; *S. a. bicanthalis,* viviparous; Guillette and Jones, 1985).

The reproductive adaptations concomitant with viviparity are well documented (see for example Packard *et al.*, 1977) and include loss of shell, increased nutrient transfer, placentation and reduction in yolk protein and, in eutherian mammals, the development of the trophoblast. The necessary precondition for these changes is that eggs be retained in the oviduct for extended

[1] From the Symposium on *Evolution of Viviparity in Vertebrates* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1990, at San Antonio, Texas.

periods of time during which embryonic development occurs. Thus, for viviparity to arise from oviparity, natural selection might favor intermediate stages in which females retain eggs for progressively longer periods of time (Shine and Bull, 1979), and it is logical to ask whether changes in hormonal control mechanisms are the primary determinants in the evolution of viviparity and subsequent yolk reduction. According to Eldredge and Gould (1972), a change in timing of expression of a single regulatory gene can lead to dramatic changes in morphology or function. In the context of the hormonal determinants of the evolution of viviparity, a change in the timing or level of expression of genes for key steroidogenic enzymes (cholesterol side chain cleavage enzyme and aromatase) controlling progesterone and estradiol synthesis secretion could result in an extended ability to retain eggs and hence viviparity.

Indeed, early researchers noted a correlation between the duration of egg retention and the longevity of the corpus luteum and suggested a causal relationship (Amoroso, 1952; Hisaw, 1959). Our studies together with others in the literature show that the essential components of the hypothalamic-pituitary-gonadal axis are in place in both oviparous and viviparous reptiles and elasmobranchs (Callard *et al.*, 1972*a, b, c*); Lance and Callard, 1978*a, b*; Callard and Ho, 1980; Dodd and Sumpter, 1984; Licht, 1984). While evidence for a secretory corpus luteum in both of these groups is strong, there appears to be a difference in progesterone secretory patterns. Whether these are due to inherent properties of the corpora lutea or to superimposed control mechanisms is not known. Moreover, the exact functions of progesterone and other products of the corpus luteum with respect to egg retention remains to be established.

Detailed below are correlative data showing that a relationship exists between the mode of reproduction (oviparous *vs.* viviparous) and the duration, pattern and quantity of progesterone in oviparous *vs.* viviparous elasmobranchs and reptiles (II-B). In addition, we review factors controlling the development and maintenance of the corpus luteum in mammals and attempt to relate this body of knowledge to scattered data in non-mammals (II-C). Finally, possible interactions of luteal hormones upon the female reproductive tract are discussed, with particular attention to contractility (II-D), vitellogenesis (II-E) and steroid hormone receptors (II-F).

LUTEAL FUNCTIONS IN VIVIPAROUS *VS.* OVIPAROUS SPECIES

Progesterone secretion

It is generally recognized that progesterone is a quantitatively important product of the corpus luteum, and the majority of studies have focused on this hormone. While both oviparous and viviparous species are capable of synthesizing progesterone, there are clear differences in the onset, duration and quantity of progesterone secretion in animals with different reproductive modes. In general, groups in which viviparity has not evolved (*e.g.*, skates, turtles, and birds) exhibit a predominantly preovulatory pattern of progesterone production. By contrast, those groups in which viviparity has evolved (*e.g.*, sharks, snakes and lizards) exhibit a predominantly post-ovulatory pattern of progesterone production (for reviews see Callard and Lance, 1977; Lance and Callard, 1980; Callard and Ho, 1980; Guillette *et al.*, 1981; Callard *et al.*, 1989).

Reptiles.—In chelonian species, plasma progesterone rises to a maximum in the preovulatory period, falling abruptly in the brief post-ovulatory luteal phase (Callard *et al.*, 1978). A similar pattern has been described for the sea turtle, *Chelonia mydas* (Callard and Lance, 1977; Licht *et al.*, 1979) and in the stinkpot turtle, *Sternotherus odoratus* (McPherson *et al.*, 1982). By contrast, in viviparous squamates, plasma progesterone begins to rise prior to ovulation but reaches a maximum 30–90 days later, depending on species (*e.g.*, the water snake, *Nerodia* (*Natrix*) [Chan *et al.*, 1973; Kleis and Callard, 1986*b*]; the garter snake, *Thamnophis sirtalis* [Highfill and Mead, 1975*a*], the iguanid lizards, *Sceloporus cyanogenys* [Callard *et al.*, 1972*a, c*] and *Chameleo pumilis* [Veith, 1974]). Removal of corpora lutea in gravid viviparous *Thamnophis* (Highfill and Mead, 1975*b*) or ovip-

arous *Anolis* (Guillette and Fox, 1985); or ovariectomy in gravid viviparous *Sceloporus cyanogenys* (Callard *et al.*, 1972*a, c*) results in a fall of plasma progesterone levels. Oviparous squamates have progesterone patterns intermediate between those of viviparous squamates and oviparous chelonia in that progesterone levels begin to rise after ovulation but terminate shortly thereafter (Bona-Gallo *et al.*, 1980; Arslan *et al.*, 1978; van Wyck, 1984). There are some exceptions to these generalizations (see Gorman *et al.*, 1981) and also species variations in progesterone titer and pattern. This is to be expected in a class like the Reptilia which exhibits a wealth of reproductive adaptations and does not negate the argument that progesterone secretion is correlated temporally with reproductive mode.

Elasmobranchs.—The data are much more limited than for reptiles (see Dodd and Sumpter, 1984). However, in the single viviparous species examined (*Squalus acanthias*), the enzyme 3BHSD has been demonstrated in the corpus luteum. High progesterone titers are maintained throughout gestation (Lance and Callard, 1969) and resemble the pattern in viviparous squamates (Tsang and Callard, 1987*a*). *In vivo* and *in vitro* studies with granulosa and lutein tissue from *Squalus* provide evidence that plasma progesterone has a luteal origin (Tsang and Callard, 1987*a, b*). Interestingly, the corpus luteum of *Squalus acanthias* is the longest-lived corpus luteum of pregnancy on record (two years), including all known mammals (see Tsang and Callard, 1987*a*).

In contrast to the dogfish, the oviparous skate, *Raja erinacea,* shows a plasma progesterone pattern characterized by a periovulatory peak, with the falling phase occurring shortly after ovulation (*i.e.*, in the early luteal stage) (Koob *et al.*, 1986). It is important to note that the temporal pattern of plasma progesterone in the skate is like that described above for the oviparous turtle, and also resembles that described for the bird, although the secretion of progesterone appears to be more abruptly terminated by ovulation in the latter (see Doi *et al.*, 1980, for the quail). More recently, we have demonstrated that luteal tissue from an oviparous species, the skate *Raja erinacea,* synthesizes progesterone (Fileti and Callard, 1988). Basal progesterone in the absence of substrate increased from stage I (recently formed corpora lutea) to stage II (mature) and decreased as corpora lutea aged (stage III). Addition of 25-OH-cholesterol substrate markedly increases progesterone synthesis in a dose-dependent fashion. These data suggest that corpora lutea from the previous cycle (mature, stage II) could contribute to the large preovulatory peak in plasma progesterone observed by Koob *et al.* (1986). Preovulatory follicles may also be an important source of progesterone; this is currently being investigated.

Other luteal hormones

Estrogen.—In some mammals (*e.g.*, rat; Elbaum and Keyes, 1976) estrogen appears to be a functionally important product of the corpus luteum, although some contribution to the estrogen pool may be made by small follicles during the luteal phase (Miller and Keyes, 1975). It may serve as both a luteotropin (Keyes and Nalbandov, 1967; Gibori and Richards, 1978) and a luteolysin (Karsch and Sutton, 1978). Several critical stages of the reproductive cycle are dependent upon estrogen and progesterone together or in the appropriate sequence; thus it seems reasonable to postulate that estrogen is also important to the evolution of viviparity, as well as progesterone, mainly because it is considered to induce progesterone (Leavitt *et al.*, 1974) and oxytocin (Fuchs *et al.*, 1983) receptors and it is essential for relaxin action (Bryant-Greenwood, 1981) on the reproductive tract. However, the role of estrogen in the regulation of luteal function and as a synergist of progesterone in the evolution of viviparity has been neglected.

In general, plasma estrogen titers rise in the preovulatory period, associated with follicular development and vitellogenesis in both oviparous and viviparous species (Licht, 1984, for review). The few reports of plasma estrogen levels during the postovulatory phase provide somewhat conflicting information for reptiles (Callard *et al.*, 1978; Arslan *et al.*, 1978; Licht *et al.*, 1979; Bona-Gallo *et al.*, 1980; McPherson *et al.*,

1982; Kleis and Callard, 1986*b*). In elasmobranchs, plasma estrogen titers fall to baseline after ovulation in the skate (Koob *et al.*, 1986) and rise toward term during pregnancy in *Squalus* (Tsang and Callard, 1987*a*). A definitive determination of estrogen synthesis by follicular and luteal tissues and its quantitative importance is required in order to evaluate the role of this hormone in the evolution of viviparity.

Relaxin.—The mammalian corpus luteum has long been recognized as a source of relaxin (Steinetz *et al.*, 1959; Schwabe *et al.*, 1978, for reviews). In collaboration with Schwabe *et al.*, we have isolated and sequenced *Squalus* ovarian relaxin, and most recently that of the skate (see Bullesbach *et al.*, 1986, 1987). Possible interactions of relaxin with ovarian progesterone and estrogen following ovulation are discussed below.

Neural lobe peptides and prostaglandins.—Although evidence for *de novo* synthesis of neural lobe peptides by the corpus luteum of non-mammals is lacking, this possibility should not be overlooked. Similarly, no evidence for prostaglandin synthesis by the corpus luteum of non-mammalian species exists. That prostaglandins may have an important role in reptilian reproduction is suggested by their actions on the reproductive tract and ovary in reptiles (see review by Guillette, 1990, and below).

REGULATION OF THE CORPUS LUTEUM

If we accept that the corpus luteum is responsible for the prolonged pattern of progesterone secretion common to viviparous species and is involved in egg retention, it is evident that a key element in the evolution of viviparity is the mechanism by which luteal function is sustained after ovulation. Information about the regulation of the mammalian corpus luteum is abundant (see Rothchild, 1981). Based on these data, control mechanisms may be classed as luteotrophic or luteolytic. Further, agents which regulate the formation (luteinization) and functional lifespan of the corpus luteum may also be categorized as extrinsic (extraovarian) or intrinsic (intraovarian). Examples of those which originate within the ovary itself are prostaglandins, estradiol and other steroids, relaxin and oxytocin, whereas those which reach the organ via the bloodstream are pituitary, fetal or decidual gonadotropins and uterine prostaglandins. It is probably a combination of several factors, operating in tandem or in sequence, which is responsible for the luteal secretory patterns unique to each species.

Extrinsic controls

Gonadotropins.—The importance of pituitary gonadotropins, specifically LH and prolactin, as regulators of the mammalian corpus luteum is undisputed (Rothchild, 1981). There is some evidence that these two hormones also affect progesterone production in elasmobranchs and reptiles when treated *in vitro.* Thus, turtle corpora lutea can convert [^{3}H]pregnenolone to progesterone but not androgen or estrogen, and progesterone synthesis is increased by LH, but not prolactin (Callard *et al.*, 1976). A similar stimulatory effect of ovine FSH and LH has been demonstrated using turtle luteal minces, FSH being most effective when newly-formed corpora lutea were used (Klosterman and Callard, unpublished). A previous study with isolated turtle luteal cells demonstrated increased progesterone synthesis in response to cAMP (Lance and Callard, 1978*a*). Using luteal minces from *Squalus acanthias,* a recent study demonstrated increased progesterone synthesis in response to extracts of the gonadotropic ventral lobe of homologous pituitary (Tsang and Callard, 1987*a*).

Patterns of peripheral gonadotropin have been reported in only a limited number of reptiles due to the shortage of suitable antisera (see Licht, 1984); for elasmobranchs, reports are limited to the studies of Dodd and co-workers (see Dodd and Sumpter, 1984). A serious gap in our knowledge is definitive information on whether hypophysectomy will terminate luteal function, or conversely, if administration of gonadotropins will prolong egg retention in oviparous species. Removal of the pituitary early in pregnancy caused resorption, or if late, abortion, in species of *Natrix, Thamnophis* and *Storeria* (Clausen, 1940). However,

Bragdon (1951) and Panigel (1956) found that the operation did not interfere with embryonic development. In viviparous *Sceloporus cyanogenys* we have shown that hypophysectomy caused disappearance of luteal 3 beta HSD within one week of the operation, coupled with a significant decline in plasma progesterone which could be maintained with ovine FSH (Callard *et al.*, 1972*c*). In addition, reproductive tract influences on the ovary have been suggested by the work of Cuellar and Cuellar (1976) in *Cnemidophorus uniparens*. Here, removal of oviductal eggs resulted in supernumerary clutches. Whether this was due to direct or indirect neural and/or hormonal effects is not known. Despite the obvious drawbacks, integration of such experiments into a more sophisticated study of luteal regulation is an essential step.

Prostaglandins (see Guillette, 1990).—There is a paucity of information on this subject. Observations by Guillette *et al.* (1984) suggest that PG F2 alpha may be luteolytic in an oviparous lizard, *Anolis carolinensis*. Further, indomethacin blocks luteolysis and delays parturition in viviparous *Sceloporus jarrovi* (Guillette *et al.*, 1989). In our hands, the effect of prostaglandin F2α on gonadotropin-induced turtle luteal progesterone synthesis *in vitro* has been variable, with both inhibitory (luteolytic) effects, or no effects, observed. This may be a function of luteal stage used for the experiments (Callard and Klosterman, unpublished).

Intrinsic controls

Inherent luteinization patterns.—In many eutherian species, preovulatory follicles have the ability to synthesize and secrete progesterone (Hsueh *et al.*, 1984) although they are not considered to be luteinized. Luteinization of granulosa cells and increased progesterone secretion are spontaneous events in tissue culture in at least nine eutherian species (see Rothchild, 1981) and progesterone secretion from luteal tissue *in vitro* may continue for a time, at least, without added gonadotropins (Gulyas and Hodgen, 1981). We infer from this that the follicular environment actively prevents luteinization of the granulosa cells and that the LH surge and subsequent change in the follicular environment allows expression of luteinization in granulosa cells.

Once the cells are luteinized, progesterone secretion is sustained by a variety of luteotropic hormones (see above). In some species (*e.g.* hystricomorphs, such as the porcupine) accessory corpora lutea form by luteinization of follicles which do not ovulate (Mossman and Judas, 1949). These are called corpora lutea atretica. It is possible that preovulatory increases in progestin secretion are accompanied by subtle morphological changes that herald luteinization, and possibly we should consider luteinization to begin when there is a definite, quantifiable increase in progesterone output. Studies of bird pre- and post-ovulatory follicles may be instructive, as avian follicles are not considered to luteinize but both pre- and post-ovulatory structures synthesize progesterone (Huang and Nalbandov, 1979). In none of the subavian species has the process of luteinization, the pattern of progesterone formation prior to ovulation or the potential of granulosa cells for progesterone secretion been carefully studied. However, in the viviparous snake, *Nerodia,* there is a sharp rise in plasma progesterone level in the immediate periovulatory period (see Lance and Callard, 1978*b*), suggesting an important change that may signal rapid luteinization. Both individual and species differences in luteal function (Fitz *et al.*, 1982) can no doubt be traced to luteal representation of functionally different granulosa cell types. Thus, the follicular granulosa of lizards and snakes is polymorphic (Hubert, 1977), and preliminary electron microscopic observations suggest the presence of different cell types in both *Squalus* (Callard *et al.*, 1989) and *Chrysemys* corpora lutea (unpublished). From an evolutionary standpoint, the relative contribution of different cell types to the corpus luteum of different species may be an important component of the oviparous-viviparous transition, by creating a variable steroid milieu for the reproductive tract.

Paracrine role of intrafollicular estradiol, progesterone and 5α-dihydrotestosterone in

follicular-luteal differentiation. — In the mammalian ovary, it is now clear that locally formed steroids have a critical role in determining the normal sequence of follicular and luteal stages, including follicular growth, granulosa luteinization, and luteolysis (for review, Hsueh *et al.,* 1984). Nonetheless, progesterone and testosterone are able to influence germinal vesicle breakdown in amphibia (Schuetz, 1974; Baulieu *et al.,* 1978) and 17-,20-hydroxyprogesterone causes oocyte maturation in teleost fish (Nagahama *et al.,* 1983). Details of the interaction of gonadotropins, steroids and growth factors in final oocyte maturation in mammals are currently being elucidated.

Prostaglandins as luteolytic agents. — Prostaglandins are important in terminating luteal function in mammals and these may originate either from the uterus or within the corpus luteum itself (Rothchild, 1981). A central argument of Rothchild's treatise on the evolution of the corpus luteum is that endogenous prostaglandin production is spontaneous and must be inhibited for the corpus luteum to synthesize and secrete progesterone. At the same time, prostaglandins through their actions on the reproductive tract enhance oviduct contractility, assisting in egg-laying. Thus, prostaglandins potentially have a two-pronged effect on critical elements in viviparity. Preliminary data obtained in turtles show differences in plasma and oviduct tissue levels of PG F2 alpha pre- and post-ovulatory in normal and AVT-induced oviposition. In pre-ovulatory turtles (*C. picta*), injections of 10 ng arginine vasotocin (AVT, *i.v.*) increased plasma prostaglandin F2α (PG F2) from a pre-injection level of 38.3 ± 10.1 to 513 ± 12 pg/0.1 ml ($P < 0.001$). Similar differences were noted in both plasma and tissue PG F2α and when pre- and post-ovulatory animals were compared (preovulatory: plasma 44.8 ± 12.48 pg/0.1 ml; tissue 1.87 ± 0.55 ng/g; postovulatory: plasma 619 ± 93 pg/0.1 ml, tissue 3.91 ± 0.42 ng/g; P (plasma) < 0.001, $n = 12$; P (tissue) < 0.05, $n = 10$. As noted above, Guillette *et al.* (1984, 1989) have presented evidence for the luteolytic action of prostaglandin F2α in lizards and have demonstrated the synthesis of prostaglandins in a viviparous lizard (Guillette *et al.,* 1988), but results from our laboratory have been inconclusive (see above).

FUNCTIONAL INTERACTIONS OF LUTEAL HORMONES ON THE FEMALE REPRODUCTIVE TRACT

Whether or not longevity of the corpus luteum is the critical factor in viviparity, it is important to ask whether the sensitivity and responsiveness of the oviduct to a particular hormonal milieu differs in oviparous *vs.* viviparous species.

Growth and development

Injections of steroid hormones maintain the thickness of the myometrium and the epithelial glands and cell height in castrate viviparous *Lacerta* (Panigel, 1956). The reproductive tract of dogfish, *Scyliorhinus* can be maintained by estradiol (Dodd and Goddard, 1961; reviewed by Callard and Klotz, 1973). Although the most effective hormone is estrogen, progesterone has a stimulatory effect on the thickness of the myometrium in the viviparous lizard (Panigel, 1956). However, estradiol, but not progesterone, stimulates the oviductal epithelium in the viviparous snake, *Thamnophis* (Meade *et al.,* 1981). In *Chrysemys picta,* marked seasonal changes of oviductal submucosa, epithelial glands and muscularis occur and the effects of exogenous estrogen in reproductively inactive animals suggest this steroid is important in the endogenous cycle (Abrams-Motz and Callard, 1991). Since both hormones are present in oviparous and viviparous species, as are estrogen and progesterone receptors (see 3, below), viviparity cannot be correlated with sensitivity to progesterone per se, but rather with the responses which are elicited by a particular pattern of steroid production.

Contractility and connective tissue properties

Eggs or embryos must exist the oviducts of both oviparous and viviparous species, and the oviducts of both types have inherent contractile activity. The pattern of rhythmicity, and the deferment of oviposition or

parturition for a species-specific time in relation to ovulation is controlled by luteal hormones to a large extent. Rothchild (1981) uses the bird model to suggest that follicular/luteal prostaglandins are important stimulators of the oviduct and oviposition, and that prolongation of luteal function and progesterone production is important in order to delay oviposition. Thus, removal of the post-ovulatory follicle (POF; Rothchild, 1946), deprivation of its blood supply (Wood-Gush and Gilbert, 1964) or granulosa cell destruction or removal (Gilbert *et al.*, 1978) delays oviposition for 1–5 days. Oviposition can also be delayed by indomethacin treatment (Hertelendy, 1973). In the case of avian species, the factors preventing the natural progression of follicular granulosa cells to luteinized progesterone-secreting cells are unknown, but luteinization does not occur and eggs are retained for extremely short periods of time. In the oviparous turtle, seasonal variations in myometrial activity have been described in our laboratory (Abrams-Motz and Callard, 1989). The peak of both *in vivo* and *in vitro* activity coincides with the highest estrogen titer and maximal follicular development, and the periods of least spontaneous myometrial activity correspond to times of low plasma steroids. We have also shown that administration of estrogen stimulates, and of progesterone, depresses, myometrial activity (see Callard and Hirsch, 1976). Progesterone also reduces the responsivity of the myometrium to both AVT and relaxin (unpublished), and also suppresses egg-laying. Thus 78% (14/18) of the ovulated animals injected with 10 ng of AVT *i.v.* oviposited compared with 0/5 animals treated with the same dose of AVT after progesterone pre-treatment (1.0 mg/kg/body weight for five days). To what extent these effects of gonadal steroids on the oviduct may be mediated by prostaglandins is not known; but see discussions of unpublished work by Guillette (1990).

Although we know nothing of the action of progesterone on the connective tissue properties of non-mammalian oviducts, seasonal differences in extensibility and tensile strength of turtle oviducts have been reported by us (Abrams-Motz and Callard, 1989). Relaxin, a luteal hormone of mammals (see Schwabe *et al.*, 1978; Bryant-Greenwood, 1981) present in shark and skate ovaries (Bullesbach *et al.*, 1986, 1987) has been shown to have potentially important physiologic actions on *Squalus* and *Raja* oviducts (Koob *et al.*, 1984; Koob and Callard, 1991). Since these effects are dependent upon estrogen priming, progesterone might be expected to influence relaxin responses indirectly by down regulation of estrogen receptors (Leavitt, 1985).

Progesterone Effects on Ovarian Growth, and Estrogen-Induced Hepatic Vitellogenin Synthesis

Among both elasmobranchs and reptiles are species in which yolk reduction and placental development are correlated. Indeed, in some species the trend to yolk reduction nears the eutherian condition of yolk elimination (Needham, 1950; Wourms, 1977; Blackburn *et al.*, 1984). In others, despite retention of young in utero, yolk is the primary source of nutrition, the placenta being undeveloped or poorly developed. The latter may be viewed as representing an intermediate condition in the transition from oviparity to true viviparity. Although it might be argued that cycles of vitellogenin synthesis can be attributed to the presence or absence of estrogen alone, data developed in this laboratory have strongly implicated progesterone as an active inhibitory agent in elasmobranchs and reptiles.

In general, estrogen/progesterone ratios in the plasma of reptiles and elasmobranchs vary in a way suggesting a physiological interaction in the normal turning on and off of ovarian follicular growth (see Tsang and Callard, 1987*b*; Callard *et al.*, 1978; Koob *et al.*, 1986; Kleis and Callard, 1986*b*). Thus, in reptiles progesterone inhibits gonadotropin-induced and normal seasonal vitellogenesis in an oviparous lizard (Callard and Zeigler, 1970; Callard *et al.*, 1972*a*) and in a viviparous lizard (Callard *et al.*, 1972*b, c*). Further, in lizards, blockade of progesterone synthesis *in vivo* by cyanoketone allows oocytes to grow beyond untreated control size when stimulated by estrogen and gonadotropin (unpublished). More directly, progesterone or testosterone injections markedly depress estrogen-induced plasma

vitellogenin levels as measured by specific vitellogenin RIA in the turtle (Ho *et al.*, 1981) and vitellogenesis cannot be induced by estrogen in lizards immediately postpartum, a period following high plasma progesterone (Callard *et al.*, 1972*c*). Furthermore, progesterone treatment blocks egg production in the little skate (Koob and Callard, 1986) and estrogen injections in the spiny dogfish do not induce the appearance of the vitellogenin band in SDS/PAGE gels until normal seasonal plasma progesterone levels have declined (Callard *et al.*, 1980). Finally, ovarian growth and, by inference, yolk protein accumulation occurs primarily during the second half of gestation in *Squalus acanthias,* when progesterone levels are low compared to the first half of gestation (Tsang and Callard, 1987*b*). In preliminary studies we have shown that progesterone delays the estrogen-induced elevation of plasma vitellogenin in intact and hypophysectomized little skates (Perez and Callard, 1989).

ESTROGEN AND PROGESTERONE RECEPTORS

That steroids act on the reptilian reproductive tract by conventional genomic mechanisms is supported by the demonstration of estrogen and progesterone receptors (ER, PR) in the oviparous turtle (Salhanick *et al.*, 1979) and the viviparous snake (Kleis and Callard, 1986*a, b*). In the snake, PR levels change during the cycle, and correlations with plasma estradiol and progesterone suggest estrogens may maintain the PR. Although less is known of elasmobranch oviduct receptors, results of Callard and Mak (1985) suggest the presence of an estrogen receptor in *Squalus* liver. More recently, we have characterized an ER in the oviduct of the little skate (Reese and Callard, 1991) and we have preliminary evidence for a PR using DNA-cellulose affinity columns in the same tissue. Immunohistochemical studies demonstrate the localization of the PR in both mucosal, submucosal and myometrial regions of the turtle oviduct (Giannoukos *et al.*, 1991). More work needs to be done on this aspect. Current hypotheses of receptor induction suggest a dynamic equilibrium between progesterone receptor induction and estrogen in the regulation of the reproductive tract (Evans *et al.*, 1980; Leavitt, 1985). In the absence of adequate estrogen, progesterone receptors are not maintained. It might therefore be anticipated that an important component of the oviparous/viviparous transition is adequate luteal phase estrogen, thus permitting continued progesterone action. A similar interaction between estradiol and progesterone receptors may be invoked to explain progesterone down regulation of hepatic vitellogenin synthesis in reptiles and elasmobranchs. Hepatic estrogen receptors have been characterized in both groups (Callard and Mak, 1985; Callard and Callard, 1987; Riley and Callard, 1988*a, b*) and progesterone receptors have been identified in the turtle liver (Riley *et al.*, 1988).

SUMMARY AND CONCLUSIONS

Many details of luteal function, regulation and physiological role remain to be elucidated in non-mammalian species. Nonetheless, we believe that persuasive evidence for a dual role of the hormone progesterone in the evolution of viviparity can be adduced. This evidence, presented above, suggests that the hormone is of central importance in the retention of eggs in the oviduct, an essential prerequisite for the transition from oviparity to viviparity. In addition, the hormone is involved in the down-regulation of hepatic vitellogenin synthesis which is a corrollary of the development of placentae and other forms of fetal nutrition in viviparous forms. These effects of the hormone do not appear *de novo* in viviparous forms, however; rather, they are part of the normal regulatory functions of the steroid in the reproductive cycles of oviparous species and may thus be considered preadaptive for the subsequent emergence of viviparity.

ACKNOWLEDGMENTS

Supported by NSF Grants PCM 8101440, DCB 8606344 and 8801301 to IPC.

REFERENCES

Abrams-Motz, V. and I. P. Callard. 1989. Seasonal variations in oviductal myometrial activity, extensibility and tensile strength in *Chrysemys picta.* Gen. Comp. Endocrinol. 72:453–460.

Abrams-Motz, V. and I. P. Callard. 1991. Seasonal

variations in oviductal morphology of the painted turtle, *Chrysemys picta*. J. Morph. 207:59–71.

Amoroso, E. C. 1952. Placentation. *In* A. S. Parkes (ed.), *Marshall's physiology of reproduction*, 2nd ed., Vol. 2, pp. 127–311. Longmans Green, London.

Amoroso, E. C. 1959. Comparative anatomy of the placenta. Ann. N.Y. Acad. Sci. 75:855–873.

Arslan, M., O. Zaidi, J. Lobo, A. A. Zaidi, and M. H. Quazi. 1978. Steroid levels in preovulatory and gravid lizards (*Uromastix hardwicki*). Gen. Comp. Endocrinol. 34:300–303.

Baulieu, E. E., F. Goleaan, M. Schorderet, and S. Schorderet-Slatkine. 1978. Steroid-induced meiotic division in *Xenopus laevis* oocytes: Surface and calcium. Nature (London) 275:593–598.

Blackburn, D. G., L. J. Vitt, and C. L. Beuchat. 1984. Eutherian-like reproductive specializations in a viviparous reptile. Proc. Natl. Acad. Sci. U.S.A. 81:4860–4863.

Bona-Gallo, A., P. Licht, D. S. MacKenzie, and B. Lofts. 1980. Annual cycles in levels of pituitary and plasma gonadotropin, gonadal steroids, and thyroid activity in the Chinese cobra (*Naja naja*). Gen. Comp. Endocrinol. 42:477–493.

Bragdon, D. E. 1951. The non-essentiality of the corpora lutea for the maintenance of gestation in certain live-bearing snakes. J. Exp. Zool. 118:419–435.

Bryant-Greenwood, G. D. 1981. Relaxin: Evidence for its action as a local hormone. *In* F. C. Greenwood, G. D. Bryant-Greenwood, and A. D. Niall (eds.), *Relaxin: Proceedings of the First International Conference*, pp. 199–203. Elsevier, Amsterdam.

Bullesbach, E. E., L. K. Gowan, C. Schwabe, B. G. Steinetz, E. O'Byrne, and I. P. Callard. 1986. Isolation, purification and sequence of relaxin from spiny dogfish, *Squalus acanthias*. Eur. Biochem. 161:335–341.

Bullesbach, E. E., C. Schwabe, and I. P. Callard. 1987. Relaxin from an oviparous species, the skate (*Raia erinacea*). Biochem. Biophys. Res. Comm. 143: 273–280.

Callard, G. V. and P. Mak. 1985. Exclusive nuclear localization of estrogen receptors in *Squalus testis*. Proc. Natl. Acad. Sci. U.S.A. 82:1336–1340.

Callard, I. P. and J. H. Leathem. 1964. *In vitro* synthesis by the ovaries and adrenals of snakes. Proc. Soc. Exp. Biol. Med. 115:567–569.

Callard, I. P. and J. H. Leathem. 1965. *In vitro* steroid synthesis by the ovaries of elasmobranchs and snakes. Arch. Anat. Microsc. Morphol. Exp. 54: 35–48.

Callard, I. P. and H. J. Ziegler. 1970. Inhibitory effects of prolactin upon gonadotropin-stimulated ovarian growth in the iguanid lizard, *Dipsosaurus dorsalis*. J. Endocrinol. 47:131–132.

Callard, I. P. and K. L. Klotz. 1973. Sensitivity of parameters of estrogen action in the iguanid lizard *Dipsosaurus dorsalis*. Gen. Comp. Endocrinol. 21: 314–321.

Callard, I. P. and M. Hirsch. 1976. The influence of estradiol-17 and progesterone on the contractility of the oviduct of the turtle, *Chrysemys picta, in vitro*. J. Endocrinol. 68:147–152.

Callard, I. P. and S.-m. Ho. 1980. Seasonal reproductive cycles in reptiles. *In* R. J. Reiter and B. K. Follett (eds.), *Progress in reproductive biology*, Vol. 5, pp. 5–38. S. Karger, Basel.

Callard, I. P., L. L. Klosterman, L. A. Sorbera, L. A. Fileti, and J. C. Reese. 1989. Endocrine regulation of reproduction in elasmobranchs: Archetype for terrestrial vertebrates. J. Exp. Zool. Suppl. 2:12–22.

Callard, I. P. and V. Lance. 1977. The control of reptilian follicular cycles. In *Reproduction and evolution. Proc. 4th Intl. Symp. Comp. Biol. Reprod.*, pp. 199–209. Australian Acad. Sci., Canberra.

Callard, I. P. and G. V. Callard. 1987. Steroid hormone receptors and nonreceptor binding proteins. *In* D. Norris and R. E. Jones (eds.), *Hormones and reproduction in fishes, amphibians and reptiles*, pp. 355–384. Plenum Press, NY.

Callard, I. P., C. C. Bayne, and W. F. McConnell. 1972*a*. Hormones and reproduction in the female lizard, *Sceloporus cyanogenys*. Gen. Comp. Endocrinol. 18:175–194.

Callard, I. P., S. W. C. Chan, and M. A. Anderson. 1972*b*. The control of the reptilian gonad. Amer. Zool. 12:273–287.

Callard, I. P., J. P. Doolittle, W. L. Banks, Jr., and S. W. C. Chan. 1972*c*. Recent studies on the control of the reptilian gonadal cycle. Gen. Comp. Endocrinol. Suppl. 3:65–75.

Callard, I. P., I. McChesney, C. Scanes, and G. V. Callard. 1976. The influence of mammalian and avian gonadotropins on *in vitro* ovarian steroid synthesis in the turtle, *Chrysemys picta*. Gen. Comp. Endocrinol. 28:2–9.

Callard, I. P., V. Lance, A. R. Salhanick, and D. Barad. 1978. The annual ovarian cycle of *Chrysemys picta*: Correlated changes in plasma steroids and parameters of vitellogenesis. Gen. Comp. Endocrinol. 35:245–257.

Callard, I. P., S.-m. Ho, D. A. Gapp, S. Taylor, D. Danko, and F. G. Wulczyn. 1980. Estrogens and estrogenic actions in fish, amphibians and reptiles. *In* J. McLachlan (ed.), *Symposium on estrogen in the environment*, pp. 213–237. Elsevier Press, Amsterdam.

Callard, I. P., D. Riley, and L. E. Perez. 1990*a*. Vertebrate vitellogenesis: Molecular model for multihormonal control of gene regulation. In A. Epple, C. G. Scanes, and M. H. Stetson (eds.), *Progress in comparative endocrinology*, pp. 343–348. Wiley-Liss, Inc., New York.

Callard, I. P., D. Riley, and L. E. Perez. 1990*b*. Vitellogenesis in reptiles as a model for mammalian sex differentiated hepatic protein synthesis. J. Exp. Zool. Suppl. 4:106–111.

Chan, S. W. C., S. Ziegel, and I. P. Callard. 1973. Plasma progesterone in snakes. Comp. Biochem. Physiol. 44A:631–637.

Clausen, H. J. 1940. The effect of ovariectomy and hypophysectomy on gestation in snakes. Endocrinology 87:700–704.

Cuellar, O. and H. S. Cuellar. 1976. Successive clutches induced by surgical excision of post-ovulatory oocytes in the lizard *Cnemidophorus uniparens*. Experientia 32:660.

Dodd, J. M. and C. K. Goddard. 1961. Some effects of oestradiol benzoate on the reproductive ducts of the female dogfish, *Scyliorhinus caniculus*. Proc. Zool. Soc. (Lond.) 137:325–331.

Dodd, J. M. and J. P. Sumpter. 1984. Fishes. *In* G. E. Lamming (ed.), *Marshall's physiology of reproduction*, Vol. 1, pp. 1–126. Churchill Livingstone, Inc., New York.

Doi, O., T. Takai, T. Nakamura, and Y. Tanabe. 1980. Changes in the pituitary, plasma and follicular progesterone and estradiol, and plasma testosterone and estrone concentrations during ovulatory cycles of the quail (*Coturnix coturnix japonica*). Gen. Comp. Endocrinol. 41:156–163.

Elbaum, D. J. and P. L. Keyes. 1976. Synthesis of 17-estradiol by isolated ovarian tissues of the pregnant rat: Aromatization in the corpus luteum. Endocrinology 99:573–579.

Eldredge, N. and S. J. Gould. 1972. Punctuated equilibria: An alternative to phyletic gradualism. *In* T. Schopf (ed.), *Models in paleobiology*, pp. 85–115. Freeman Press, San Francisco.

Evans, R. W., T. J. Chen, W. J. Hendry, III, and W. W. Leavitt. 1980. Progesterone regulation of the estrogen receptor in the hamster uterus during the estrous cycle. Endocrinology 107:383–390.

Fileti, L. and I. P. Callard. 1988. Corpus luteum function and regulation in the skate, *Raja erinacea*. The Bulletin (MDIBL) 27:37–39.

Fitz, T. A., M. H. Mayan, H. R. Sawyer, and G. D. Niswender. 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol. Reprod. 27:703–711.

Fuchs, A.-R., S. Periyasamy, M. Alexandrova, and M. S. Soloff. 1983. Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: Effects of ovarian steroids. Endocrinology 113:742–749.

Giannoukos, G., O. Toft, and I. P. Callard. 1991. Immunorecognition of progesterone receptors (pr) in the turtle, *Chrysemys picta* by avian monoclonal antibodies. Amer. Zool. 31:73A.

Gibori, G. and J. S. Richards. 1978. Dissociation of two distinct luteotropic effects of prolactin: Regulation of luteinizing hormone-receptor content and progesterone secretion during pregnancy. Endocrinology 102:767–774.

Gilbert, A. B., M. F. Davidson, and J. W. Wells. 1978. Role of the granulosa cells of the postovulatory follicle of the domestic fowl in oviposition. J. Reprod. Fertil. 52:227–229.

Gorman, G. C., P. Licht, and F. McCollum. 1981. Annual reproductive reproductive patterns in three species of marine snakes from the central Philippines. J. Herpetol. 15:335–354.

Guillette, L. J., Jr. 1990. Prostaglandins and reproduction in reptiles. *In* A. Epple, C. G. Scanes, and M. H. Stetson (eds.), *Comparative endocrinology*, pp. 603–607. Wiley-Liss, Inc., New York.

Guillette, L. J., Jr., S. Spielvogel, and F. L. Moore. 1981. Luteal development, placentation and plasma progesterone concentration in the viviparous lizard, *Sceloporus jarrovi*. Gen. Comp. Endocrinol. 43:20–29.

Guillette, L. J., Jr., L. A. Lavia, N. J. Walker, and D. K. Roberts. 1984. Luteolysis induced by prostaglandin F2 alpha in the lizard, *Anolis carolinensis*. Gen. Comp. Endocrinol. 56:271–277.

Guillette, L. J., Jr. and S. L. Fox. 1985. Effect of deluteinization on plasma progesterone concentration and gestation in the lizard, *Anolis carolinensis*. Comp. Biochem. Physiol. 90A:303–306.

Guillette, L. J., Jr. and R. E. Jones. 1985. Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard, *Sceloporus aeneus*. J. Morphol. 184:85–98.

Guillette, L. J., Jr., C. A. Herman, and D. A. Dickey. 1988. Synthesis of prostaglandins by tissue of the viviparous lizard *Sceloporus jarrovi*. J. Herpetol. 22:180–185.

Guillette, L. J., Jr., A. Hensley, P. Jaffee, and J. Matter. 1989. Indomethacin influences vasotocin-induced parturition and oviposition in lizards. Theriogenology. (In press)

Gulyas, B. J., R. L. Stouffer, and G. D. Hodge. 1979. Progesterone synthesis and fine structure of dissociated monkey (*Macaca mulatta*) luteal cells maintained in culture. Biol. Reprod. 20:779–792.

Hertelendy, F. 1973. Block of oxytocin-induced parturition and oviposition by prostaglandin inhibitors. Life Sci. 13:1581–1589.

Highfill, D. R. and R. A. Mead. 1975*a*. Sources and levels of progesterone during pregnancy in the garter snake, *Thamnophis elegans*. Gen. Comp. Endocrinol. 27:389–400.

Highfill, D. R. and R. A. Mead. 1975*b*. Function of the corpora lutea of pregnancy in the viviparous snake, *Thamnophis elegans*. Gen. Comp. Endocrinol. 27:401–407.

Hisaw, F. L. 1959. Endocrine adaptations of the mammalian estrous cycle and gestation. *In* A. Gorbman (ed.), *Comparative endocrinology*, pp. 533–552. John Wiley and Sons, Inc., New York.

Ho, S.-m. and I. P. Callard. 1984. High efficiency binding of ^{3}H-progesterone by putative progesterone receptors in cytosol and nuclear extract of turtle oviduct. Endocrinology 114:70–79.

Ho, S.-m., D. Danko, and I. P. Callard. 1981. Effect of exogenous estradiol 17 on plasma vitellogenin levels in male and female *Chrysemys* and its modulation by testosterone and progesterone. Gen. Comp. Endocrinol. 43:413–421.

Ho, S.-m., S. Kleis, R. McPherson, G. J. Heisermann, and I. P. Callard. 1982. Regulation of vitellogenesis in reptiles. Herpetologica 38:40–50.

Hsueh, A. J. W., E. Y. Adashi, P. B. C. Jone, and T. H. Welsh, Jr. 1984. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr. Rev. 5:76–127.

Huang, E. S-R. and A.V. Nalbandov. 1979. Steroidogenesis of chicken granulosa and theca cells: *In vitro* incubation system. Biol. Reprod. 20:442–453.

Hubert, J. 1977. Facteure et modalites de la differentiation des trois types cellulaires de la granulosa

du follicule ovarian chez les Lacertiliens. Bull. Soc. Zool. de France 102:151–155.
Karsch, F. and G. P. Sutton. 1978. An intraovarian site for the luteolytic action of estrogen in the rhesus monkey. Endocrinology 98:553–561.
Keyes, P. L. and A. V. Nalbandov. 1967. Maintenance and function of the corpora lutea in rabbits depend on estrogen. Endocrinology 80:938–946.
Kleis-San Francisco, S. M. and I. P. Callard. 1986*a*. Identification of a putative progesterone receptor in the oviduct of the viviparous snake, *Nerodia*. Gen. Comp. Endocrinol. 61:490–498.
Kleis-San Francisco, S. M. and I. P. Callard. 1986*b*. Progesterone receptors in the oviduct of a viviparous snake (*Nerodia*): Correlations with ovarian function and plasma steroid levels. Gen. Comp. Endocrinol. 63:220–229.
Koob, T. J., J. J. Laffan, and I. P. Callard. 1984. Effects of relaxin and insulin on reproductive tract size and early fetal loss in *Squalus acanthias*. Biol. Reprod. 31:231–238.
Koob, T. J., P. Tsang, and I. P. Callard. 1986. Steroid hormone correlates of the ovulatory cycle of the skate, *Raia erinacea*. Biol. Reprod. 35:267–275.
Koob, T. J. and I. P. Callard. 1986. Progesterone treatment causes early oviposition in *Raja erinacea*. The Bulletin (MDIBL) 25:138–139.
Koob, T. J. and I. P. Callard. 1991. Reproduction in female elasmobranchs. *In* R. Kinne and E. Kinne (eds.), *Comparative physiology,* pp. 155–209. S. Karger, Basel.
Lance, V. and I. P. Callard. 1969. A histochemical study of ovarian function in the ovoviviparous elasmobranch, *Squalus acanthias*. Gen. Comp. Endocrinol. 13:255–267.
Lance, V. and I. P. Callard. 1978*a*. Steroidogenesis by enzyme-dispersed turtle (*Chrysemys picta*) ovarian cells in response to ovine gonadotropins (FSH and LH). Gen. Comp. Endocrinol. 34(3): 304–311.
Lance, V. and I. P. Callard. 1978*b*. *In vivo* responses of female snakes (*Natrix fasciata*) and female turtles (*Chrysemys picta*) to ovine gonadotropins (FSH and LH) as measured by plasma progestonone, testosterone and estradiol levels. Gen. Comp. Endocrinol. 35:295–301.
Lance, V. and I. P. Callard. 1980. Phylogenetic trends in the hormonal control of gonadal steroidogenesis. *In* P. Pang and A. Epple (eds.), *Evolution of the vertebrate endocrine system,* pp. 167–231. Texas Tech. Press, Lubbock, TX.
Leavitt, W. W. 1985. Hormonal regulation of myometrial estrogen, progesterone, and oxytocin receptors in the pregnant and pseudopregnant hamster. Endocrinology 116:1079–1084.
Leavitt, W. W., D. O. Toft, C. A. Strott, and B. W. O'Malley. 1974. A specific progesterone receptor in the hamster uterus: Physiological properties and regulation during the estrous cycle. Endocrinology 94:1041–1053.
Licht, P. 1984. Reptiles. *In* G. E. Lamming (ed.), *Marshall's physiology of reproduction,* Vol. 1, pp. 206–282. Churchill Livingstone, Inc., New York.
Licht, P., J. Wood, D. W. Owens, and F. Wood. 1979. Serum gonadotropins and steroids associated with breeding activities in the green sea turtle *Chelonia mydas*. Gen. Comp. Endocrinol. 39:274–289.
Matthews, L. H. 1955. The evolution of viviparity in vertebrates. Mem. Soc. Endocrinol. 4:129–148.
McPherson, R. J., L. R. Boots, R. MacGregor, III, and K. R. Marion. 1982. Plasma steroids associated with seasonal reproductive changes in a multiclutched freshwater turtle, *Sternotherus odoratus*. Gen. Comp. Endocrinol. 48:440–451.
Meade, R. A., V. P. Eroschenko, and D. R. Highfill. 1981. Effects of progesterone and estrogen on the histology of the oviduct of the garter snake, *Thamnophis elegans*. Gen. Comp. Endocrinol. 45:345–354.
Miller, J. B. and P. L. Keyes. 1975. Progesterone synthesis in developing rabbit corpora lutea in the absence of follicular estrogens. Endocrinology 97: 83–90.
Mossman, H. W. and I. Judas. 1949. Accessory corpora lutea, lutein cell origin and the ovarian cycle in the Canadian porcupine. Am. J. Anat. 85:1–39.
Nagahama, V., K. Hirose, G. Young, S. Adachi, K. Suzuki, and B.-I. Tamaoki. 1983. Relative *in vitro* effectiveness of the 17α, 20β-dihydroxy-4-pregnen=3-one and other pregnene derivatives on germinal vesicle breakdown in oocytes of ayu (*Plecoglossus altivelis*), amago salmon (*Oncorhyncus rhodurus*), rainbow trout (*Salmo gairdneri*), and goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 51:15–23.
Needham, J. 1950. *Biochemical morphogenesis.* Cambridge University Press, Cambridge.
Packard, G. C., C. R. Tracy, and J. J. Roth. 1977. The physiological biology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. Biol. Rev. (Cam. Philos. Soc.) 52:71–105.
Panigel, M. 1956. Controbution a l'etude de l'ovoviviparite chez les reptiles. Gestation et parturition chex le lezard vivipare, *Zootoca vivipara*. Ann. Sci. Nat. Zool. 18:569–668.
Perez, L. E. and I. P. Callard. 1989. Evidence for progesterone inhibition of vitellogenesis in the skate (*R. erinacea*). Amer. Zool. 29:357A.
Reese, J. C. and I. P. Callard. 1991. Biochemical characterization of an estradiol receptor in the oviduct of the little skate, *Raia erinacea*. Gen. Comp. Endocrinol. (In press)
Riley, D. and I. P. Callard. 1988*a*. Characterization of turtle liver nuclear estrogen receptors, seasonal changes and pituitary dependence of cytosolic and nuclear forms. J. Exp. Zool. 245:277–285.
Riley, D. and I. P. Callard. 1988*b*. An estrogen receptor in the liver of the viviparous water snake, *Nerodia*: Characterization and seasonal changes in binding capacity. Endocrinology 123:753–761.
Riley, D., J. C. Reese, and I. P. Callard. 1988. Hepatic progesterone receptors: Characterization in the turtle (*Chrysemys picta*). Endocrinology 123:1195–1201.
Rothchild, I. 1946. The effect of hypophysectomy on the time of lay of the hen's egg. Endocrinology 39: 82–83 #65.

Rothchild, I. 1981. The regulation of the mammalian corpus luteum. Rec. Prog. Horm. Res. 37:183–298.

Salhanick, A. R., C. Vito, T. O. Fox, and I. P. Callard. 1979. Estrogen binding proteins in the oviduct of the turtle, *Chrysemys picta*: Evidence for a receptor species. Endocrinology 105:1388–1395.

Schuetz, A. W. 1974. Role of hormones in oocyte maturation. Biol. Reprod. 10:150–178.

Schwabe, C., B. Steinetz, D. Weiss, A. Segaloff, J. K. McDonald, E. M. O'Byrne, J. Hochman, V. Carriere, and L. Goldsmith. 1978. Relaxin. Rec. Prog. Horm. Res. 34:123–199.

Shine, R. and J. J. Bull. 1979. The evolution of live-bearing in lizards and snakes. Am. Nat. 113:905–923.

Shine, R. and L. Guillette, Jr. 1988. The evolution of viviparity in reptiles: A physiological model and its evolutionary consequences. J. Theor. Biol. 132: 43–50.

Steinetz, B. F., V. H. Beach, and R. L. Kroc. 1959. The physiology of relaxin in laboratory animals. *In* C. H. Lloyd (ed.), *Recent progress in the endocrinology of reproduction*, pp. 384–423. Academic Press, New York.

Tinkle, D. W. and J. W. Gibbons. 1977. The distribution and evolution of viviparity in reptiles. Misc. Publ. Mus. Zool., University of Michigan, #154.

Tsang, P. and I. P. Callard. 1987*a*. Luteal progesterone production and regulation in the viviparous dogfish, *Squalus acanthias*. J. Exp. Zool. 241:377–382.

Tsang, P. and I. P. Callard. 1987*b*. Morphological and endocrine correlates of the reproductive cycle of the aplacental viviparous dogfish, *Squalus acanthias*. Gen. Comp. Endocrinol. 66:182–189.

van Wyk, J. H. 1984. Physiological changes during the ovarian cycle of the female rock lizard, *Agama atra* (Sauria: Agamidae). S. Afr. J. Zool. 19:253–260.

Veith, W. J. 1974. Reproductive biology of *Chamaeleo pumilus pumilus* with special reference to the role of the corpus luteum and progesterone. Zool. Africana 9:161–183.

Weekes, H. C. 1935. A review of placentation among reptiles with particular regard to the function and evolution of the placenta. Proc. Zool. Soc. Lond. 3-4:625–645.

Wood-Gush, D. G. M. and A. B. Gilbert. 1964. The control of nesting behavior in the domestic hen. II. The role of the ovary. Anim. Behav. 12:451–453.

Wourms, J. P. 1977. Reproduction and development in chondrichthyan fishes. Amer. Zool. 17:473–515.