J. Steroid Biochem. Molec. Biol. Vol. 40, No. 4–6, pp. 571–575, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

THE ROLE OF STEROIDS IN REPRODUCTION IN FEMALE ELASMOBRANCHS AND REPTILES

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Summary—Adequate evidence exists to suggest the importance of temporal changes in steroid hormone ratios in the normal reproductive/vitellogenin cycle in oviparous and viviparous elasmobranchs and reptiles. In oviparous species, where the cycle is relatively short, secretion of gonadal hormones is synchronous; thus inhibitory actions of progesterone (P) on hepatic or reproductive tract functions would be offset by stimulatory actions of estradiol (E), resulting in appropriate vitellogenin secretion and reproductive tract development. In viviparous species, temporal asynchrony of E and P secretion occurs, and the actions of the individual hormones can be more easily dissected out. Thus, during gestation, where P is the dominant hormone, antagonistic or stimulatory actions of E may be prevented, and the inhibitory action of P on vitellogenesis dominant. Hence vitellogenesis is limited to the follicular phase and eggs are retained.

Although the elasmobranch and reptilian species discussed here do not form a continuum through phylogenesis, but rather are extant forms of a particular line of evolution, it is possible to extrapolate from these observations to the probable endocrine interactions in a species as viviparity evolves from oviparity. The theoretical intermediate stage would involve; (a) egg retention, (b) extension of the luteal phase and increased P secretion and (c) resulting in E/P asynchrony and potential expression of "independent" P action, egg retention and yolk suppression.

INTRODUCTION

Viviparity has evolved from oviparity many times in vertebrates [1, 2] and in eutherian mammals viviparity is the only mode of reproduction. In contrast, in other vertebrate groups with the exception of birds (100% oviparous) related forms may be either oviparous or viviparous depending upon habitat, latitude, and other factors. Despite the importance of the oviparous/viviparous transition in vertebrate evolution, the endocrine controls are poorly understood even though mammalian viviparity is totally steroid dependent. Viviparity involves the birth of live young, as opposed to the laying of shelled eggs. A critical initial step in the transition is egg retention followed by intrauterine development. Once this has occurred viviparity can progress from early yolk-dependant stages through intermediate stages involving an initial dependency upon reduced yolk stores and subsequent utilization of placental homologs, to the condition best exemplified by eutherian mammals in which yolk is absent and developing embryos are totally dependent upon uterine modes of nutrition. In elasmobranchs and reptiles the evolution of viviparity from oviparity has occurred many times and these groups provide suitable models with which to probe for probable hormonal functions in the process. In both of these groups, species are found in which viviparity has evolved to near-eutherian status with reference to yolk-reduction and placental development [3, 4]. Since the process of yolk-reduction is linked to the development of alternative uterine mechanisms of fetal nutrition, it is likely that the processes are co-ordinately linked by similar endocrine factors. The primary hormone targets are; (a) the liver, the site of synthesis of the yolk protein precursor vitellogenin [5], (b) the ovary, site of vitellogenin uptake by oocytes [6] and (c) the reproductive tract [7]. Our hypothesis is that while follicular estrogen is essential for both hepatic vitellogenin synthesis and reproductive tract development, progesterone (P) through both synergistic and antagonistic actions with estrogen is necessary for the continued maintenance of oviductal embryos and the simultaneous

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.

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inhibition of vitellogenin synthesis [8]. These processes occur during the reproductive cycles of individual animals to differing degrees, depending upon their mode of reproduction (oviparous/viviparous). Investigation of selected species which exemplify these reproductive modes is our best chance to understand the operation of these endocrine controls as animals evolved from the oviparous to viviparous condition, with the subsequent development of placental structures and loss of vitellogenin synthetic capacity by the liver.

RESULTS AND DISCUSSION

I. Comparison of Reproductive Cycles

(A) Oviparous (elasmobranch: Raja erinacea; reptile: Chrysemys picta)

Raja erinacea. During repetitive reproductive periods, this species exhibits sequential (5–7 day) ovulatory cycles with a short (2–3 day) luteal phase. The follicular phase is characterized by high levels of P (16 ng/ml plasma) and testosterone (T, 16 ng/ml) coincident with lower levels of estradiol (E, 2–3 ng/ml). Plasma P and T levels drop sharply after ovulation, and plasma E levels are reduced [9]. After ovulation, oocytes receive albumen from the proximal region of the oviduct and the eggs are shelled in the complex E-dependent shell gland [10]. Shelled eggs remain in the proximal muscular portion of the oviduct 1–2 days prior to expulsion. A sex specific protein (200 kDa) is present in the plasma during periods of egg formation. The protein reacts with an antibody made to yolk protein on Western blots, is absent in normal males and inducible in males by E. Plasma levels in normal cycling females are 10 mg/ml [11, 12].

Chrysemys picta. This species exhibits 1–6 ovulatory cycles each spring (No. eggs/clutch ≈ 10) depending upon latitude. As in *R. erinacea*, the follicular phase is characterized by an early increase in E which induces vitellogenesis, E rising to a peak (1.0 ng/ml) at ovulation. This ovulatory peak of E is coincident with peaks of T (5.0 ng/ml) and P (2.5 ng/ml). Ovulation is followed by a 10–14 day luteal phase during which P levels are declining [13]. Ovulated eggs receive albumen in the proximal regions of the oviduct, followed by shelling [14]. Eggs remain in the oviduct for the duration of the luteal phase. As for *R. erinacea*, vitellogenin is estrogen—inducible, sex specific and plasma levels determined by RIA are well correlated with plasma E levels [15].

(B) Viviparous (elasmobranch: Squalus acanthias; reptile: Nerodia sipedon)

Squalus acanthias. This species has a well-known 23 month cycle in which mature females are pregnant for all but a short post-partum period. Fetal development is totally yolk-dependent and aplacental. Due to the length of the cycle, follicular growth is co-incident with the second year of pregnancy. In this species, E (1.0 ng/ml) and T (0.8 ng/ml) peak towards the end of pregnancy as follicles reach ovulatory size (5–6 cm). In contrast to E and T, P begins to rise after ovulation in early pregnancy and peaks when other steroid levels are still low (4.5 ng/ml) [16]. A sex-specific plasma protein coinciding with vitellogenin is absent during early pregnancy (luteal phase) and cannot be induced by E. In contrast, when P is low (follicular phase) and E levels are rising, vitellogenin is present and induced to greater levels by E injection [17].

Nerodia sipedon. As in other viviparous temperate squamates, follicular development occurs during a 4–6 week period in spring, with a May–June ovulation followed by a 9–12 week gestation period, birth being as early as late August–early September depending upon latitude. Large yolked eggs are retained without shells and a rudimentary chorio-allantoic placenta develops. As with *S. acanthias*, E (≈1.0 ng/ml) and T (≈0.5 ng/ml) are the dominant follicular phase plasma steroids [18]. Plasma P levels begin to rise in the periovulatory period and peak at around mid-gestation, declining to term (≈2.0 to 0.5 ng/ml). Vitellogenin is present during the follicular phase and is inducible by E [19].

By comparison of the steroid patterns in oviparous vs viviparous species, it can be seen (Fig. 1) that in the shorter cycle, egg laying species that plasma steroids peak quite synchronously during the follicular growth phase, and are basal or declining during the short luteal phase. In contrast, in the viviparous species, E dominates in the follicular phase, and P dominates the luteal phase as in mammalian gestation. Thus, with regard to the patterns of E vs P, these cycles may be classed as synchronous (oviparous) or asynchronous (viviparous). Since key reproductive processes are governed by the overlapping actions and interactions of these gonadal steroids, the

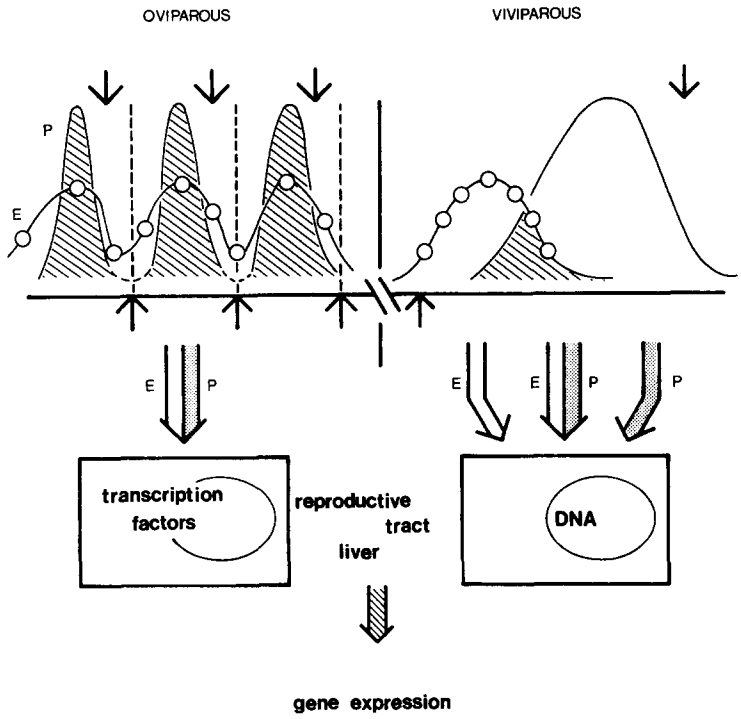

Fig. 1. Comparison of patterns of E and P observed in oviparous vs viviparous elasmobranch and reptilian species (see the text for details). Programmed ovarian differentiation during each cycle leads to repetitive patterns of hormone synthesis and secretion based on differential and sequential expression of genes for steroidogenic enzymes, ovarian peptides and target tissue steroid hormone receptors. Differing ratios of plasma steroids result in appropriate end-organ function for the species and reproductive mode (i.e. oviparous or viviparous). Thus, depending upon the ratio of E/P, their effects may be synergistic or antagonistic; this in turn leads to appropriate oviductal activity and hepatic synthesis of vitellogenin, or its modulation or inhibition. ↓ = oviposition/parturition; ↑ = cycle onset.

observed cycle and all its manifestations are a function of the time-integrated ratios of the steroids and their effects upon tissue specific gene expression. Thus one can explain differences in cycles based on observed ratios of the steroids. For the purposes of this discussion, these interactions are translated into the appropriate synthesis of vitellogenin, oviduct albumen, avidin, myosin and shell formation (if appropriate) and proper egg retention times for the species followed by oviposition or parturition.

II. Steroid Actions in the Regulation of Vitellogenesis

(A) Oviparous species

In both species, vitellogenin is inducible by E. In the turtle, E induced vitellogenesis is inhibited by both P and T suggesting that the observed levels of vitellogenin in plasma are not solely due to estrogen induction, but also to the opposing effects of the other two steroids. Hepatic receptors for both E and P have been characterized [20, 21] suggesting their role in the regulation of the vitellogenin gene. Similar but less complete information is available for the skate, where P appears to retard E-induced vitellogenesis [11].

(B) Viviparous species

In *S. acanthias* vitellogenin levels are low and undetectable on PAGE during early gestation when P levels are highest, and cannot be induced by E at this stage. In contrast, vitellogenin is present in later gestation when E levels are rising and P levels have declined. At this stage, E injection will further increase plasma levels of vitellogenin. Thus far neither E nor P receptors have been characterized in the liver, although an estrogen receptor has been characterized in the oviduct (see below). In some pregnant viviparous squamates, vitellogenin is inhibited during pregnancy and cannot be induced by gonadotropins [22] possibly due to high P levels. Changes in hepatic estrogen during the vitellogenin cycle have been reported in *N. sipedon* [23] but hepatic P receptors have not yet been demonstrated.

III. Steroid Actions in the Regulation of the Oviduct

(A) Oviparous species

The shell gland of *R. erinacea* is a suitable model for the study of steroid hormone regulation of shell gland proteins [24]. Estrogen receptors have been demonstrated and characterized in the albumen secreting portion of the tract [25]; no data are available on myometrial activity in this species. In the turtle, we have demonstrated that seasonal oviduct contractile activity [26] is dependent upon E/P levels which peak in the periovulatory period. In this species, P injection increases egg retention time [27] and significantly inhibits AVT-induced oviposition. Seasonal changes in oviductal morphology and responses to E suggest that this steroid dominates changes in reproductive tract morphology [28]. Both E [29] and P [30,31] receptors are present in the reproductive tract of *C. picta* and PR-B is estrogen-dependent [31]. By immunocytochemistry, the P receptor is detectable in both the myometrium and the secretory epithelium and glandular regions [32]. In studies of myometrial activity, E increases and P decreases contractile activity [33].

(B) Viviparous species

No data are currently available on steroid receptors in the oviduct of *S. acanthias*. However, we have noted that E injections in pregnant *S. acanthias* allow the development of sensitivity to relaxin which appears to be important in the accommodation of the reproductive tract to developing embryos, as well as altering the diameter of the cervix and in so doing allowing spontaneous abortion [34]. Myometrial activity in this species is less in early pregnancy when P levels are high and more in the second half of pregnancy when P has declined and E levels are increasing. P injection also inhibits relaxin effects (reduction in myometrial activity) [35]. In pregnant *N. sipedon*, both E and P receptors have been characterized in the oviduct, and the P receptor appears to be controlled by E; P receptor levels peak following the follicular phase E and slowly decline during gestation [18].

Acknowledgements—This work was supported by NSF Grants PCM 8101440 and DCB 8606344 and 8801301 to I. P. Callard.

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