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Secretory proteins in the reproductive tract of the snapping turtle, *Chelhydra serpentina*

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Abstract

SDS-polyacrylamide gel electrophoresis was used to separate the secretory proteins produced by the epithelial and endometrial glands of the uterine tube and uterus in the snapping turtle *Chelydra serpentina.* The proteins were analyzed throughout the phases of the reproductive cycle from May to August, including preovulatory, ovulatory, postovulatory or luteal, and vitellogenic phases. The pattern of secretory proteins is quite uniform along the length of the uterine tube, and the same is true of the uterus, but the patterns for uterine tube and uterus are clearly different. We identify 13 major proteins in *C. serpentina* egg albumen. Bands co-migrating with 11 of these are found in the uterine tube, but at most 4 are found in the uterus, suggesting that the majority of the albumen proteins are most likely secreted in the uterine tube, not in the uterus. Although some of the egg albumen proteins are present in the uterine tube only at the time of ovulation, most of the bands corresponding to albumen proteins are present throughout the breeding season even though the snapping turtle is a monoclutch species. These results suggest that the glandular secretory phase in the uterine tube is active and quite homogeneous in function regardless of location or phase of the reproductive cycle.

Keywords: Albumen; Monoclutch; Oviduct; Reptiles; SDS-PAGE; Shell membranes; Uterine tube; Uterus

1. Introduction

The female reproductive physiology of the common snapping turtle, *Chelydra serpentina*, is of special interest because unlike most turtles it is a monoclutch species. It ovulates all of its mature follicles simultaneously in late May to mid-June (Mahmoud and Licht, 1997).

The present investigation focuses on the secretory proteins of the female reproductive tract, also referred to as the oviduct. The anatomically distinguishable parts of the oviduct include the infundibulum, uterine tube, isthmus, uterus and vagina (Fox, 1977). Our specific goal is to gain information about the proteins of the snapping turtle egg albumen and where they are produced in the oviduct, and to examine how the secretion of these proteins varies throughout the reproductive cycle. To date, no detailed investigations relative to oviductal protein synthesis have been conducted on any turtle with a monoclutch mode. The secretory glands have been localized in only a few turtles and these were polyclutch species (Palmer and Guillette, 1991).

The composition of albumen proteins in reptiles exhibits substantial differences among orders (Palmer and Guillette, 1991). Moreover, detailed study of albumen proteins in reptiles is still very limited and more investigation is needed. In the case of bird eggs, previous studies have shown that there is great variability in the composition of

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egg albumen proteins between different avian species, and these observations have led to the conclusion that protein composition and properties suitable to nest and incubation conditions may have a selective advantage (Sibley, 1970; Sibley and Ahlquist, 1972). In comparison with birds, reptiles have more ancient origins, more diverse habitats and exhibit both oviparity and viviparity, suggesting an even less conserved evolutionary pattern of reproductive morphology and physiology. Reptiles in general exhibit large differences in the functional anatomical features of the reproductive tract, as well as diverse eggshell structures and nest conditions (Palmer and Guillette, 1991). Thus their eggs may be expected to exhibit even greater variation in the albumen proteins than is seen with avian eggs.

In this study, we have used SDS-polyacrylamide gel electrophoresis to study the secretory proteins produced by the epithelial and the tubular endometrial glands of the female reproductive tract during all phases of the ovarian cycle of the snapping turtle, *C. serpentina*.

2. Materials and methods

2.1. Animals

Adult female snapping turtles, *C. serpentina*, were collected from central Wisconsin during each phase of the reproductive cycle: pre-ovulation phase (May), ovulation phase (late May to mid-June), post-ovulatory or quiescent phase (early post-ovulation, mid-June to mid-July), vitellogenic phase (mid-July to late August), and inactive phase (fall and winter). The time periods given in parentheses are only approximate, however, and reproductive phases of individual turtles were identified by noting the following reproductive parameters, as previously described by Mahmoud and Licht (1997): ovarian weight, follicle size, presence of corpora lutea, presence (and location) of eggs in the oviduct, and vitellogenesis. Prior to ovulation, the ovaries are at their maximum weight and contain both mature (18–22 mm diameter) and immature (5 mm diameter and smaller) follicles. All of the mature follicles ovulate at the same time during a period of 48 h, after which ovarian weight is minimal. After albumen coating, the eggs are retained in the uterus for about 2 weeks for the process of shelling. The corpora lutea remain steroidogenically active while the eggs are in the reproductive tract, but begin to degenerate just prior to oviposition. After egg laying, degeneration of the corpora lutea proceeds rapidly until they all disappear. The vitellogenic phase then begins and follicles at the 5 mm size grow rapidly until they attain their mature size (18–22 mm) by late August. During the fall and winter, the ovaries remain unchanged until the following spring when the cycle once again commences.

Over the course of 3 years, a total of 29 turtles were studied. These were identified as follows: 4 in preovulation phase, 7 in ovulation, 9 post-ovulatory, 6 vitellogenic, and 3 in the inactive phase. Freshly captured turtles were sacrificed, and the uterus and uterine tube were removed and prepared for protein analysis by SDS-polyacrylamide gel electrophoresis.

2.2. Tissue preparation for SDS-polyacrylamide gel electrophoresis

Immediately after sacrificing the turtle, the uterine tube and uterus were excised and washed with isotonic saline or distilled water. The uterine tube and uterus, each about 30 cm long, were separated, blotted from the outside with paper towels and cut into three segments 10 cm long. Each segment was cut lengthwise and exposed inside out and the excised segments were washed twice with physiological saline to remove blood from the tissue.

For the actual preparation of gel samples, the protein-containing mucosa in the uterine tube or uterus were removed by scraping. However, in order to find the best way of doing this, several slightly different approaches were tested. First, the lining of the oviduct was either scraped vigorously with a sharp scalpel, scraped gently with a scalpel, or scraped with a blunt instrument. Then, for each of these methods, the scrapings were either placed directly into SDS final sample buffer or extracted with saline. In the latter case, the scrapings were placed into isotonic saline and centrifuged. The supernatant was subsequently mixed with an equal volume of 2× concentrated SDS final sample buffer (consisting of 0.125 M Tris–Cl pH 6.8, 2% SDS, and 20% glycerol, with 0.05% Phenol Red as a tracking dye and pH indicator) and the pellet was resuspended in 1× SDS final sample buffer. Finally, 2-mercaptoethanol was added to each sample up to a final concentration of 10% (v/v) and samples were heated at 100 °C for 2 min. Samples were stored frozen at −20 °C until analyzed by electrophoresis.

2.3. SDS-polyacrylamide gel electrophoresis

Proteins in the scrapings from turtle uterine tube and uterus were analyzed by SDS-polyacrylamide gel electrophoresis using standard methods (Laemmli and Favre, 1973). Proteins from turtle egg albumen and molecular mass markers (see below) were run on each gel for comparison. Gels 7.3 cm long were run using a Mini-Protean II slab gel apparatus (BioRad) and typically contained 10 or 16% acrylamide. Gel solutions were prepared from a stock solution containing 30% (w/v) acrylamide, with 0.8% (w/v) piperazine diacrylamide (BioRad) as cross-linker. Gels were stained with 0.1% (w/v) Coomassie blue R250 in methanol:acetic acid: water (5: 1: 4) and destained in methanol/acetic acid/water (1:2:17). Note that since the focus of this study was the major proteins of the egg albumen, it was not necessary to use a more sensitive detection technique, such as silver staining. For a permanent record, the gels were dried between sheets of cellophane (Juang et al., 1984) and photographed on a

light box using Kodak technical pan film. Two sets of molecular mass standards for SDS gels were used. Kaleidoscope prestained standards (BioRad) included myosin (202 kDa), β-galactosidase (133 kDa), bovine serum albumin (71 kDa), carbonic anhydrase (41.8 kDa), soybean trypsin inhibitor (30.6 kDa), lysozyme (17.8 kDa) and aprotinin (6.9 kDa). Dalton Marker VII-L (Sigma) included bovine serum albumen (66 kDa), egg albumen (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa).

3. Results

3.1. Preparation of secretory proteins from mucosa of snapping turtle oviduct

Our basic approach to gaining information about the secretory proteins in the female reproductive tract of *C. serpentina* was to dissect the uterine tube and uterus from freshly sacrificed females, remove the protein-containing mucosa from the inner lining of the lumen of the oviduct by scraping, and finally prepare the proteins for analysis by SDS-polyacrylamide gel electrophoresis. However, several alternative ways of doing this were tested (Fig. 1, and see Materials and methods).

For the uterine tube, the gel shown in Fig. 1a demonstrates that the method of scraping has no significant effect on the pattern of Coomassie-stained bands, though more vigorous scraping gives a higher yield of protein (Fig. 1a, lanes 1–3). Extraction of the scrapings with saline (Fig. 1a, lanes 7–9) gives both a smaller yield of protein and a restricted set of bands, but the pellet after saline extraction (Fig. 1a, lanes 4–6) has virtually the same protein pattern as the samples placed directly into SDS gel sample buffer (lanes 1–3). This suggests that none of the proteins we observe on gels is preferentially soluble in saline, and thus that none of them were preferentially removed by our technique of washing the tissue twice with physiological saline to remove blood (see Materials and methods). However, we cannot rule out the possibility that some proteins may have been completely removed by the two saline washes and thus are not observed on the gels.

Similar results are obtained with the scrapings from the uterus (Fig. 1b). Hemoglobin of *C. serpentina* runs as a band (apparent molecular mass about 12 kDa) that is clearly distinct from any proteins found in the uterine tube, uterus or egg albumen. No hemoglobin bands were observed, either in samples of uterine tube or uterus, indicating that the samples were not contaminated with turtle blood (data not shown).

Based on these results, all subsequent work used the method represented in lane 1 of Fig. 1a and b. The oviductal segments were scraped vigorously with a sharp scalpel and the scrapings were placed directly into SDS final sample buffer, treated with 10% 2-mercaptoethanol, boiled for 2 min, and analyzed by gel electrophoresis. Note that the immediate treatment of the samples with SDS in this way obviated the need for protease inhibitors. Moreover, the fact that samples extracted with saline show the same banding patterns as those dissolved immediately in

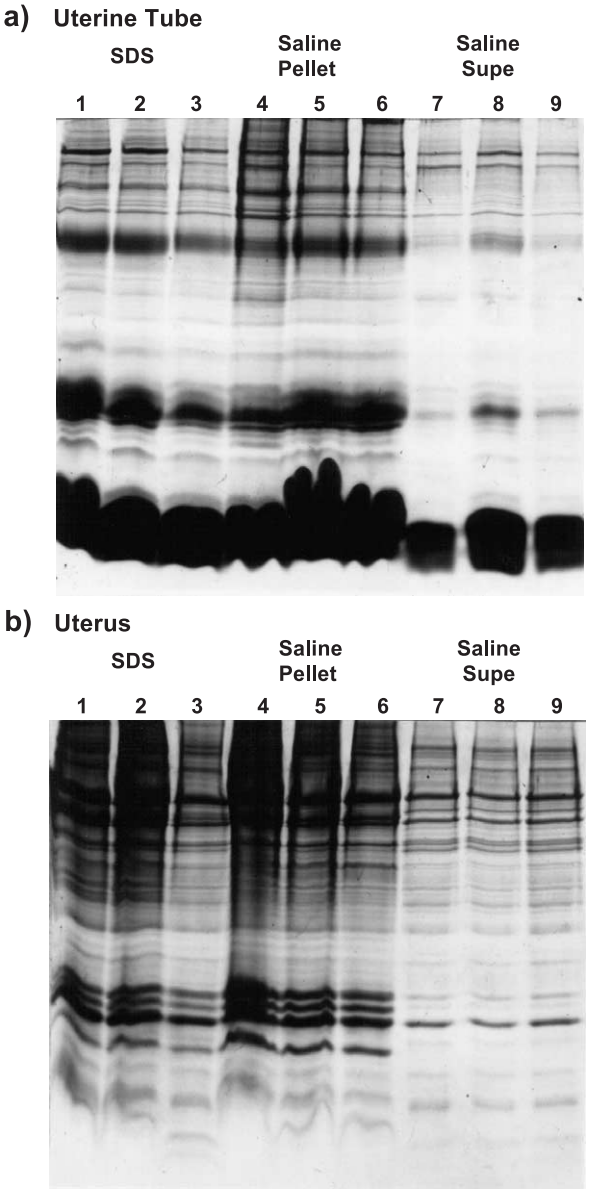

Fig. 1. Test of methods for preparing secretory proteins from the linings of the snapping turtle oviduct for analysis by SDS-polyacrylamide gel electrophoresis. The lining of the uterine tube (a) or uterus (b) was either scraped vigorously five to six times with a sharp scalpel (lanes 1, 4, 7); scraped gently one or two times with a sharp scalpel (lanes 2, 5, 8); or scraped with a blunt instrument (lanes 3, 6, 9). The scrapings obtained were either placed directly into SDS final sample buffer and immediately boiled with 2-mercaptoethanol (lanes 1–3), or placed into isotonic saline. In the latter case, the tissue in saline was centrifuged and the pellet (lanes 4–6) and supernatant (lanes 7–9) were separately mixed with SDS final sample buffer and boiled with 2-mercaptoethanol before loading onto gels containing 16% acrylamide.

SDS final sample buffer indicates that no significant proteolysis is occurring.

3.2. Uniformity of secretory proteins along the length of the uterine tube and uterus of the common snapping turtle

Using the approach described above, we first asked whether the protein pattern varies along the length of the oviduct. The uterine tube from each turtle was cut into three segments along its length, and similarly the uterus was cut into three segments. The proteins from the various segments were prepared and analyzed separately by SDS-polyacrylamide gel electrophoresis.

Examples from two different turtles are shown in Fig. 2, one at ovulation (Fig. 2a) and one post-ovulation (Fig. 2b). The same experiment has been done with 15 different turtles representing all stages of the reproductive cycle, and in all cases the three segments of the uterine tube are virtually identical to one another and the three segments of the uterus are identical to one another. Although minor variations in the relative intensity of bands in the three segments are occasionally seen, no consistent differences have been detected.

We conclude that the pattern of secreted proteins does not vary along the length of the uterine tube, or along the length of the uterus. All further experiments were therefore carried out using pooled samples, i.e., samples representing the total secretory proteins from the entire length of the uterine tube, or from the entire length of the uterus.

3.3. Comparison of secretory proteins in the uterine tube and the uterus with the egg albumen proteins of the snapping turtle

It is already evident from Figs. 1 and 2 that there are differences between the protein patterns of uterine tube and uterus. We therefore asked, which portion of the oviduct is the most likely source of the proteins in the egg albumen of the snapping turtle, the uterine tube or the uterus? To answer this question, protein samples were prepared from the uterine tube and the uterus and compared with egg albumen proteins by SDS gel electrophoresis, both in gels containing 16% acrylamide (Fig. 3) and in gels containing 10% acrylamide (Fig. 4).

We identify 13 major protein bands in the egg albumen of *C. serpentina* (e.g., Fig. 3a, lane 1 and Fig. 4a, lane 1). The estimated molecular masses of these proteins in kDa, indicated at the left side of each panel in Figs. 3 and 4, were determined by the standard method of plotting the logarithms of the molecular masses of marker proteins as a function of mobility (R_f) and then interpolating from this standard curve (Weber and Osborn, 1975).

Neither the uterine tube nor the uterus protein patterns are identical to the egg albumen. Both have some bands that co-migrate with albumen proteins and some bands that do not. However, the protein pattern from the egg albumen generally resembles the uterine tube more than the uterus, particularly at the time of ovulation (Figs. 3a

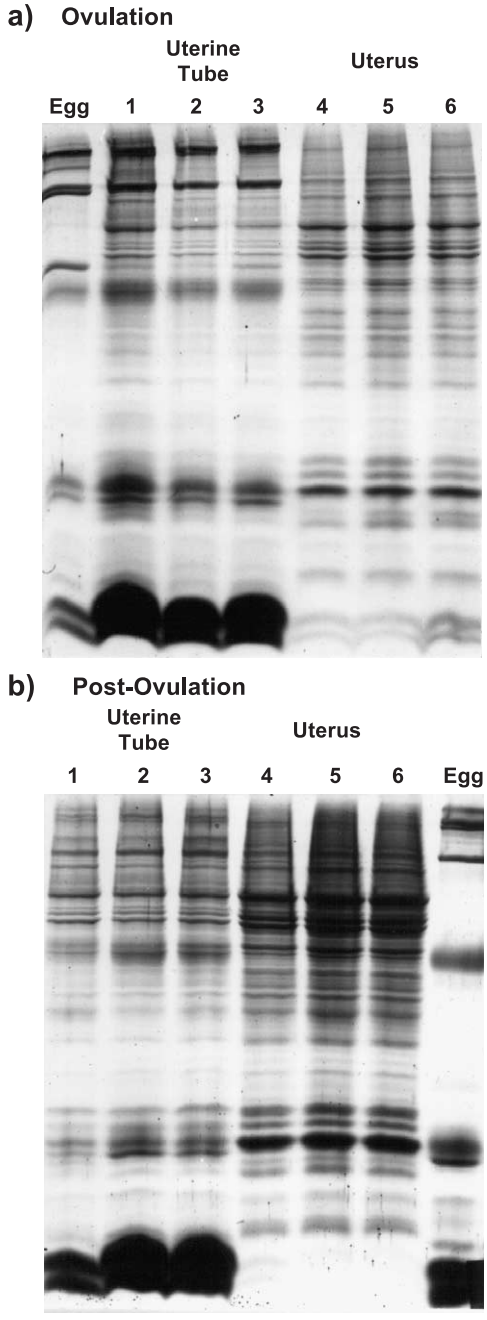

Fig. 2. Uniformity of secretory proteins in each of the compartments, uterine tube and uterus, of the snapping turtle oviduct. Protein patterns from two different turtles are shown, one of which was in the ovulation phase (late May) and the other post-ovulation (late June). For each turtle, the uterine tube was cut into three segments along its length, and similarly the uterus was cut into three segments. The secretory proteins from the oviductal linings were prepared separately for each of the segments and analyzed by electrophoresis in SDS gels containing 16% acrylamide. The segments are shown in order for each turtle with sample 1 being the segment proximate to the ovaries. Samples 1–3 are from the uterine tube and samples 4–6 are from the uterus. The lanes marked "Egg" show the proteins from snapping turtle egg albumen for comparison.

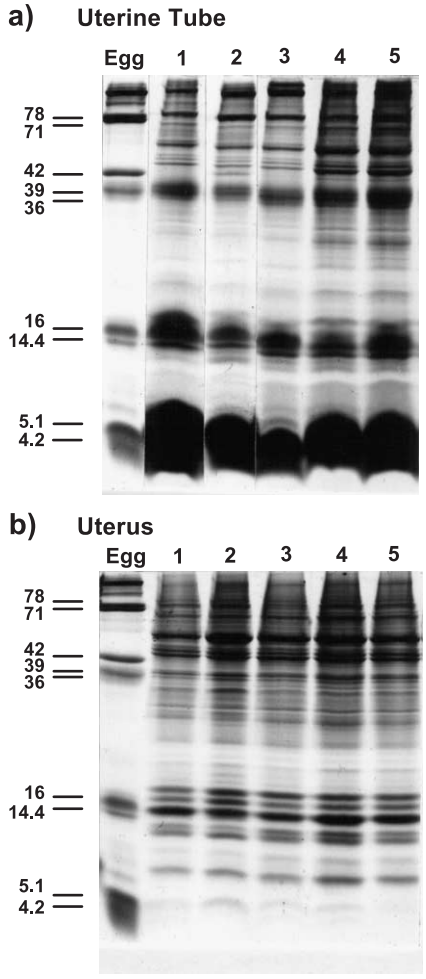

Fig. 3. Secretory proteins of the snapping turtle oviduct throughout the reproductive cycle: (a) uterine tube, (b) uterus. Samples were analyzed on SDS-polyacrylamide gels containing 16% acrylamide. Samples 1–5 represent each of the major stages in the ovarian cycle: preovulatory phase (sample 1), ovulation (sample 2), early postovulatory phase with corpus luteum active (sample 3), later postovulatory phase with corpus luteum degenerative (sample 4), and vitellogenesis (sample 5). The positions of the major egg albumen proteins ("Egg") are indicated at the left along with their molecular masses in kDa.

and 4a, sample 2). The uterine tube shows bands co-migrating with all of the egg albumen proteins except those at 120 and 142 kDa, although the bands at 42 and 71 kDa are weak (Figs. 3a and 4a). By contrast, the uterus clearly lacks bands corresponding to the egg albumen bands at 4.2, 5.1, 14.4, 36, 42, 120, 142, 168 and 182 kDa, and the band at 71 kDa is weak (Figs. 3b and 4b).

Thus, the uterine tube may have as many as 11 of the 13 albumen protein bands, whereas the uterus has at most four. Although there are many similarities between the protein patterns of the uterine tube and the uterus, the uterine tube clearly has more bands that co-migrate with egg albumen proteins. This suggests that the uterine tube is the most likely site for secretion of the egg albumen proteins.

3.4. Secretory proteins of the uterine tube and the uterus throughout the reproductive cycle of the common snapping turtle

The common snapping turtle, *C. serpentina*, is unusual among turtles in that it is a monoclutch species that oviposits 70 to 80 eggs just once a year, in mid-June. This stands in contrast to most other turtles, which are polyclutch species and lay smaller numbers of eggs several times throughout the summer. Since *C. serpentina* is a monoclutch species, one might expect significant variation in the proteins found in the linings of the uterine tube and/or uterus throughout the breeding season.

To test this possibility, the secretory proteins of the female reproductive tract were analyzed by SDS-polyacrylamide gel electrophoresis throughout the reproductive cycle. The gels shown in Figs. 3 and 4 compare the proteins from each of the major stages: preovulatory phase (sample

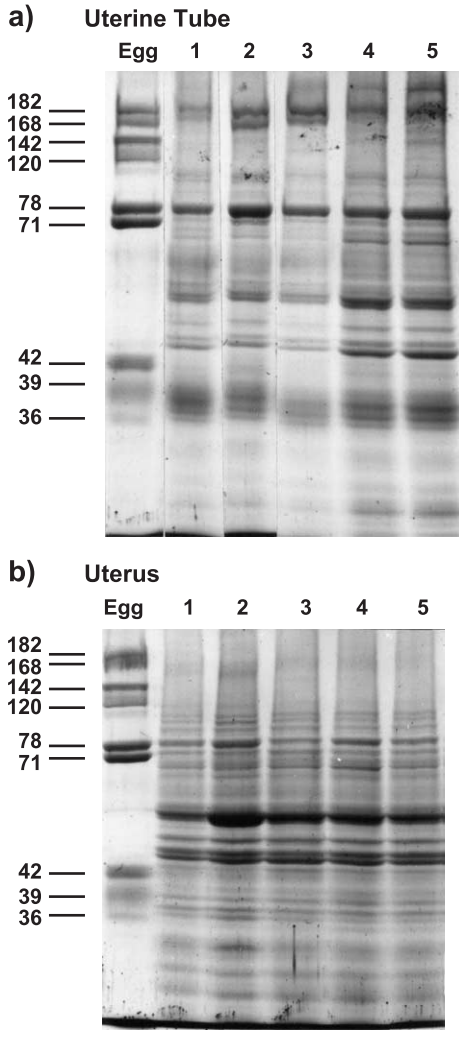

Fig. 4. Secretory proteins of the snapping turtle oviduct throughout the reproductive cycle: (a) uterine tube, (b) uterus. The samples are the same as in Fig. 3 but were analyzed on SDS-polyacrylamide gels containing 10% acrylamide.

1), ovulation (sample 2), early postovulatory phase with corpus luteum active (sample 3), later postovulatory phase with corpus luteum degenerative (sample 4), and vitellogenesis (sample 5). Progesterone determinations were done on all turtles and the results were consistent with previous studies (Mahmoud and Licht, 1997).

In the uterine tube (Figs. 3a and 4a) there are surprisingly few variations in the protein pattern throughout the reproductive cycle. However, some of these changes may be important. As mentioned above, bands have been found in the uterine tube that co-migrate with 11 of the 13 major egg albumen proteins, namely those at 4.2, 5.1, 14.4, 16, 36, 39, 42, 71, 78, 168 and 182 kDa. Of these 11 protein bands, eight appear consistently throughout the cycle, namely those at 4.2, 5.1, 14.4, 16, 36, 39, 78 and 182 kDa. However, the band at 42 kDa is observed only at the time of ovulation, and then rather weakly (Fig. 3a, sample 2). The band at 168 kDa is observed only at or near the time of ovulation (Fig. 4a, samples 1–3). The band at 71 kDa is present weakly throughout the cycle but appears to be much stronger at the time of ovulation (Fig. 4a, sample 2).

In the uterus, we have not detected any consistent pattern of changes in the secretory proteins throughout the reproductive cycle (Figs. 3b and 4b).

4. Discussion

We have monitored the proteins present in the lining of the uterine tube and uterus during the reproductive cycle of the snapping turtle, *C. serpentina*. This work is of interest for two reasons. First, *C. serpentina*, in contrast to most other turtles, is a monoclutch species and the secretory proteins of monoclutch turtle species have not previously been investigated. Second, we have used a novel approach to monitor the secretory activity of the oviductal glands, which could as well be applied to other species. The method involves scraping the lining of the oviduct and dissolving the secretory proteins directly in SDS-containing buffer for analysis by gel electrophoresis.

We are able to draw three main conclusions from this work. First, the major source of proteins for the snapping turtle egg albumen is most likely to be the uterine tube, not the uterus. We find 13 major proteins in the egg albumen, and bands corresponding to 11 of these are found in the lining of the uterine tube (Figs. 3a and 4a). By contrast, at most four of the egg albumen bands co-migrate with proteins found in the uterus (Figs. 3b and 4b). Based on these results and the published work of others on other turtle species, we conclude that the uterus is not the primary site of egg albumen production but is probably involved in synthesis of the proteinaceous eggshell membranes (Palmer, 1988, 1990; Perkins and Palmer, 1996).

This result is in conformity with other reptiles. Others have argued that the uterine tube in reptiles morphologically resembles the avian magnum in birds and is the site for albumen synthesis (Aitken and Solomon, 1976; Solomon, 1983; Palmer and Guillette, 1988, 1990, 1992; Perkins and Palmer, 1996). Moreover, the endometrial glands of the uterus have been identified in various chelonians and squamates, including the snapping turtle, as the site of eggshell membrane formation (Palmer and Guillette, 1988, 1992; Guillette et al., 1989; Cree et al., 1996; Perkins and Palmer, 1996; I.Y. Mahmoud, unpublished data). Palmer (1988, 1990), using [^{3}H]-leucine and explant cultures in the turtle, *Pseudemys s. scripta,* showed that albumen proteins are synthesized and secreted in vitro by the uterine tube and that the endometrial glands of the uterus contain numerous spherical, electron dense secretory granules, similar to those of the avian isthmus that secrete the proteinaceous fibers of the eggshell membranes. The dualistic functions of the reptilian uterus in producing both membranous and calcareous layers of the eggshell suggest strong dependence on hormonal signals to initiate secretory functions (Palmer and Guillette, 1990, 1991). Although the reptiles and avian eggs are structurally alike, the structure of the reproductive tract and the regulation of its functions are significantly different.

Our second conclusion is that the secretion of proteins is uniform along the length of the uterine tube, and also along the length of the uterus. In all snapping turtles we have examined, from all stages of the reproductive cycle, samples from different segments of the uterine tube have always had virtually identical protein patterns (Fig. 2, samples 1–3). The same is true for different segments of the uterus (Fig. 2, samples 4–6). A similar conclusion, that protein secretion is uniform along the length of the uterus, was reached by Palmer (1988, 1990) for the turtle *P. s. scripta*.

Our third conclusion is that, with a few exceptions, the secretory proteins from the uterine tube and uterus of the snapping turtle do not vary throughout the breeding season. This is surprising because *C. serpentina* is a monoclutch species. It ovulates all of its eggs at one time, between late May and mid-June. One might therefore expect the secretory glands to be active only during gravidity while the eggs are still in the uterus, a period of approximately 2 weeks. Instead, the glands apparently remain active throughout the summer. Of the 11 major albumen protein bands that are present in the uterine tube, eight appear to be present at constant levels throughout the reproductive cycle. Exceptions are the band at 71 kDa, which is more intense at the time of ovulation, and the bands at 42 and 168 kDa, which are only present at the time of ovulation.

Thus, physiologically the snapping turtle behaves like a polyclutch species. The purpose of such continuous glandular activity is unclear, but one possible explanation may be the high and stable plasma levels of progesterone, estradiol, testosterone and follicle stimulating hormone throughout the reproductive cycle (Mahmoud and Licht, 1997). Progesterone levels rise after ovulation to 0.92 ± 0.1 ng/mL at the time when the corpora lutea are steroidogenically active and eggs are still in the oviduct, and this rise may be essential for stimulating glandular activity such as

albumen and shelling secretions (Mahmoud et al., 1980). Starting just before oviposition, and continuing afterwards, progesterone levels fall slightly to 0.81±0.12 ng/mL, and in fact this drop may be the trigger for oviposition in the snapping turtle. During the rest of the summer, progesterone levels gradually rise again to a peak of 1.2±0.21 ng/mL at vitellogenesis, and then fall dramatically to 0.42±0.1 ng/mL shortly after the maturation of the follicle in September. Estradiol, testosterone and FSH also remain high and rise gradually throughout the summer. The continuous rising values of steroids and gonadotropins throughout the summer may be valuable to the snapping turtle since it has a brief reproductive cycle where the maturation of follicles and the development of the oviductal glands must take place rapidly prior to the significant drop in hormone levels, and ambient temperature, in the fall (Mahmoud and Licht, 1997).

The endocrine system plays an important role in regulating the glandular tissue in the reproductive tract in reptiles. However, little is known concerning the mechanism of steroid action on the reproductive tract ultrastructure and protein synthesis and secretion. Estradiol is clearly involved in the growth and hypertrophy of the reproductive tract. Ovariectomy in squamates results in both reduction in oviductal mass and regression of endometrial glands, whereas ovariectomy followed by sex steroid replacement results in oviductal hypertrophy, development of endometrial glands, and increased vascularization (Wilkinson, 1965; Yaron, 1972; Browning, 1973; Veith, 1974; Ortiz and Morales, 1974; Fawcett, 1975; Mead et al., 1981; Jones and Guillette, 1982; Masson and Guillette, 1987). Progesterone acts synergistically with estradiol in causing hypertrophy of the oviduct in lizards (Fawcett, 1975; Guillette et al., 1989).

The role of steroid receptors in regulating oviductal structure and function has been documented in many studies. Estradiol promotes the activity of progesterone receptors (Ho and Callard, 1984; Mahmoud et al., 1986; Kleis-San Francisco and Callard, 1986; Reese and Callard, 1989) indicating that the oviduct needs to be primed with estradiol to respond to progesterone. Progesterone has been shown to inhibit its own receptors in estrogen primed turtle oviducts (Selcer and Leavitt, 1991). Deluteinization of the lizard *Cnemidophorus uniparens* immediately following ovulation resulted in the disruption of eggshell production (Cuellar, 1979), suggesting that progesterone, or some other luteal agent, is involved in regulating eggshell formation by the oviduct.

One question raised by our study is why the egg albumen and uterine tube protein patterns do not match more closely. More specifically, why are the 120 and 142 kDa egg albumen proteins not found in the uterine tube? Several hypotheses can be suggested which should be amenable to experimental investigation. First, these proteins may be easily lost during the initial saline washes of the material, or they may for some reason not be solubilized in the SDS gel sample buffer. Second, the proteins may be subject to post-translational modification (proteolytic processing, for example) after their incorporation into the egg. Third, production of these proteins may be very transitory. Perhaps we have simply not taken the turtles at exactly the right time.

This has been a preliminary study, and many important questions remain to be answered. We hope that this research will form a basis for further investigation on the reproductive physiology of *C. serpentina.* The snapping turtle is widely used as a source of freshwater turtle meat, but is on the endangered species list in some states. More precise knowledge of its reproductive physiology might be useful in reproductive management so that the turtles can be better protected.

The method we have employed is novel and may also be applicable to other turtle species. The analysis could in principle be done on live turtles, using an appropriate endoscopic device, and this might be quicker and more reliable than hormone assays. Such an approach may be particularly valuable for studying the reproductive physiology of the large sea turtles and other endangered species.

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References

Aitken, R.N.C., Solomon, S.E., 1976. Observations on the ultrastructure of the oviduct of the Costa Rican green turtle (*Chelonia mydas* L.). J. Exp. Mar. Biol. Ecol. 21, 75–90.

Browning, H.C., 1973. The evolutionary history of the corpus luteum. Biol. Reprod. 8, 128–157.

Cree, A., Guillette, L.J., Reader, K., 1996. Eggshell formation during prolonged gravidity of the tuatara *Sphenodon punctatus*. J. Morphol. 230, 129–144.

Cuellar, H., 1979. Disruption of gestation and egg shelling in deluteinized oviparous lizards *Cnemidophorus uniparens* (Reptilia: Teiidae). Gen. Comp. Endocrinol. 39, 150–157.

Fawcett, J.D., 1975. Effects of season, ovariectomy and hormone replacement therapy on the oviduct of *Anolis carolinensis* (Reptilia: Iguanidae). PhD thesis, Univ. Colorado.

Fox, H., 1977. The urinogenital system of reptiles. In: Gans, C. (Ed.), Biology of the Reptilia vol. 6. Academic Press, New York, pp. 1–157.

Guillette, L.J., Fox, S.L., Palmer, B.D., 1989. Oviductal morphology and egg shelling in the oviparous lizards *Crotaphytus collaris* and *Eumeces obsoletus*. J. Morphol. 20l, 145–159.

Ho, S.M., Callard, I.P., 1984. High affinity binding of [^{3}H]-R5020 and [^{3}H]-progesterone by putative progesterone receptors in cytosol and nuclear extract of turtle oviduct. Endocrinology 144, 70–79.

Jones, R.E., Guillette, L.J., 1982. Hormonal control of oviposition and parturition in lizards. Herpetologica 38, 80–93.

Juang, R.-H., Chang, Y.-D., Sung, H.-Y., Su, J.-C., 1984. Oven-drying method for polyacrylamide gel slab packed in cellophane sandwich. Anal. Biochem. 141, 348–350.

Kleis-San Francisco, S.E., Callard, P., 1986. Progesterone receptors in the oviduct of a viviparous snake (Nerodia): Correlations with

ovarian function and plasma steroid levels. Gen. Comp. Endocrinol. 63, 220–229.
Laemmli, U.K., Favre, M., 1973. Maturation of the head of bacteriophage T4: I. DNA packaging events. J. Mol. Biol. 80, 575–599.
Mahmoud, I.Y., Licht, P., 1997. Seasonal changes in gonadal activity and the effects of stress on reproductive hormones in the common snapping turtle, *Chelydra serpentina*. Gen. Comp. Endocrinol. 107, 359–372.
Mahmoud, I.Y., Klicka, J., Cyrus, R., Colás, A.E., 1980. The rate of conversion of (4–14C) progesterone by corpora lutea of the snapping turtle, *Chelydra serpentina*. Gen. Comp. Endocrinol. 41, 274–277.
Mahmoud, I.Y., Colas, A.E., Woller, M.J., Cyrus, R.V., 1986. Cytoplasmic progesterone receptors in uterine tissue of the snapping turtle (*Chelydra serpentina*). J. Endocrinol. 109, 385–392.
Masson, G.R., Guillette, L.J., 1987. Changes in oviductal vascularity during the reproductive cycle of three oviparous lizards *Eumeces obsoletus*, *Sceloporus undulatus* and *Crotaphytus collaris*. J. Reprod. Fertility. 80, 361–371.
Mead, R.A., Eroschenko, V.P., Highfill, D.R., 1981. Effects of progesterone and estrogen on the histology of the oviduct of the garter snake, *Thamnophis elegans*. Gen. Comp. Endocrinol. 45, 345–354.
Ortiz, E., Morales, M.J., 1974. Development and function of the female reproductive tract of the tropical lizard, *Anolis pulchellus*. Physiol. Zool. 47, 207–217.
Palmer, B.D., 1988. Eggshell and albumen formation in turtles. Am. Zool. 28, 49A.
Palmer, B.D., 1990. Functional morphology and biochemistry of reptilian oviducts and eggs: Implications for the evolution of reproductive modes in tetrapod vertebrates. PhD thesis, Univ. Florida.
Palmer, B.D., Guillette, L.J., 1988. Histology and functional morphology of the female reproductive tract of the tortoise *Gopherus polyphemus*. Am. J. Anat. 183, 200–211.
Palmer, B.D., Guillette, L.J., 1990. Morphological changes in the oviductal endometrium during the reproductive cycle of the tortoise, *Gopherus polyphemus*. J. Morphol. 204, 323–333.
Palmer, B.D., Guillette, L.J., 1991. Oviductal proteins and their influence on embryonic development of birds and reptiles. In: Ferguson, M.W.J., Deeming, D.C. (Eds.), Physical Influences on Embryonic Development in Birds and reptiles. Cambridge Univ. Press, Cambridge, U.K, pp. 29–46.
Palmer, B.D., Guillette, L.J., 1992. Alligators provide evidence for the evolution of an archosaurian mode of oviparity. Biol. Reprod. 46, 39–47.
Perkins, M.J., Palmer, B.D., 1996. Histology and functional morphology of the oviduct of an oviparous snake, *Diadophis punctatus*. J. Morphol. 227, 67–79.
Reese, J.C., Callard, I.P., 1989. Two progesterone receptors in the oviduct of the freshwater turtle *Chrysemys picta*: Possible homology to mammalian and avian progesterone receptor systems. J. Steroid Biochem. 33, 297–310.
Selcer, K.W., Leavitt, W.W., 1991. Progesterone downregulates progesterone receptor, but not estrogen receptor, in the estrogen-primed oviduct of a turtle (*Trachemys scripta*). Gen. Comp. Endocrinol. 83, 316–323.
Sibley, C.G., 1970. A comparative study of the egg-white proteins of passerine birds. Bulletin vol. 32. Peabody Museum of Natural History (Yale Univ.), New Haven.
Sibley, C.G., Ahlquist, J.E., 1972. A comparative study of the egg-white proteins of non-passerine birds. Bulletin vol. 39. Peabody Museum of Natural History (Yale Univ.), New Haven.
Solomon, S.E., 1983. Oviduct. In: Freeman, B.M. (Ed.), The Physiology and Biochemistry of the Domestic Fowl, vol. 4. Academic Press, London, pp. 379–419.
Veith, W.J., 1974. Reproductive biology of *Chamaeleo pumilus pumilus* with special reference to the role of the corpus luteum and progesterone. Zool. Afr 9, 161–183.
Weber, K., Osborn, M., 1975. Proteins and sodium dodecyl sulfate: Molecular weight determination on polyacrylamide gels and related procedures. In: Neurath, H., Hill, R.L., Boeder, C.L. (Eds.), The Proteins. Academic Press, New York, pp. 179–223.
Wilkinson, R.F., 1965. Seasonal and hormonal changes in the oviducts of *Diadophis punctatus*. PhD thesis, Univ. Missouri.
Yaron, Z., 1972. Endocrine aspects of gestation in viviparous reptiles. Gen. Comp. Endocrinol. Suppl. 3, 663–674.