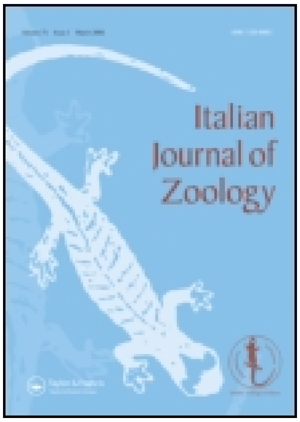

Italian Journal of Zoology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tizo20

Uterine dynamics of the southern eagle ray Myliobatis goodei (Chondrichthyes: Myliobatidae) from the southwest Atlantic Ocean

J. H. Colonello [a] , H. E. Christiansen [a] , M. B. Cousseau [b] & G. J. Macchi [a c]
[a] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) , Mar del Plata , Argentina
[b] Departamento de Ciencias Marinas de la Universidad Nacional de Mar del Plata , Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) , Argentina
Published online: 15 Feb 2013.

Italian Journal of Zoology, 2013, 187–194
Vol. 80, No. 2, http://dx.doi.org/10.1080/11250003.2012.742146

Taylor & Francis
Taylor & Francis Group

Uterine dynamics of the southern eagle ray *Myliobatis goodei* (Chondrichthyes: Myliobatidae) from the southwest Atlantic Ocean

J. H. COLONELLO[1]*, H. E. CHRISTIANSEN[1], M. B. COUSSEAU[2], & G. J. MACCHI[1,3]

[1]*Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, Argentina,* [2]*Departamento de Ciencias Marinas de la Universidad Nacional de Mar del Plata, Argentina, and* [3]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

Abstract
Uterine layer changes and their relationship with the reproductive cycle were analyzed for the southern eagle ray *Myliobatis goodei* (Chondrichthyes: Myliobatidae), based on histological and histometric studies. The specimens were collected from bottom-trawl surveys conducted in the coastal southwest Atlantic ecosystem between latitude 34 and 42°S. Oviduct samples were removed and fixed in 10% buffered formalin until processing for histological analysis. Both oviducts are developed in mature females, but only the left is functional. After ovulation, the size of the right posterior oviduct decreases and the left develops into a complex uterus that occupies almost the entire coelomic cavity. This uterus undergoes successive morphological and functional changes related to reproductive stages. Mucosa was characterized by temporal villous extensions or trophonemata with high variability depending on reproductive events. Invaginations or crypts associated with presumably lipid secretion (histotrophe) were observed between the trophonematum bases. The muscular layer develops into a complex muscular-vascular-conjunctive structure. The nutrition of early-gestation embryos is lecithotrophic and later they receive nutrients from the yolk sac and histotrophe. After yolk depletion, embryos become entirely dependent on histotrophe. Uterolactation, presumptive water imbibition of the muscular layer, and uterine hypertrophy observed in this study reinforce the "similarities" between Myliobatiformes and mammals.

Keywords: *Myliobatiformes, histotrophy, uterus, trophonemata, gestation*

Introduction

Chondrichthyans are the oldest Gnathostomata and probably the most successful in terms of evolutionary history (Compagno 1990). The success of this group may be attributed in part to their complex reproductive adaptations developed over 400 million years of evolution, which in many cases are more advanced than those of some higher tetrapods (Carrier et al. 2004; Luer et al. 2007).

Reproduction of chondrichthyans could be grouped by whether the embryo's development is external to the mother's body (oviparity) or internal (viviparity), and may be further divided by the way in which the mother supplies nutrients to the embryo (Wourms 1977; Compagno 1990; Hamlett et al. 2005; Musick & Ellis 2005). Oviparity is a lecithotrophic mode, while viviparity includes a variety of lecitotrophic and matrotrophic modes (Wourms 1977; Compagno 1990; Hamlett et al. 2005; Musick & Ellis 2005). In the first case, stored yolk provides all nutritional requirements of the embryo. In matrotrophic species, the maternal organism supplements yolk from other sources, such as uterine secretions (histotrophy), unfertilized oocytes (ovatrophy), embryos with arrested development (adelphotrophy) or through the formation of a pseudo-placenta (placentatrophy) (Hamlett et al. 2005; Musick & Ellis 2005).

Histotrophy reaches its maximum development in stingrays and eagle rays (Chondrichthyes: Myliobatiformes) in which several uterine specializations are related to this reproductive mode (Hamlett et al. 1985, 2005; Compagno 1990; Hamlett & Hysell 1998). Throughout the reproductive cycle the endometrium, through villous extensions or trophonemata, is involved in diverse physiological processes such as gas exchange, nutrient provision, osmoregulation (Babel 1967; Hamlett et al.

*Correspondence: J. H. Colonello, Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo Nro 1 Escollera Norte, Mar del Plata B7602HSA, Argentina. Tel: 54 223 4862586. Fax: 54 223 4861830. Email: jcolonello@inidep.edu.ar

1985, 1996; Smith & Marriner 1986; Johnson & Snelson 1996; Hamlett & Hysell 1998) and possibly endocrine functions (Hamlett et al. 1996). In addition to the yolk sac, embryos receive nutrients rich in lipids and proteins from a substance called histotrophe or "uterine milk" (Hamlett et al. 1985), which is secreted by trophonemata and absorbed by the embryo through its external gill filaments (Hamlett et al. 1985, 1996), mouth and spiracles (Babel 1967).

Various aspects related to the ultrastructure, histochemistry and function of trophonemata have been previously analyzed (Hamlett et al. 1985, 1996); however, the information on uterine changes and their relationship to the reproductive cycle is scarce.

In the southwestern Atlantic Ocean, eight species of Myliobatiformes in the families Dasyatidae and Myliobatidae occur south of 35°S (Menni & Stehmann 2002; Cousseau et al. 2007; Figueroa 2011). The southern eagle ray *Myliobatis goodei* Garman 1885 (Chondrichthyes: Myliobatidae) reaches a maximum size of 900 mm (disc width) and occurs in coastal waters less than 70 m from southern Brazil to Argentine Patagonia (Menni & Stehmann 2002; Cousseau et al. 2007). The aim of this study is to describe the uterine dynamics and their relationship with the reproductive cycle of *M. goodei* based on histological and histometric analysis.

Materials and methods

Individuals of *M. goodei* were collected from three scientific bottom trawl surveys carried out by the Instituto Nacional de Investigación y Desarrollo Pesquero (National Institute for Fisheries Research and Development, Argentina), during December 2003 and 2005, and July 2004, between 34 and 42°S. Disc width, total weight, sex and maturity stage were registered for each specimen sampled. Mature females were determined by the presence of yolked oocytes. Mature females were sorted into four reproductive stages according to ovarian and uterine condition: (1) pre-ovulation – batch of large oocytes in the left ovary and the left oviduct increasing in size; (2) post ovulation – fertilized oocytes (i.e. eggs) in the left posterior oviduct wrapped together by a thin membrane (Figure 1); (3) gestation – developing embryos in the uterus and ovaries with diverse oocytes condition, from the atretic until the starting-vitellogenesis condition; (4) post partum – yolked oocytes in the ovary and reduced uterus in comparison with gestation stage. Three gestation stages were also arbitrarily considered according to embryo development: (a) early gestation –embryos characterized by open gill slits with buds of gill filaments on arches, and the external yolk sac is greater than disc width; (b) mid gestation – embryos showed external aspect similar to adults but external yolk is still present as well as external gill filaments; (c) late gestation –embryos were entirely developed and neither external yolk sac nor external gill filaments were observed.

Samples of all uteri were removed from 30 mature females (Stage 1: 4 individuals; Stage 2: 4 individuals; Stage 3: 20 individuals; Stage 4: 2 individuals) and fixed in 10% buffered formalin until processing for histological analysis. Following fixation, tissues were removed, dehydrated in a gradate series of ethanol, cleared in xilol and embedded in paraffin. Segments of 3 μm thickness were sectioned and stained with haematoxylin and eosin and examined under a microscope.

In order to assess the magnitude of the uterine changes associated with the reproductive cycle, the percent volume occupied by uterine layers was estimated. For this purpose, uterus slides were analyzed on a 25-point grid Zeiss/Oberköchen at 10× through a point-counting system from an image in contiguous areas of the uterine wall. The percentage of the tissue components was related with each reproductive stage of mature females.

Results

Uterus anatomy

Only the left reproductive tract is functional in mature females of *M. goodei*. Both ovaries and oviducts are similar morphologically before sexual maturation, but then only the left ovary develops yolked oocytes and the left oviduct receives eggs. After ovulation, the size of the right posterior oviduct decreases and the left one develops into a uterus (Figure 1), which occupies the greater portion of the coelomic cavity. Anatomically, the uterine wall is composed of mucosa, submucosa, muscle and serosa.

Morphological modifications are observed in the mucosa, submucosa and serosa without significant size increase. The most significant change in the uterine wall throughout the reproductive cycle is observed in the muscular layer (Figure 2.A). The mucosa presents villous extensions (trophonemata) characterized by a dynamic epithelium that change depending on the stage of the reproductive cycle. Under the epithelium, vascular and capillary systems are surrounded by basal membrane and conjunctive tissue (Figure 2B.1). Invaginations or crypts (here termed "basal crypts") observed between each pair of trophonemata are associated with histotrophe

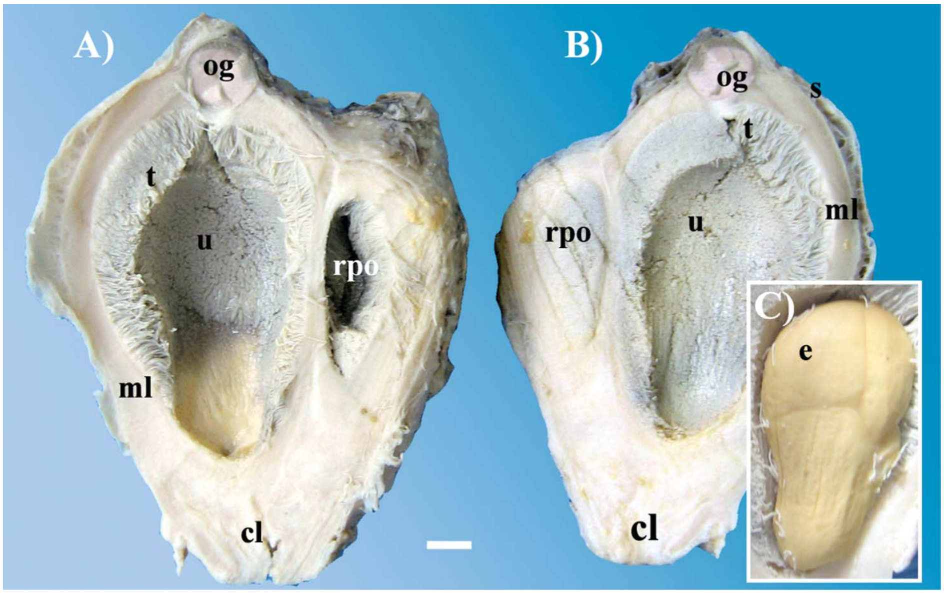

Figure 1. Longitudinal section of the mid-posterior oviducts of *Myliobatis goodei* at ovulation stage showing **A**) portion adhered to the vertebral column, **B**) portion contiguous to ventral, and **C**) eggs envelop by a thin membrane. References: rpo, right posterior oviduct; u, uterus, og, oviducal gland; t, trophonemata; ml, muscular layer; s, serosa; cl, cloacae; e, eggs. Scale bar: 1 cm.

secretion (Figure 2B.2). The muscular layer develops into a complex muscular-vascular-conjunctive structure, which can be described in relation to a superficial stratum and a deeper stratum (Figure 2B.3 and 2B.4). Uterine wall size increases correlatively with the muscular layer due to vascularity increases (diameter of blood vessels) and presumably water imbibition of conjunctive tissue. The concept of "water imbibition" refers to a process analogous to that in the superior mammalians (Christiansen pers. obs.).

Uterine dynamics

Morphological and functional uterine changes related to stages of the reproductive cycle can be described in relation to trophonemata, basal crypts, and muscular (superficial and deeper stratum) modifications (Figure 3).

Stage 1: Pre-ovulation

The epithelium of trophonemata is simple and pseudo-stratified with little development and no secretion activity. The corium is reticular, characterized by the presence of undifferentiated cells, fibroblasts and free elements; vascularity is scarce and confined to a capillary system adjacent to the epithelium. Prominent epithelial invaginations or basal crypts are observed contiguous to each trophonematum. These basal crypts are covered by simple epithelium, showing synthesis and secretion of lipid droplets. The muscular layer is containing the slightly dense conjunctive tissue and the muscular stratum, conformed by small arterial-venous formations and compacted smooth cells (Figure 3).

Stage 2: Post-ovulation

The epithelium of trophonemata is cubic, with signs of synthesis and secretion activity evidenced by the acidophil cytoplasm. Vascularity is reduced but the vessels' diameter is larger compared with pre ovulation. The corium is reticular and mainly composed of fibroblasts and histiocytes. Secretion activity is observed close to the basal crypt epithelium. The muscular layer is thin and formed by fibers and a modest arterial-venous system (Figure 3).

Stage 3: Early gestation

Each trophonematum begins to enlarge and develop invaginations into its surface. The epithelium is cubic with scarce synthesis and secretory activity (Figure 3). The corium is reticular, shows fibroblasts and is infiltrated by dilated blood vessels. Basal crypts show an epithelium with many folds and much secretory activity, presumably related to lipid droplets

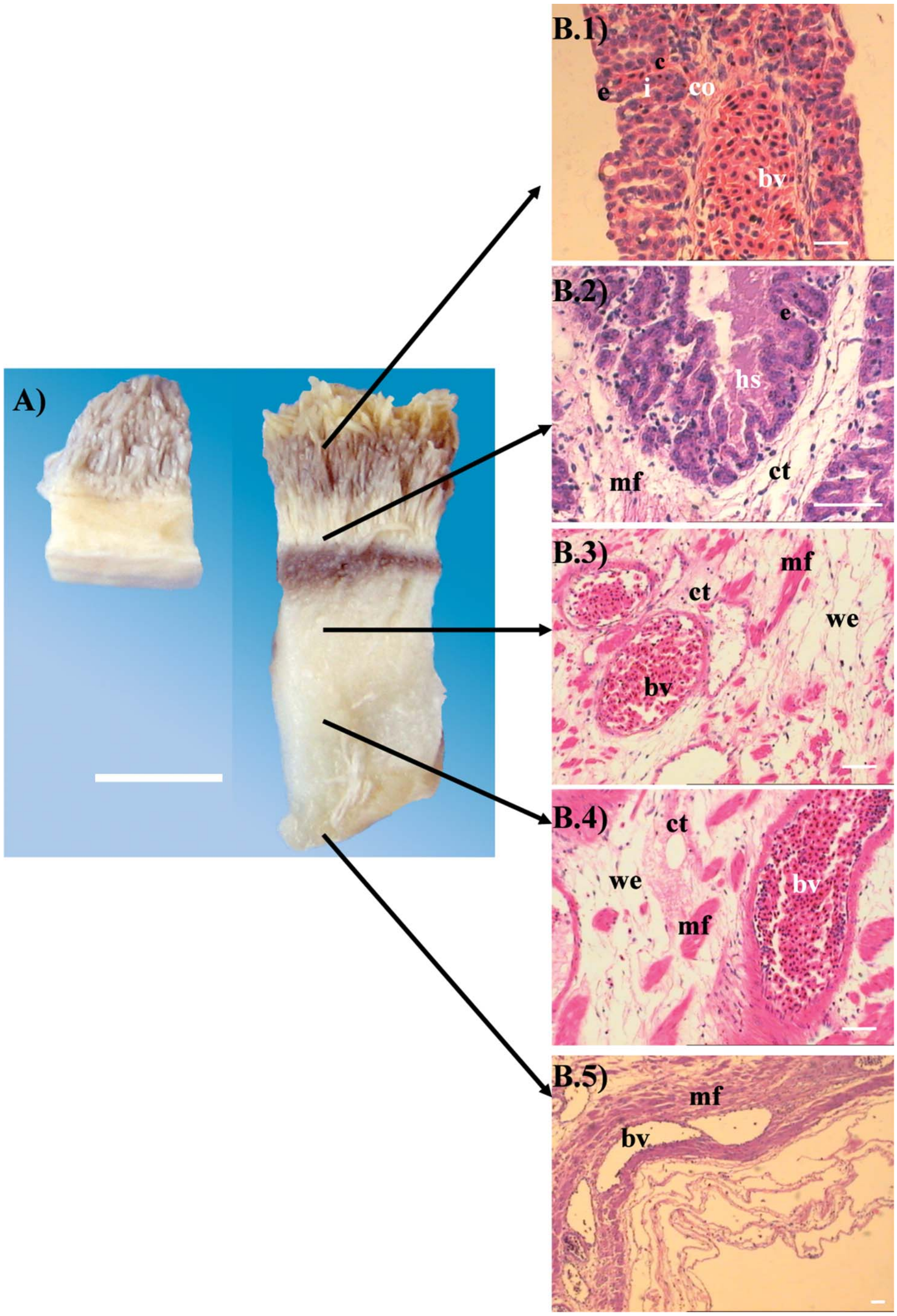

Figure 2. **A**) Longitudinal section of *Myliobatis goodei* uterus at early (left) and mid (right) gestation stage; **B**) photomicrography of **B.1**) trophonematum, **B.2**) secretory crypts, **B.3**) superficial and **B.4**) deeper muscular layer, and **B.5**) serosa. References: e, trophonematum epithelium; co, corium; i, trophonematum invaginations; bv, blood vessel; hs, histotrophe secretion; ct, connective tissue; mf, muscular fibbers; we, presumptive water imbibition. Scale bars: A, 1 cm; B, 0.012 cm.

and descamative cells. The submucosa is constituted by lax conjunctive tissue. The muscular stratum is characterized by fascicles of muscle fibers separated by lax tissue, infiltrated by an arterial-venous net (Figure 3).

Stage 3: Mid-gestation

The epithelium of each trophonematum changes from cubic to plane and the surface increases as a result of folds or invaginations. The corium is reduced to a basal membrane in some regions and

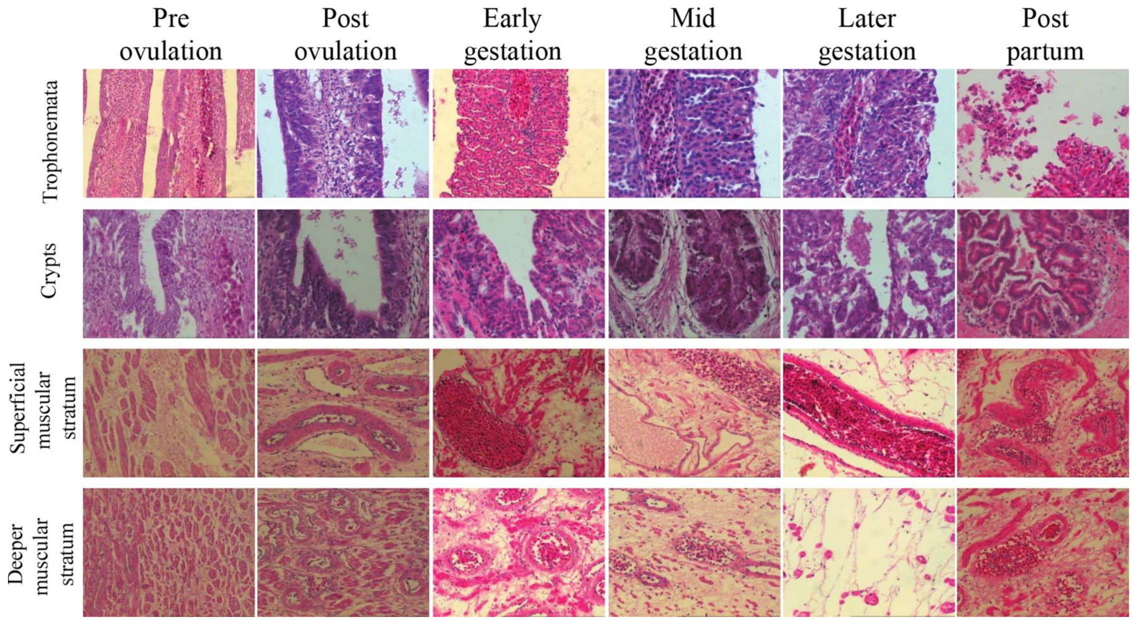

Figure 3. Uterine changes of *Myliobatis goodei* during reproductive stages related to trophonemata (T), basal crypts (C), superficial (SML) and deeper (DML) muscular stratum. T and C: 20×; SML and DML: 10×.

is adventitial to sub-epithelial capillaries. This capillarity is the result of an afferent-efferent system derived from a central vessel of the trophonematum. Histotrophe secretion is related to basal crypts located between the trophonemata. The muscular layer becomes a "vascular-muscular-conjunctive" structure (Figure 2A). Fiber cells are arranged as an isolated bundle, irrigated by an arterial-venous system and presumptive water imbibition (Figure 3).

At mid-gestation, embryos show an external aspect similar to adults but external yolk is still present and external gill filaments are finalizing their process of regression (Figure 4A). These filaments are characterized by a central core of the conjunctive-arterial-venous system covered by cubic epithelium (Figure 4B).

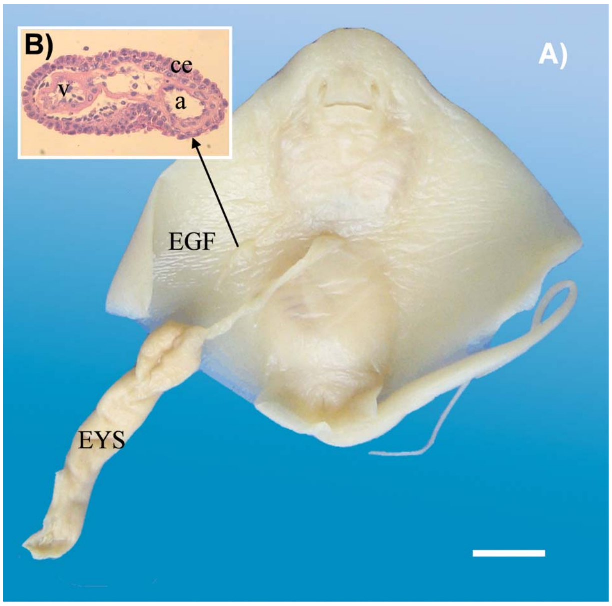

Figure 4. **A**) Photography of *Myliobatis goodei* embryo with external yolk sac (eys) and residual external gill filaments (egf); **B**) photomicrography of egf (20×) showing the arterial (a) venous (v) system covered by cubic epithelium. Scale bar: 1 cm.

Stage 3: Late gestation

The epithelium of the trophonemata is plane. Arterial-venous vascularity and secretory activity of trophonemata and basal crypts are diminished. Presumptive water imbibed from conjunctive tissue is increased into the muscular layer, which produces the dispersion of muscular fibers. The volume of blood vessels into the arterial-venous system is also diminished (Figure 3).

Stage 4: Post-partum

The size and thickness of the uterus are notably reduced in comparison with the late gestation stage and trophonemata are reduced to small flattened filaments. A necrobiotic and descamative process joined with corium mobilization and congestive blood vessels give support to the senescence phase. These processes are less visible in the crypts, where a new regenerative cycle is evident. The muscular layer is compact as result of a dehydration process, which

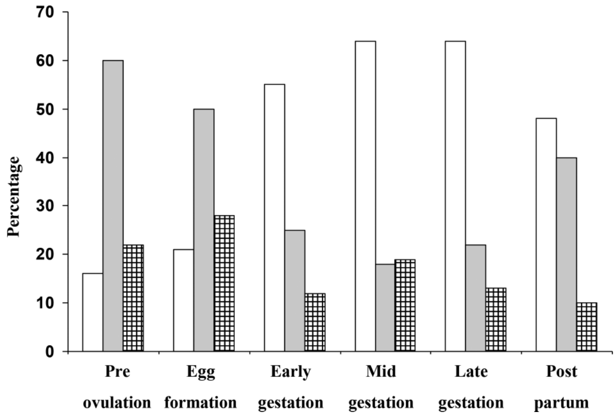

Figure 5. Variation in the percentage of volume occupied by muscular fibbers (filled bars), conjunctive tissue and water imbibed (empty bars) and vascular system (grid bars) within muscular layer during reproductive stages.

produces re-orientation of fiber cells in bundles, with scarce conjunctive tissue. The size of the arterial-venous system is slightly reduced (Figure 3).

Uterine wall size increase throughout the reproductive cycle is a consequence of vascularity and presumptive water imbibition of the muscular layer (Figure 2A). These modifications become evident by the proportion occupied by the muscular fibers, the conjunctive tissue and the vascular system (Figure 5). Before ovulation, fiber cells represent over 60% of the total tissue, while conjunctive tissue and water imbibed represent less than 20%. After embryo isolation, water imbibition increases and consequently muscular fibers become scattered; the proportion of the arterial-venous system remains stable although differences are observed in blood volume. During mid-gestation, the proportion of the conjunctive tissue is higher than 60% while muscular fibers represent less than 20%. After the embryo's birth, water imbibition diminishes and the proportion occupied by muscular fiber increases again (Figure 5).

Discussion

Reproductive asymmetry, although not a rule [e.g. reproductiove symetry observed in the common eagle ray *Myliobatis aquila* (Capapé et al. 2007)], is very widespread in Myliobatiformes. Most species show reproductive asymmetry related to development and functionality of ovaries and uteri (Hamlett et al. 1985; Smith & Merriner 1986; Johnson & Snelson 1996; White et al. 2001; Neer & Thompson 2005). Although both ovaries and uteri are functional in some species, asymmetric fertility and fecundity has been observed (Babel 1967; Charvet-Almeida et al. 2005; Fahy et al. 2007) and even the bat ray *Myliobatis californica* has a nearly non-functional right ovary but fully functioning right and left uterus (Martin & Cailliet 1988). In the yellow stingray *Urobatis jamaicensis*, as the animal produces small broods, ova typically enter into the left reproductive tract and the right tract apparently remains unused until larger broods demand the simultaneous use of both uteri (Fahy et al. 2007). Non-pregnant females of *Myliobatis goodei* exhibit externally similar oviducts, but after ovulation the right posterior oviduct is reduced to a rudimentary structure and the left one develops into a unique uterus that occupies a great portion of the coelomic space. This extreme kind of asymmetry is the first such documented for chondrichthyans.

The uterus of *M. goodei* shows a dynamic epithelium, with morphological and vascular modifications throughout the reproductive cycle. After ovulation, secretory activity of trophonemata is dominant. This secretion seems to have structural and mechanical functions instead of nutritional, because embryos are not yet differentiated. The egg's movement into the uterus can be accomplished by the secretion and "ciliary" action of trophonemata jointly with muscular contraction. During early to mid gestation, the surface of the trophonemata is characterized by invaginations related to vascular activity. Hamlett et al. (1985) demonstrated through electronic microscopy that these invaginations are composed of simple columnar cells with vesicles involved in lipid secretion. In *M. goodei* the main function of trophonemata invaginations appears to be related more to gaseous exchange than to histotrophe secretion. The prominent vascularity and the thin epithelium aid gaseous exchanges to supply the oxygen demands of embryos. The source of histotrophe is mainly related to the basal crypts observed between the trophonemata, especially later in gestation when embryo requirements increase.

Muscular layer modifications during the reproductive cycle can be related to transport (ovulation), mechanical protection (gestation) and contractile capacity (birth). Based on differences of the muscular layer between recently post-partum and mature but not pregnant females, as well as the necrobiotic and descamative process of trophonemata after birth, a resting or recovery period before a new reproductive cycle can be suggested. This hypothesis contrasts with observations made of the stingrays *Dasyatis americana* (Chapman et al. 2003), *Dasyatis chrysonota* (Ebert & Cowley 2009) and the cownose ray *Rhinoptera bonasus* (Smith & Merriner 1986), where mating and ovulation take place soon after parturition. Seasonal analyses based on macroscopical and histological observations of reproductive organs of *M. goodei* are required to give precise ovulation, gestation and parturition events.

Embryo nutrition of *M. goodei* appears to be triphasic. For a short period of time, early gestation nutrition is lecithotrophic. During mid-gestation embryos receive nutrients from the yolk sac and histotrophe through external gill filaments. After yolk depletion, embryos became entirely dependent on histotrophe, which is ingested orally because external filaments are completely absorbed. Furthermore, in some species, enlarged trophonemata have been observed entering into the gill slits, mouth (Babel 1967; Smith & Merriner 1986) and even spiracles (White et al. 2001). This pattern was also corroborated in other Myliobatids such as *Urolophus halleri* (Babel 1967) and *Rhinoptera bonasus* (Smith & Merriner 1986). A nutritive function of the external gill filament appears to be more frequent in matrotrophic than lecitotrophic species, where embryos depend solely on yolk reserves. By the end of the gestation period, uterus lumen is characterized by a mechanical disruption caused by the embryo's movement, descamative trophonematum and histotrophe secretion; this condition may support the passive ingestion of trophonematum.

The continuous supply of nourishment increases more than 3000% in weight from ripe ova to term embryo in histotrophic species (Babel 1967; Hamlett et al. 1985; Capapé 1993), but the interpretation of these values as a sign of reproductive efficiency must be made with caution. More detailed analysis and other factors such as litter size, age and size at maturity, and duration of gestation period should be considered. The size of litters, the substantial increase in embryonic weight, fecundity and the short gestation period of about four to six months may suggest a high reproductive efficiency of histotrophy in Myliobatiformes with regards to other viviparous reproductive modes of elasmobranchs, such as lecitotrophy, oophagy, adelphophagy or even placentophagy.

Hamlett et al. (1996) introduced the term uterolactation to refer to the production of nutrient histotroph by uterine tissues. The process of synthesis and secretion of milk is reminiscent of mammary gland function in other vertebrates (Hamlett et al. 2005). The presumptive water imbibition process of the muscular layer and uterine hypertrophy observed in this study reinforce the "similarities" to mammals. However, most of the changes in the maternal-fetal relationship of chondrichthyan fishes are not well understood. Future studies on histotrophic species are required to clarify topics related to osmoregulation, excretion, endocrine regulation and immunology, as well as the uterine water imbibition. These studies will be important from an evolutionary standpoint, because chondrichthyan fishes occupy a pivotal position in comparative and evolutionary studies of vertebrate reproduction and development.

Acknowledgements

The authors are grateful to the Histology Laboratory of INIDEP (Gabinete Histología) for assistance in technical support, and to Lic. A.M. Massa for providing access to material. We thank D.F. Figueroa and C. Capapé for comments on the manuscript. Dr. H.E. Christiansen was supported by the PIP CONICET 5009. INIDEP Contribution Number 1750.

References

Babel JJ. 1967. Reproduction, life history, and ecology of the round stingray *Urolophus halleri* cooper. California Fish and Game 1371–104.

Capapé C. 1993. New data on the reproductive biology of the thorny stingray, *Dasyatis centroura* (Mitchill, 1815), from off the Tunisian coasts. Environmental Biology of Fishes 38:73–80.

Capapé C, Guélorget O, Vergne Y, Quignard JP. 2007. Reproductive biology of the common eagle ray *Myliobatis aquila* (Chondrichthyes: Myliobatidae) from the coast of Languedoc (southern France, northern Mediterranean). Vie et Milieu – Life and Environment 57:1–6.

Carrier JC, Pratt Jr HL, Castro JI. 2004. Elasmobranch reproduction. In: Carrier JC, Musick JA, Heithaus MR, editors. Biology of sharks and their relatives. CRC Press. pp. 269–286.

Chapman DD, Corcoran MJ, Harvey G, Malan S, Shivji MS. 2003. Mating behaviour of southern stingrays. Environmental Biology of Fishes 68:241–245.

Charvet-Almeida P, Góes de Arújo ML, Almeida MP. 2005. Reproductive aspects of freshwater stingrays (Chondrichthyes: Potamotrygonidae) in the Brazilian Amazon Basin. Journal of the Northwest Atlantic Fisheries Science 35:135–171.

Compagno LJV. 1990. Alternative life-history styles of cartilaginous fishes in time and space. Environmental Biology of Fishes 28:33–75.

Cousseau MB, Figueroa DE, Díaz de Astarloa JM, Mabragaña E, Lucifora LO. 2007. Rayas chuchos y otros batoideos del atlántico sudoccidental (34°S–55°S). Argentina: National Institute of Fisheries Research and Development (INIDEP).

Ebert DA, Cowley PD. 2009. Reproduction and embryonic development of the blue stingray, *Dasyatis chrysonota*, in southern African waters. Marine Biological Association of the United Kingdom 89:809–815.

Fahy DP, Spieler RE, Hamlett WC. 2007. Preliminary observations on the reproductive cycle and uterine fecundity of the yellow stingray, *Urobatis jamicensis* (Elasmobranchii: Myliobatiformes: Urolophidae) in southeast Forida, USA. The Raffles Bulleting of Zoology 14:131–139.

Figueroa DE. 2011. Clave ilustrada de agnatos y peces cartilaginosos de Argentina y Uruguay. In: Wöhler OC, Cedrola P, Cousseau MB, editors. Contribuciones sobre la biología, pesca y comercialización de tiburones en la Argentina. Aportes para la colaboración del Plan de Acción Nacional. Buenos Aires: Consejo Federal Pesquero. pp. 25–74.

Hamlett WC, Hysell MK. 1998. Uterine specializations in elasmobranchs. The Journal of Experimental Zoology 282:438–459.

Hamlett WC, Kormanik G, Storrie M, Stevens B, Walker TI. 2005. Chondrichthyan parity, lecitotrophy and matotrophy. In: Hamlett WC, editor. Reproductive biology and phylogeny of Chondrichthyes, sharks, batoid and chimaeras, Vol. 3. Enfield, NH: Science Publishers Incorporated. pp. 395–434.

Hamlett WC, Musick JA, Eulitt AM, Jarrell RL, Kelly MA. 1996. Ultrastructure of uterine trophonemata, accommodation for uterolactation, and gas exchange in the southern stingray, *Dasyatis americana*. Canadian Journal of Zoology 74:1417–1430.

Hamlett WC, Wourms JP, Smith JW. 1985. Stingray placental analogues: Structure of trophonemata in *Rhinoptera bonasus*. Journal of Submicroscopic Cytology 17:541–550.

Johnson MR, Snelson FF. 1996. Reproductive life history of the atlantic stingray, *Dasyatis Sabina* (Pisces, Dasyatidae), in the freshwater St. Johns River, Florida. Bulletin of Marine Science 59:74–88.

Luer CA, Walsh CE, Bodine AB, Wyffels JT. 2007. Normal embryonic development in the clearnose skate, *Raja eglanteria*, with experimental observations on artificial insemination. Environmental Biology of Fishes 80:239–255.

Martin LK, Cailliet GM. 1988. Aspects of the reproduction of the bat ray, *Myliobatis califorica* in Central California. Copeia 1988:754–762.

Menni RC, Stehmann MFW. 2002. Distribution, environment and biology of batoid fishes off Argentina, Uruguay and Brazil: A review. Revista del Museo de Ciencias Naturales (nueva serie) 2:69–109.

Musick JA, Ellis JK. 2005. Reproductive evolution of Chondrichthyans. In: Hamlett WC, editor. Reproductive biology and phylogeny of Chondrichthyes, sharks, batoid and chimaeras, Vol. 3. Enfield, NH: Science Publishers Incorporated. pp 45–71.

Neer JA, Thompson BA. 2005. Life history of the cownose ray, *Rhinoptera bonasus*, in the northern Gulf of Mexico, with comments on geographic variability in life history traits. Environmental Biology of Fishes 73:321–331.

Smith JW, Merriner JV. 1986. Observations on the reproductive biology of the cownose ray, *Rhinoptera bonasus*, in Chesapeake Bay. Fishery Bulletin 84:871–877.

White WT, Platell ME, Potter IC. 2001. Relationship between reproductive biology and age composition and growth in *Urolophus lobatus* (Batoidea: Urolophidae). Marine Biology 138:135–147.

Wourms JP. 1977. Reproduction and development in Chondrichthyan fishes. American Zoologist 17:379–410.