Live-bearing manta ray: how the embryo acquires oxygen without placenta and umbilical cord

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We conducted an ultrasonographic experiment on a pregnant manta ray, *Manta alfredi* (Chondrichthyes, Batoidei). This study showed how the embryo of the live-bearing elasmobranchs respires in the body of the female. In the embryonic stage, the manta ray embryo takes in uterine fluid by buccal-pumping. After birth, the manta ray shifts its respiratory mode from buccal-pumping to ram-ventilation. The rapid reduction of the spiralce size in the young manta ray may reflect this shift of respiratory mode.

Unlike mammals or some carcharhinid sharks that acquire oxygen through a placenta and umbilical cord, the manta ray embryo does not have a direct connection with the mother. Thus, the manta ray embryo obtains oxygen by buccal-pumping of the uterine fluid, in the same way that the embryos of egg-laying species obtain oxygen from the water in the egg case. This finding extends our understanding of the diversity of embryonic respiratory systems in live-bearing vertebrates.

Keywords: buccal-pumping; echosonography; embryo; live-bearing; manta ray; respiration

1. INTRODUCTION

In viviparous (live-bearing) vertebrates, embryos obtain oxygen from the mother during the entire gestation period. Acquisition of oxygen by the embryo is accomplished in various ways. For example, mammals and a few terminal taxa of the carcharhini sharks exhibit convergent evolution of a ‘placenta’: the oxygen is supplied to the foetal blood from the mother’s body via the placenta, and the oxygenated blood is carried to the embryo through the umbilical cord [1]. However, most viviparous fishes, amphibians and reptiles have evolved different live-bearing systems in which a placenta is not formed. In this study, we examined the embryonic respiratory system of the manta ray, *Manta alfredi* (Chondrichthyes, Batoidei), the largest live-bearing ray, reaching a disc width of over 5.0 m [2]. The embryonic respiratory mechanism of the manta ray may yield an understanding of the mystery of how the embryos of most viviparous elasmobranchs (sharks and rays) respire in the body of the female without a placenta or umbilical cord. Embryonic respiration of the viviparous elasmobranchs has been studied using the ultrastructure of the uterus [3–5], but the respiratory system of the embryo itself is still poorly understood.

In June 2009, a female manta ray was born at Okinawa Churaumi Aquarium in Japan. This event provided us the first opportunity to access the embryonic respiratory behaviour of viviparous elasmobranchs. Myliobatiform stingrays, including the manta ray, have a unique viviparous reproductive mode, which is called a ‘lipid histotroph’ [6,7]. In this reproductive mode, a placenta is not formed, but the embryo is grown to large sizes in the uterus mostly by the lipid-rich ‘milk’ secreted from the uterine wall [1,3,4,6,7]. However, it is not understood how this milk, as well as oxygen, is taken in by embryos, in the absence of an umbilical cord.

The purposes of the present paper are (i) to describe the kinematic and morphological characteristics associated with the respiratory system of the viviparous manta ray embryo and (ii) to compare the embryonic respiratory system of the manta ray with those of other viviparous and oviparous elasmobranchs.

2. MATERIAL AND METHODS

Ultrasonographic imaging was performed in a pregnant manta ray (*M. alfredi*) at Okinawa Churaumi Aquarium. Diagnostic sonography is an ultrasound-based imaging technique, which can visualize movement inside a body [8,9].

The pregnant manta ray (3.5 m in disc width) was obtained by set net off Okinawa Main Island, Japan. Before the experiment, the individual was transported to the portable pool (3.7 m diameter, 0.6 m depth) placed in a reserve tank at the Okinawa Churaumi Aquarium (see the electronic supplementary material for details). For the sonographic observation, we used a sonography diagnostic imaging system FAZONE M (FUJIFILM Co). The transducer of the sonograph was placed on the dorsal surface of the body above the uterus. This individual safely gave birth six months after the sonographic experiment (22 June 2009) at Okinawa Churaumi Aquarium. This was the second successful manta ray birth in captivity.

In addition to the sonographic experiment, we collected morphological data from dead specimens (see the electronic supplementary material for details). We examined five specimens of the giant manta (*Manta birostris*) and four specimens of the manta ray (*M. alfredi*). The specimens included two newborn juveniles of the manta ray, which were born in captivity at Okinawa Churaumi Aquarium (one individual died 5 days after its birth in 2007; the other died 16 days after its birth in 2010). The specimens measured in this study are preserved at Okinawa Churaumi Aquarium, except one specimen of the giant manta embryo which is preserved at the National Museum of Natural History (USA). The taxonomy of the genus *Manta* follows the study of Marshall et al. [10].

3. RESULTS

Sonographic data showed the manta ray had a single live embryo in the uterus. The embryo was positioned in the same direction as the mother, with its pectoral fins folded dorsally. The yolk-sac was absent. During the experiment, the mouth of the embryo was rhythmically opened and closed (figure 1). Average duration of the single mouth opening/closing cycle was 1650 ± 58 s.d. ms. This cycle was divided into expansion [figure 1a(i)] and compression [figure 1a(ii)] phases. During the expansion phase, the lower jaw was depressed until the gape height reached...
approximately 10 per cent of the mouth width (480 ± 50 s.d. ms after the mouth starts opening) [figure 1a(ii)]. In the compression phase, the lower jaw was elevated and the oral cavity was compressed. During the first one-sixth of the compression phase, the oral valve was rapidly raised [figure 1a(iii)] and covered the mouth opening (730 ± 50 s.d. ms after the mouth starts opening).

Observation of the dead specimens revealed that the morphology of the spiracles of the embryo and the newborn juveniles is different from those of adult individuals. In the embryo and the newborn juveniles, the spiracle opening is elliptical [figure 2a], with a spiracular valve just inside. In contrast, the spiracle opening of adult specimens is not elliptical but slit-shaped, sometimes almost completely closed [figure 2b]. The difference in spiracular morphology between young and adult is also confirmed in several photographs in the previous literatures [2,10]. Proportions of the spiracle opening decrease ontogenetically (figure 2c).
Antero–posterior widths of the spiracle opening of the embryo and newborn juveniles are 0.7–1.1% of the disc width, whereas those of adults are 0–0.3%. Considering that the reduction of the spiracles occurs between 2.0 and 3.0 m in disc width, the spiracles are closed in a relatively short period after birth. The oral valves are well developed along maxilla and mandibula. The maxillary and mandibular valves touch each other when the maxillary valve fully rises during the compression phase of buccal cavity.

4. DISCUSSION

The buccal-pumping behaviour of the manta ray embryo is probably related to the oxygen-transfer strategy from mother to embryo. Unlike the embryos of mammals or some carcharhinid sharks that respire through a placenta, the manta ray embryo does not have a direct connection with the mother’s body [2,6,7]. Therefore, the only available source of oxygen is the uterine fluid surrounding the embryo. The historical studies on the uterus of the cownose ray (Rhinoptera bonasus) and the southern stingray (Dasatias americana), which have a similar uterine structure to the manta ray, suggest that oxygen would be supplied to the uterine fluid from the surface of the uterus by diffusion [3,4]. Viviparous elasmobranchs show wide variation in the method of embryonic nutrition [6]. However, the embryonic respiratory behaviour observed in the manta ray is probably widely shared among viviparous elasmobranchs, because embryos of many viviparous elasmobranchs do not have a direct connection with the mother. The embryonic respiratory behaviour of the manta ray resembles those of oviparous (egg-laying) species; the embryo of oviparous elasmobranchs actively takes in water into the mouth by buccal-pumping [11]. The water in the egg case is taken in from the external environment through the slits on the sides of the egg-case by the embryo’s tail fanning [11,12]. However, unlike the water in the egg case of oviparous elasmobranchs, the uterine fluid of the viviparous manta ray is not only the source of oxygen, but also the source of nutrients. Considering the fact that the intestine of a stillborn giant manta was filled with uterine fluid, the manta ray embryo probably drinks uterine fluid for nutrition as well.

The manta ray embryo shows several morphological features associated with this buccal-pumping behaviour. The manta ray embryo has well-developed spiracles and breathing valves (oral and spiracular valves). These structures are used for the generation of a respiratory current in benthic sharks and batoids [13,14]: In these animals, water is drawn into the buccal cavity via the mouth and/or spiracles by the expansion of the buccal cavity, and the oral and spiracular valves prevent water from exiting through the buccal cavity during the increase in oral pressure [13,14]. In fact, the oral valve of the manta ray embryo does not move during the expansion phase of the buccal cavity, whereas it rises during the compression phase. Interestingly, the spiracles of the manta ray become rapidly reduced after birth. This phenomenon probably reflects the fact that the manta ray switches its main respiratory system from buccal-pumping to ram-ventilation. The adult manta ray is known to funnel water into the buccal cavity by continuously swimming with its mouth open [14]. In general, during ram-ventilation, the spiracle does not function as an inlet for respiratory current, thus the spiracles are greatly reduced or lost in many active swimmers (e.g. lamniform sharks and carcharhinid sharks) [15]. According to the observation on the newborn manta ray in Okinawa Churaumi Aquarium in 2009, the shift in respiratory system in the manta ray might occur within a few days after birth.

Consequently, the manta ray embryo shares several features with the respiratory systems of both placental viviparity and oviparity; the uterus of the manta ray has the capability to supply oxygen to the embryo in the same way that the placenta does in viviparous mammals, but, because of the lack of a direct connection with the mother, the manta ray embryo must take in the surrounding fluid in the same way as embryos of egg-laying species do. This finding extends our understanding of the diversity in embryonic respiratory systems among live-bearing vertebrates.

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