

## SHORT COMMUNICATION

**Anembryonic Gestation in Wild South American Sea Lion, *Otaria flavescens***M. F. Grandi<sup>1\*</sup>, E. A. Crespo<sup>1,2</sup> and S. L. Dans<sup>1,2</sup>

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With 3 figures and 1 tables

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**Summary**

We present the first record and description of an anembryonic gestation in a wild South America sea lion, *Otaria flavescens* (Carnivora, Pinniped). This is the first report of an anembryonic gestation in a wild marine mammal species. This description furthers the knowledge of general aspects of the reproduction of an otariid species, which presents the particularities of delayed implantation and polygynic breeding system, and adds information on a reproductive abnormality in marine mammals.

In humans, anembryonic gestation or empty sac (formerly called blighted ovum) is an abnormal pregnancy, where there is a first-trimester intrauterine gestational sac/placenta with the absence of an embryonic pole, or there is minimal embryonic debris without heart rate activity (Farquharson et al., 2005; Casikar et al., 2010). It occurs as a result of early embryonic death with continued development of the trophoblast. Miscarriages from an empty sac are mainly due to chromosomal or structural abnormalities of the zygote (Hakvoort et al., 2006), and this abnormality is one of the main causes for human pregnancy loss (Hsu, 1990; Pandya et al., 1996).

This type of abnormality was studied in some farm and domestic mammalian species (i.e. James et al., 1976; Hsu, 1990; Vanderwall et al., 2000; Pomar et al., 2005; among others). Nevertheless, such abnormal development has not yet been documented in wild species.

Among marine mammals, pinnipeds are seasonal breeders, and their oestrous cycles are synchronized by parturition (Atkinson, 1997). Pinnipeds present an annual reproductive cycle where the complete gestation last 12 months and comprises an embryonic diapause (a delay in implantation of the fertilized egg, which can last for 3–5 months) and a placental gestation of around 9 months (Boshier, 1981; Boness, 2009). After ovulation and fertilization, obligate delayed implantation is characterized by differentiation of the zygote up to the blastocyst stage. Further differentiation of the blastocyst then stops and blastocyst is free-floating in the uterine horn for the

period of dormancy (Boshier, 1981). During delayed implantation, pregnancy is indicated, macroscopically, by the presence of a *corpus luteum*, and increased diameter of a uterine horn. Usually, implantation of the blastocyst occurs in the uterine horn corresponding to the ovary containing the *corpus luteum* (ipsilateral). Once implanted, the blastocyst begins a process of differentiation of tissues and organs, and remains in the uterus for the duration of its foetal phase (Stewart and Stewart, 2009).

The South American sea lion, *Otaria flavescens*, is a sexually dimorphic and polygynous species that forms breeding colonies during the austral summer (December–March) when mating and pupping activities occur (Vaz-Ferreira, 1982; Cappozzo and Rosas, 1991; Campagna, 1985; Campagna and Le Boeuf, 1988). During this period, females give birth to a single pup, copulate with territorial males 6–8 days after parturition and are pregnant for another year (Vaz-Ferreira, 1950; Cappozzo and Perrin, 2009). Even though there are several studies of breeding behaviour and breeding system of *O. flavescens*, there is a lack of information related to some aspects associated with reproduction (e.g. embryonic diapause, embryonic implantation; see Cappozzo and Perrin, 2009; Grandi et al., 2012). Therefore, for the purpose of increasing the knowledge of Otariid reproduction, the aim of this note is to record and describe an anembryonic gestation in a wild South America sea lion.

On 26th June 2009, a female South American sea lion was found dead in Punta Norte (42.07°S; 63.78°W)

Península Valdés, Chubut Province, Argentina. The animal presented a small external abdominal hole that corresponded with diaphragm, intestine and liver perforated, and with an internal bleeding. That the specimen was, fortunately, in fresh condition facilitated performance of a complete necropsy in the field. Standard body length (SL) was measured to the nearest centimetre using standard methods (Committee on Marine Mammals, 1967), giving a total length of 156 cm. The animal was in poor body condition, with the thickness of ventral blubber varying between 4 and 5 mm. Individual age was estimated from counts of incremental growth layers in the dentine of canine teeth. A sagittal thin ground section of the left maxillary canine was obtained and polished with wet sandpaper (Crespo, 1988; Grandi et al., 2010). Growth layer groups (GLGs) were observed with a stereomicroscope ( $\times 30$ ) under transmitted light. For this species, 1 GLG represents a 1-year period (Laws, 1962; Crespo, 1988; Rosas et al., 1993; Grandi et al., 2010). The animal was an adult female, 14 years old. The reproductive tract was excised and preserved in 10% formaldehyde solution. Once in the laboratory, ovaries were measured, weighed (Table 1) and then sliced parallel to the attachment of the ovarian ligament at 2-mm intervals. A *corpus luteum* and a *corpus albicans* were noticed in the right ovary, and two *corpora albicantia* were observed in the left ovary. Measurements of these ovarian corpora were taken on preserved tissues using a vernier calliper (Table 1). Paraffin-embedded samples of the main corpora were sectioned and stained with hematoxylin and eosin for microscopic analysis.

On an external inspection, the right uterine horn presented a dorsal thickening of 28.7 mm about midway between the external junction of the cornua and the uterotubal junction. This uterine horn was dissected longitudinally along the anterior aspect, and an empty sac was found (Fig. 1a).

The blighted ovum was removed and observed with a stereomicroscope ( $\times 30$ ), perceiving that there appeared

Table 1. Length, width, depth, weight and record of corpora from the ovaries of the *O. flavescens* studied

	Left ovary	Right ovary
External measurements (mm)*	28.55 $\times$ 20.80 $\times$ 18.40	34.65 $\times$ 18.70 $\times$ 14.40
Weight (g)	6	7.5
<i>Corpus luteum</i> (mm)**	–	14.86 $\times$ 13.16
<i>Corpora albicantia</i> (mm)**	9.80 $\times$ 6.87 4.61 $\times$ 2.94	3.27 $\times$ 1.90

\*Measured along three axes. \*\*Two corpora albicantia in left ovary and a corpus luteum and a corpus albicans in right ovary were noticed. Measurements are length  $\times$  width.

to be a yolk sac, with vitreous consistency and a maximum diameter of 30.25 mm, but no tissue of embryo proper (Fig. 1b). There were neither development of other embryonic membranes, such as amnion or allantois, nor placental scars or tissues attached to the uterine horn wall (Fig. 1b). Hsu (1990) recognized several types of abnormalities in the development of *in vitro* cultured mice embryos. Following Hsu's (1990) criteria, the description of the specimen studied is consistent with a Group 2 category of abnormal development: an empty yolk sac without tissue of embryo proper (similar to an empty chorion only in human, see Hsu, 1990). In mice, an empty sac results from an abnormal development, where embryoblasts disappear from the conceptus while the remaining extra embryonic membranes (chorion or yolk sac) continue to develop as fast as normal development (Hsu, 1990).

When we compare the specimen found with other records of stranded South American sea lion females with normal pregnancies (Grandi, 2010; Grandi et al., 2010), it was evident that the *corpus luteum* observed in the right ovary corresponded to an active *corpus luteum*. Also its average diameter was slightly smaller than *corpus luteum* of pregnancies found the same month of the year (Fig. 2a).

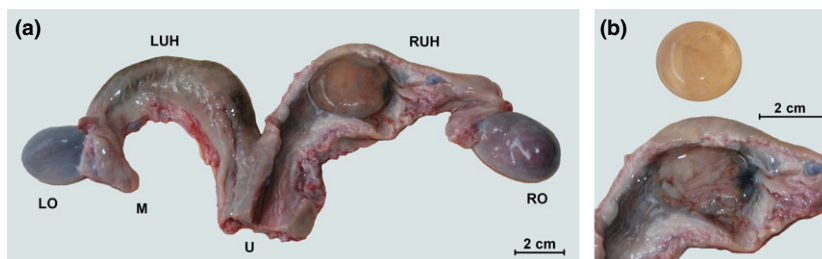


Fig. 1. (a) Photograph of the female reproductive organs of an *Otaria flavescens* dissected, showing the position of the blighted ovum. LO, left ovary; M, mesosalpinx; LUH, left uterine horn; U, uterine body; RUH, right uterine horn; RO, right ovary. (b) Photograph detailing the blighted ovum and the opened right uterine horn of the specimen studied.

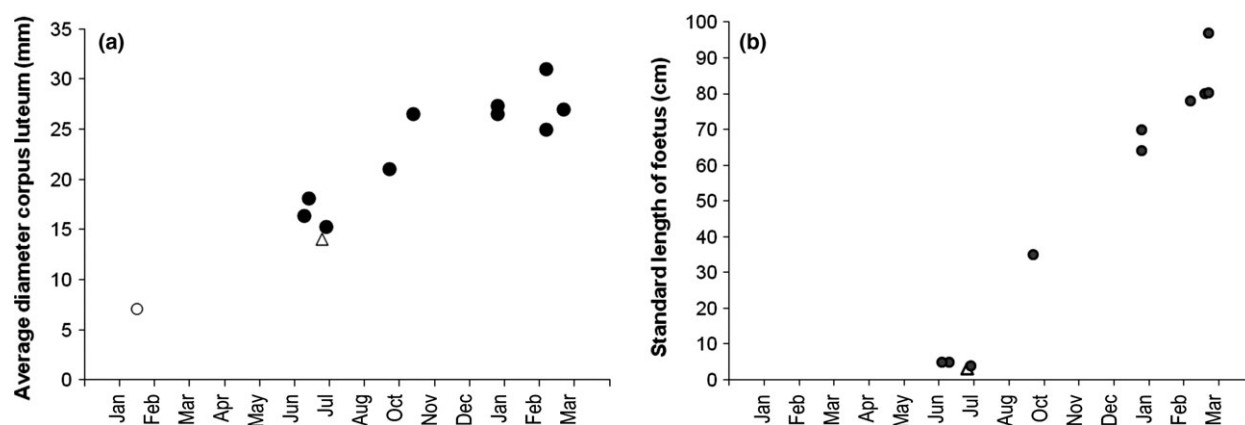


Fig. 2. (a) Average diameter of *corpus luteum* of the specimen studied ( $\Delta$ ), *corpus luteum* of normal pregnancies of *O. flavescens* by month ( $\bullet$ ), and average diameter of ovulatory main antral follicles ( $\circ$ ). (b) Standard length of foetus of *O. flavescens* by month ( $\bullet$ ) and empty sac size found ( $\Delta$ ) (data of stranded South American sea lion females with normal pregnancies,  $n = 11$  from Grandi, 2010).

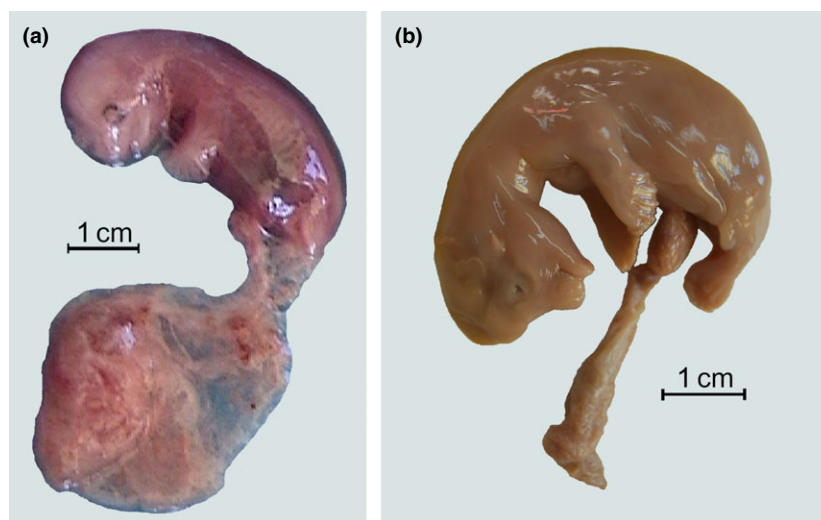


Fig. 3. Photographs of *O. flavescens* foetuses from 9 June 2002 (a) and 30 June 2008 (b) with part of their umbilical cord.

Considering that the median of mating occurs during the third week of January (1 week after the peak of births, Campagna and Le Boeuf, 1988) and diapause lasts 3 months in this species (Cappozzo and Perrin, 2009), cessation of diapause and embryo implantation in a normal pregnancy would begin in late April, during the southern decreasing photoperiod. By the time the studied specimen was found (end of June), a foetal size of approximately 4 cm would be expected in a normal pregnancy (Fig. 3a,b). This is similar to the ovum size found (Fig. 2b). Considering the size and activity of *corpus luteum* found in the ipsilateral ovary, and the size of the empty sac, it is likely that in the studied animal, the development of a normal pregnancy began (with trophoblast growth), but no further development of the embryo or placental implantation took place.

This is the first record and description of an anembryonic gestation for pinnipeds and, to the best of our knowledge, it is the first report of an anembryonic gestation in a wild marine mammal species. The present description represents a contribution to the knowledge of general aspects of the reproduction of an otariid species, and a record of a reproductive abnormality in marine mammals.

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