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Early embryo development in the elephant assessed by serial ultrasound examinations

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Abstract

The elephant has an extraordinary long pregnancy, lasting 21 months. However, knowledge on embryo development is limited. To date, only single morphological observations of elephant embryo development associated with placentation are available, all lacking correlation to gestational age. The present study describes morphological characteristics of early embryo development in the elephant with exact biometric staging. Six pregnancies in five Asian and one African elephants with known conception dates were followed by 2D and 3D ultrasound, covering the embryonic period from ovulation to day 116 post-ovulation. The embryonic vesicle was earliest observed was on day 50 p.o. The proper embryo was not detected until day 62 p.o. Embryonic heartbeat was first observed on day 71 p.o. The allantois, which became visible as a single sacculation on day 71 p.o. was subdivided in four compartments on day 76 p.o. By day 95 p.o., head, rump, front and hind legs were clearly distinguished. Between days 95 and 103 p.o. the choriovitelline placenta was replaced by the chorioallantoic placenta. A physiological midgut herniation was transiently present between days 95 and 116 p.o. On the basis of the late appearance of the embryonic vesicle, delayed implantation in the elephant is discussed. The study provides a coherent description of elephant embryonic development, formation of the extraembryonic organs and their role in placenta formation, all of which are of interest for both comparative evolutionary studies and the improvement of assisted reproduction techniques.

Keywords: Elephant; Reproduction; Embryogenesis; Ultrasound; Extraembryonic organs

1. Introduction

The situation of the African (*Loxodonta africana*) and Asian elephant (*Elephas maximus*) in their respective native countries is quite different. In Asia, the ever-growing human population repels the wild-elephant population to restricted areas. To ensure their survival, wild-elephant herds are thus forced to raid crops, causing severe conflict with man. Furthermore, natural habitats are more and more fragmented, separating subpopulations and impeding genetic exchange [1].

From historical reports it is known that the African elephant once inhabited the whole continent [2]. Extensive ivory trade dating back as far as in Roman times [3], expanding human settlements [4] and civil wars [5] are associated with a decline of the African elephant population. Elephants have long vanished from North Africa and populations are greatly diminished in West, Central and East Africa [6].

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In Southern Africa, where the elephant population had almost gone extinct due to ivory poaching in the 19th century, numerous National parks have been founded to save the species. Here, elephant numbers in protected areas continuously build up and lead to local overpopulations. In the Kruger National Park, an estimated population of 10 individuals in 1908 augmented to approximately 6500 elephants 60 years later [7]. According to the South African National Parks Board, this number overstrained the natural capacities of the park. In 1965 it was decided to limit the elephant population to 7000 animals. In the following 18 years, about 16,200 elephants were culled. Due to political and public pressure, the culling policy was abandoned in 1994. Since then, elephant numbers in the park increase by 7% per annum and culling is reconsidered [8].

In captivity, breeding success is still insufficient to come by Wiese reported that without imports from the wild or dramatically improved fecundity, the captive Asian elephant population in North America will decline to 10 individuals in 50 years [9]. The prospect for the captive North American African elephant population, with a decline of 2% per year, is almost equally alarming [10]. Reasons for poor reproduction in captivity are numerous. Proven breeder bulls are rare and the majority of captive females that were once imported from the wild are now post reproductive age. To improve captive breeding, ultrasound guided artificial insemination was developed [11–13].

In light of the elephant situation in the wild and in captivity it is obvious that more knowledge about its reproductive biology is needed. Our understanding of its embryogenesis in particular will enhance our abilities to manage *in situ* and *ex situ* populations appropriately.

Foetal specimens collected during culls provided for our knowledge on elephant prenatal development [14] and placentation [15–18]. However, the age of the specimens described was not known and the development of the embryo could not be correlated to the development of its extraembryonic organs and placenta formation. Since gestational age was not known, specimens were classified according to their body mass [19,20]. Growth curves derived from newly published data showed a systematic error in previously published growths graphs of up to 60 days [21].

Transrectal ultrasound was already employed in field contraception studies conducted between 1996 and 1998 in the Kruger National Park in South Africa [22]. Using ultrasound, the reproductive status of the elephant was determined prior subcutaneous implantation of contraceptive hormones to avoid hormone treatment of pregnant animals. However, 3 of 57 animals that were diagnosed as non-pregnant and treated with contraceptives turned out to be pregnant when a second ultrasound exam was performed 1 year later. These preliminary findings suggested a delayed implantation in the elephant and prompted us to conduct further study of elephant embryo development presented here. This study aimed to describe the early embryonic development in elephants as seen by transrectal 2D and 3D ultrasound in correlation with gestational age with a special focus on depicting the topographic relationship of the extraembryonic organs and their function in placental formation.

2. Materials and methods

2.1. Elephants

Five Asian elephants (*E. maximus*) and one African elephant (*L. africana*) were examined (Table 1). All elephants were kept in free contact management setting. Gestational age in the six females was known from artificial insemination ($n = 1$) or observed mating ($n = 5$) and corresponded with LH and progesterone measurements [23,24]. In addition to hormonal data, the rupture of the leading follicle was monitored by ultrasound. The day of ovulation was defined as day 0 of gestation.

Table 1
Pregnant elephants examined by 2D and 3D ultrasound

Scanned elephant	Species	Studbook #	Institution	No. of exams	Gestational age (days p.o.)
Elephant 1	La[a]	143	Indianapolis Zoo, Indianapolis, USA	4	37, 44, 58, 62
Elephant 2	Em[b]	356	African Lion Safari, Cambridge, CA	12	67–112
Elephant 3	Em	264	African Lion Safari, Cambridge, CA	17	12–115
Elephant 4	Em	347	African Lion Safari, Cambridge, CA	28	38–116
Elephant 5	Em	8403	Whipsnade Wild Animal Park, GB	3	74, 89, 116
Elephant 6	Em	424	African Lion Safari, Cambridge, CA	46	52–116

[a] African elephant (*Loxodonta africana*).
[b] Asian elephant (*Elephas maximus*).