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Early primary-rather than primordial follicles constitute the main follicular reserve in the African elephant (*Loxodonta africana*)

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ABSTRACT

Information on the ovarian follicle reserve in the African elephant (*Loxodonta africana*) is lacking. This study set out to determine the ratios of early preantral follicles and their relative dimensions in the ovaries of 16 African elephant aged 10–34 years. The ovaries were sectioned histologically. Follicles were counted and classified according to expansion of the pre-granulosa cells. Early primary follicles were the most common (75.8% ± 11.8%), followed by true primary follicles (23.8% ± 11.8%), whereas primordial follicles were the most rare (<2%). Measurements made on at least 100 early preantral follicles from each animal ($n = 1464$) indicate that growth in oocyte and nuclear diameters started with transition to the true primary stage $P < 0.01$. This, together with the observed ratios between the three types of early preantral follicles suggest that both classical primordial and early primary follicles contribute to the ovarian reserve in the African elephant.

1. Introduction

For the management of the African elephant (*Loxodonta africana*), methods of contraception as well as assisted breeding are required (Brown et al., 2004; Delsink, 2006). The duration of the female elephants reproductive life is about 50 years (Perry, 1953; Freeman et al., 2009). Along with humans and whales they have the longest reproductive lifespan of mammals, requiring individual oocytes to remain meiotically competent for over 40 years (te Velde and Pearson, 2002) which exposes them to prolonged arrested development and structural damage (Faddy and Gosden, 1995).

The last culling of wild elephant took place in Kruger National Park in South Africa in 1995 (Whyte, 2004). Since then there has been limited reproductive physiological research carried out on wild elephant, although endocrinological, ultrasound and behavioural studies have continued in captive elephant. Despite well-meaning efforts and investment, these animals are kept in unnatural environmental and social conditions and are showing worrying levels of acyclicity, particularly in older animals. In North American zoos the level of acyclicity/irregular cyclicity increased significantly between surveys in 2002 and 2005 (Proctor et al., 2010). It therefore becomes important to obtain biological samples from wild elephant populations and study their natural reproductive cycle when the opportunities arise.

Mammalian maternal ageing is accompanied by reduced oocyte numbers (Faddy et al., 1992), increased oocyte aneuploidy (te Velde and Pearson, 2002) and a progressive loss of ovarian follicles (Picton, 1998). Once oocytes have been lost from the ovarian reserve there is little or no renewal and, according to present dogma, the ensuing infertility is irreversible (Gosden, 2004). In 2004 interest was rekindled in neo-oogenesis following experiments in mice (Johnson et al., 2004; Johnson, 2005) and the debate continues (Tilly and Johnson, 2007; Begum et al.,

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2008; Bukovsky et al., 2009; Gougeon, 2010). Knowledge of the ovarian follicle reserve in the elephant may form the basis for investigations on the cause of early reproductive failure (Brown et al., 2004), reproductive senescence (Freeman et al., 2009) and the effect of contraceptives on the follicle pool (Perdok et al., 2007).

Primordial follicles have been described as the most abundant follicle in the ovary and are commonly referred to as the building blocks of the ovarian reserve (Picton, 2001), supplying the female with oocytes throughout reproductive life. Studies in rats (Hirshfield, 1989; Oktay, 1995), cattle (Wandji et al., 1996; Braw-Tal and Yossefi, 1997), humans (Picton, 2001) and other mammals (Fortune et al., 2000) have shown that the transition from flattened pre-granulosa cells to cuboidal granulosa cells and the accompanying rounding-up of the nuclei signals that the follicle has left the ovarian reserve and is irreversibly committed to growth that may end in ovulation or atresia. The resting pool of follicles, however, may have pre-granulosa cells that range from flat to cuboidal in shape (Lintern-Moore et al., 1974; Gougeon and Chainy, 1987; Hirshfield, 1992; Faddy and Gosden, 1995; Van Wezel and Rodgers, 1996; Meredith et al., 2000; Moss, 2001; de Bruin et al., 2002; Sawyer et al., 2002; Rodgers and Irving-Rodgers, 2009). The biological consequences of the differences in somatic cell morphology between species is unclear and very little is known about the follicle population dynamics in long-lifespan species such as elephants. Hence, the four aims of the current study were; (i) to compare the dimensions of early pre-antral follicles at different stages of development; (ii) to compare the numbers of early pre-antral follicles at different stages of development; (iii) to determine the relative abundance of early pre-antral follicles at different stages of development and; (iv) to determine the type of early preantral follicle constituting the follicular reserve in sub-adult and adult wild African elephant.

2. Materials and methods

2.1. Tissue collection

The ovaries of 14 African elephant cows shot by professional hunters working under annual authorization granted by The Parks and Wildlife Management Authority of Zimbabwe were used to measure and count early preantral follicles at different stages of development. Subsequently, the ovaries of another two elephant, sourced in the same way as the other 14, were used in follicle counts only. Fifteen of the 16 animals were tuskless. The ovaries were recovered within 3 h of death, bisected and immersed in 4% buffered formalin (Zimvet, Harare, Zimbabwe) in a labelled 650 ml wide-necked glass jar. The mandible of each animal was also collected in order to estimate its age (Laws, 1966; Jachmann, 1988).

2.2. Tissue analysis

The fixed ovaries were weighed whole to the nearest 0.1 g using a Mettler AJ 100 scale, and again after any large corpora lutea (Fig. 1a) were removed by careful dissection.

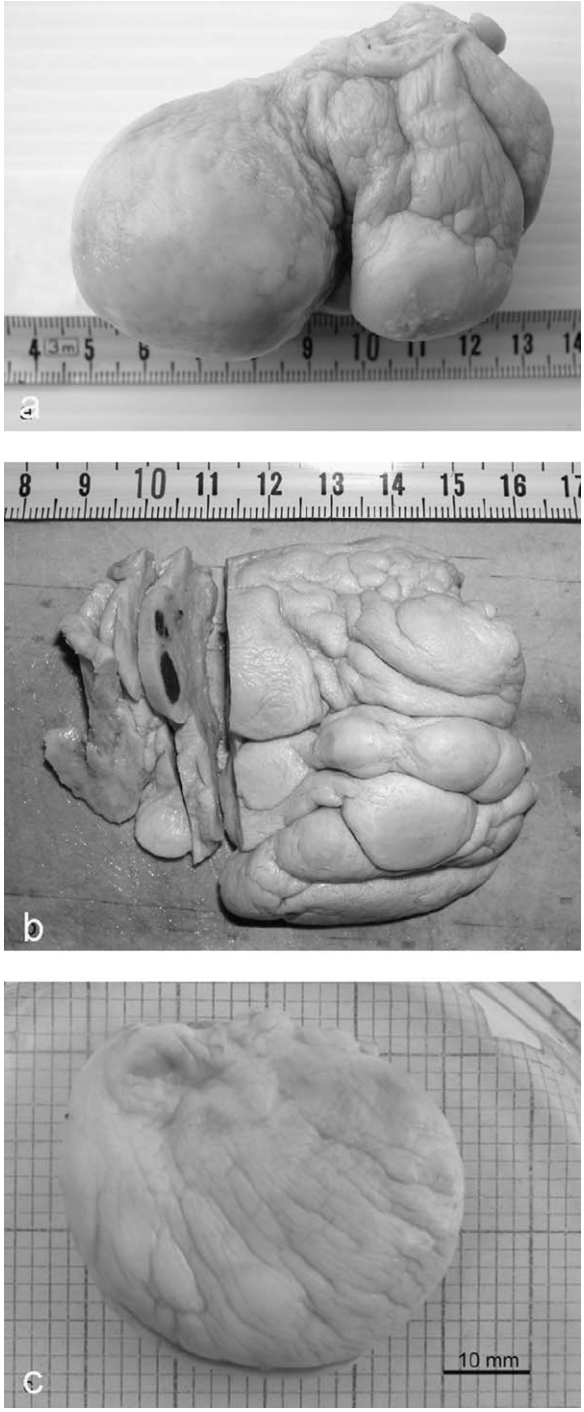

Fig. 1. Examples of elephant ovaries (a) from a pregnant elephant showing the large corpora lutea that develop during pregnancy, (b) from a non-pregnant elephant showing a cut segment revealing two corpora rubra, and (c) from a pre-pubertal elephant demonstrating the smooth and regular ovarian surface.