Ontogeny of the ovarian follicular reserve of the

African elephant (*Loxodonta africana*)

By

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### List of Abbreviations

2n2c  Two chromosomes, 2 DNA strands, the genetic constitution of oogonia

2n4c  Two chromosomes, 4 DNA strands, the genetic constitution of primary oocytes

3β-HSD 3β-hydroxysteroid dehydrogenase

5α-DHP 5α-dihydroprogesterone

AMH  anti-mullerian hormone

Ap  Area associated with a point

BCL  B cell lymphoma/leukemia

BV  Biological variation

CE  Coefficient of error

CITES  The Convention in International Trade in Endangered Species

CL  One corpus luteum or more corpora lutea, as would be clear from the context

CN  Corpora nigra

CRL  Crown rump length

CV  Coefficient of variation

D  Dimension, eg. 0–D or 3–D

D1  Diameter 1

D2  Diameter 2

E  For example, E80, embryonic day 80

elPL  Elephant placental lactogen

EP  Early primary follicle, early primary follicles, or the early primary stage of development of a follicle, as would be clear from the context

FGF  Fibroblast-like growth factor

Fig  Factor in germline

FOV  Fields of view

FOX  Forkhead box
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GDF</td>
<td>Growth differentiation factor</td>
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<tr>
<td>GSC</td>
<td>Germline stem cells</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>HEC</td>
<td>Human elephant conflict</td>
</tr>
<tr>
<td>IMS</td>
<td>Industrial methylated spirits</td>
</tr>
<tr>
<td>KIT</td>
<td>Tyrosine protein kinase</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinising hormone</td>
</tr>
<tr>
<td>LIF</td>
<td>Leucocyte inhibitory factor</td>
</tr>
<tr>
<td>M</td>
<td>Molar tooth eg MII or MVI</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>n</td>
<td>Number of a sample</td>
</tr>
<tr>
<td>NGF</td>
<td>Non-growing follicle</td>
</tr>
<tr>
<td>NP</td>
<td>National Park</td>
</tr>
<tr>
<td>Nv</td>
<td>Number in volume</td>
</tr>
<tr>
<td>Oct4</td>
<td>Octamer binding transcription factor 4</td>
</tr>
<tr>
<td>OSE</td>
<td>Ovarian surface epithelium</td>
</tr>
<tr>
<td>PAC</td>
<td>Problem animal control</td>
</tr>
<tr>
<td>pZP</td>
<td>Porcine zona pellucida</td>
</tr>
<tr>
<td>P</td>
<td>Value of statistical significance</td>
</tr>
<tr>
<td>P13k</td>
<td>Phosphotidylinositol 3 kinase</td>
</tr>
<tr>
<td>PGC</td>
<td>Primordial germ cell</td>
</tr>
<tr>
<td>sec</td>
<td>Seconds</td>
</tr>
<tr>
<td>SF</td>
<td>Small follicle or small follicles, as would be clear from the context</td>
</tr>
<tr>
<td>SVC</td>
<td>Savé Valley Conservancy</td>
</tr>
<tr>
<td>Σ</td>
<td>Sum of</td>
</tr>
<tr>
<td>t−</td>
<td>Segment thickness</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>TP</td>
<td>True primary follicle, true primary follicles or a follicle that is at the true primary stage of development, as would be clear from the context</td>
</tr>
<tr>
<td>TPM</td>
<td>True primordial follicle, true primordial follicles, or a follicle that is at the true primordial stage of development, as would be clear from the context</td>
</tr>
<tr>
<td>UCF</td>
<td>Unbiased counting frame</td>
</tr>
<tr>
<td>Vol.dis</td>
<td>Volume of disector</td>
</tr>
<tr>
<td>Vref</td>
<td>Reference volume</td>
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SUMMARY

Ontogeny of the ovarian follicular reserve of the African elephant (*Loxodonta africana*)

By

FIONA JANE STANSFIELD

Promoter: Professor J O Nöthling

Co-promoter: Professor J Soley

Department: Production Animal Studies

Degree: PhD

The aim of this study was to define the ovarian follicular reserve of wild African elephants in terms of its type of small follicles (SF), its establishment and distribution throughout the ovaries, and the change in numbers of SF in the embryo and fetus as well as throughout prepubertal and adult life.

The large elephant population in Zimbabwe provided the opportunity to collect ovaries from elephants culled for management reasons and hunted professionally. In total, gross morphological and histological studies were done on the gonadal ridges from 5 embryos (76–96 days post conception) and ovaries from 11 fetuses (4.8–22.2 months), 29 prepubertal females (2 months–10 years), 24 adult females (11–55 years) and 7 aged females (56–70 years). Specimens were fixed in 4% buffered formalin before a series of 25 µm thick sections were cut and examined using stereological protocols to count SF numbers in each section and thereby calculate the follicle reserve of the whole ovary. Prior to counting SF numbers, their distribution throughout the ovary was studied and the repeatability of counts was validated.

Numbers of SF were highest in mid-term fetuses, lower in fetuses during the second half of gestation, even lower in calves younger than 4½ years, whereas the numbers in calves aged 4½–9 years were significantly higher than those in younger calves, and similar to
what they were in late-term fetuses. The numbers of SF were substantially and highly significantly lower in elephant 10–15 years in age compared to calves aged 4½9 years, suggesting a reduction around puberty. Thereafter the ovarian reserve fell steadily until depletion around the age of 70 years. During adult life the ovarian reserve was composed of early-primary (EP) and true-primary (TP) follicles. By 45 years of age only TP follicles remained although these enabled oestrous cyclical activity for many more years; of 7 sets of ovaries recovered from females aged 57–70 years, 6 showed evidence of cyclical activity or pregnancy within the preceding 6 years.

The study shows that EP and TP form the follicular reserve from before birth until 45 years, with TP forming the reserve thereafter, which depletes in some old elephants and persists to maximum life span in others.