Placentation in the African elephant, *Loxodonta africana*. IV. Growth and function of the fetal gonads

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Abstract

The gonads, both ovaries and testes, of 44 elephant fetuses weighing 0.09–112 kg (6.1–21.3 months gestation) were examined grossly and histologically. As in equids, elephant fetal gonads undergo a phase of marked growth and enlargement during the second half of gestation, which is more pronounced in ovaries than testes due to growth and antrum formation of numerous follicles in the former. Stromal cells undergo hypertrophy and transformation to form zones of interstitial cells that are associated with the enlarged follicles in the ovaries and in which the primitive seminiferous tubules are embedded in the testes. The interstitial cells have the capacity to synthesize 5α-dihydroprogesterone and other 5α-reduced progestagens from cholesterol and pregnenelone and the hypothesis is raised that these fetal gonadal progestagens may supplement significantly the progestagens secreted by the multiple large corpora lutea of pregnancy in the elephant.

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Introduction

Interstitial gland cells, which possess the organelles associated with steroid hormone synthesis (Davies & Broadus 1968, Christensen & Gillim 1969), are present in the gonads of many mammalian fetuses, including humans and equids (Mossman & Duke 1973). In the mare these interstitial cells undergo tremendous hypertrophy and hyperplasia during the second half of gestation (Gonzalez-Angulo et al. 1975, Hay & Allen 1975), causing the gonads themselves, both ovaries and testes, to enlarge greatly and then regress again in the final stages of pregnancy (Cole et al. 1933, Amoroso & Rowlands 1951, Allen 1970). The interstitial cells secrete large quantities of androstenedione, dehydroepiandrosterone and other 7α-hydroxylated C19 steroids (Pashen & Allen 1979, Tait et al. 1983, 1985), which the placenta then aromatizes to the common phenolic oestrogens, oestrone and oestriol, and the equine-specific ring β unsaturated oestrogens, equilin and equilenin (Bhavnani et al. 1969, 1971, Bhavnani & Short 1973a, 1973b). These oestrogens are present in high concentrations in the blood and urine of pregnant equids between 100 and 320 days of gestation (Cox 1975, Raeside & Liptrap 1975).

The gonads of the elephant fetus undergo a similar interstitial cell-driven enlargement during the second half of gestation (Perry 1964, Hanks 1971) and these cells have been shown recently to possess the steroid synthetic enzymes, P450 side-chain-cleavage enzyme (SCC 450) required for the conversion of cholesterol to pregnenolone, and 3β-hydroxysteroid dehydrogenase (3βHSD) involved in the metabolism of pregnenolone to progesterone and other progestagens (Allen et al. 2002). Furthermore, elephant fetal gonad tissue recovered after the tenth month of gestation metabolized tritium-labelled cholesterol and pregnenelone to 5α-dihydroprogesterone and other 5α-pregnane derivatives in vitro (Allen et al. 2002).

An opportunity was afforded to examine the phenomenon of gonadal enlargement in the elephant fetus more closely in 44 fetuses recovered from pregnant elephant culled for management reasons in South Africa. This paper describes the morphological changes in these fetal ovaries and testes throughout gestation and discusses the possible role(s) of the fetal gonads in the maintenance of pregnancy in this species.

Materials and Methods

Collection of fetal gonads

For 2 weeks in each of three successive years (1993–1995 inclusive) the authors joined the annual
management-based cull of elephant in the Kruger National Park in the Western Transvaal region of South Africa. One complete elephant family, consisting usually of an aged matriarch, between four and six of her adult daughters and their respective calves, was shot each day and the entire reproductive tract from each pregnant adult female was brought to a makeshift laboratory established at the edge of the culling area. Here the conceptus bulge was incised and, after recovering pieces of placenta and endometrium for histological and histochemical studies (Allen et al. 2002, 2003, Wooding et al. 2005), the fetus was removed from the uterus and weighed. A flank laparotomy incision was performed through which the testes or the ovaries plus attached uterus were removed. Most specimens were photographed before the gonads themselves were dissected free, trimmed of excess fat and other attached tissues and weighed individually on a Mettler PC 2200 pan balance (Mettler Instrument AG, Zurich, Switzerland).

**Histological preparation of tissues**

Small blocks (approximately 2 cm³) of tissue cut from one of each pair of gonads were fixed in Bouin’s fluid for histological examinations, snap-frozen in OCT Embedding Compound (Raymond Lamb, East Sussex, UK) in liquid nitrogen for immunocytochemical staining or immersed in cold PBS for steroid hormone synthesis and conversion experiments (Allen et al. 2002). The Bouin’s fixed samples were dehydrated by passing them through increasing concentrations of alcohol followed by xylene. They were embedded in paraffin wax and sectioned at 5–8 μm for staining with haematoxylin and eosin.

**Results**

**Gross features**

A total of 23 pairs of ovaries showing a mean individual weight of 0.24–35.2 g were collected from female fetuses that weighed 0.65–98.5 kg and were therefore calculated, using the equations designed by Craig (1984), to range in gestational age from 7.6 to 20.6 months (Table 1a, Fig. 1). Similarly, 21 pairs of testes with a mean individual weight of 0.063–22.5 g were recovered from male fetuses weighing 0.09–112 kg (6.1–21.3 months’ gestation; Table 1b, Fig. 1).

Both the ovaries and the testes exhibited a greyish/dark blue/brown coloration from the earliest stages of gestation examined (7.6 and 6.1 months respectively). The ovaries assumed a slightly kidney-shaped outline with the indentation occurring at the point of attachment of the ovary to the tip of the uterine horn (Fig. 2a), whereas the testes remained essentially spherical in shape throughout pregnancy (Fig. 3a). The gonads of both genders increased markedly in size from around the eleventh or twelfth month of gestation, with the growth spurt being more pronounced in the ovaries (e.g. 2.2 rising to 14.1 g between 11.3 and 13.5 months’ gestation; Table 1a) than the testes (e.g. 2.1 rising to 5.8 g between 12.3 and 13.7 months’ gestation; Table 1b). Thereafter, the ovaries grew rapidly to reach a maximum mean weight of 30–35 g, at around 16–17 months (Table 1a, Fig. 1) compared with a maximum mean testis weight of only 20–23 g, again around 16–17 months (Table 1b, Fig. 1). Beyond this stage of maximum growth, the ovaries showed a much more pronounced decline in size than the testes until the point was reached when the weights of the gonads of both genders levelled off and fluctuated around 15–23 g during the last 2–3 months of gestation (Fig. 1).

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many of the follicles throughout the cortex and medullary
notably, considerable enlargement and antral formation in
months; Table 1a) showed some marked changes. Most
agement or interstitial cell development at this early stage.
organ (Fig. 2c) and there was no sign of any follicular enlar-
follicles in the cortical region of the ovary. Cords of these
putative granulosa cells, to create primitive or primordial
large, lightly stained oocytes (Fig. 2c), most of which were
large domestic species. Namely, a dense accumulation of
ovaries of a late-stage fetus or newborn offspring of any
overall picture was much as one would expect to see in the
Histological findings
Fetal ovaries
At the earliest stage examined histologically (cull no. E70/95, 650 g fetus, 7.6 months’ gestation; Table 1a) the
interstitial cells at the periphery of the zones could be
degenerating.
Fetal testes
At the earliest stage examined histologically (cull no. E34/95, 90 g fetus, 6.1 months’ gestation; Table 1b) the fetal
testes already exhibited an organized arrangement of small
lobular accumulations of interstitial cells in which sparsely
arranged primitive seminiferous tubules were embedded,
with the lobes separated by loosely arranged fibrous trabec-
ulothelial transformation and hypertrophy of the fibroblast-like
stromal tissue, leaving only sparse trabeculae of normal
fibrous stroma scattered throughout the organ.
As gestation advanced to 10–18 months, in which the
fetal ovaries showed a more marked increase in growth
compared with the fetal testes (cull no. E74/95, 17 kg, 13.5 months; Table 1a), the enlarged antral follicles
appeared both bigger and more numerous within the ovar-
ian stroma and the zones of interstitial cells were now
much broader due to increased recruitment and hypertro-
phy of the interstitial cells. Most of the antral follicles
exhibited a thin wall (Fig. 2d) composed of a single layer
of primitive fibroblast-like cells but large oocytes were
occasionally seen within the follicles, each enveloped in a
typically multi-layered cumulus and usually attached to a
more multi-layered follicle wall.
Continuing through gestation towards term, the number
and size of enlarged antral follicles declined steadily from
around 18 months of gestation (Fig. 2f; cull no. E51/94, 98.5 kg, 19.9 months; Table 1a) and patches of lympho-
ncyte-like mononuclear cells could be seen infiltrating into
the spaces occupied by the previously enlarged antral fol-
licles (Fig. 2g; 98.5 kg). The interstitial tissue was still
the most prominent component of the ovarian stroma but, in
this latest stage ovary examined (cull no. E51/94, 98.5 kg),
interstitial cells at the periphery of the zones could be
clearly discerned to be degenerating.
regions of the ovary, interspersed with discrete zones of
mononucleate interstitial cells (Fig. 2d), which appeared
to be originating by hypertrophy and rounding up of the
fibroblast-like stromal cells (Fig. 2e). This enlargement
caused the interstitial cells to cluster tightly together in
distinct groups or zones between the enlarging follicles
(Fig. 2d). In this manner the interstitial cell zones and the
antral follicles quickly came to constitute the bulk of the
ovarian tissue, leaving only sparse trabeculae of normal
fibrous stroma scattered throughout the organ.

Whereas the fetal testes remained smooth in contour
throughout gestation (Fig. 3a), the ovaries developed a
markedly cobbled external appearance in mid-gestation
due to the enlargement and antrum formation in many fol-
licles in both the medullary and cortical regions of the
ovary (Fig. 2a and b). This external roughness tended to
smooth out and disappear in the final stages of gestation,
however, in parallel with a decline in the number and size
of antral follicles (Fig. 2f). It was evident that the faster
and more pronounced increase in weight of the ovaries
over the testes in mid-gestation, followed by the more
rapid decline in the former, was caused by the growth and
fluid accumulation in the antral follicles in the ovaries
followed by their regression and atresia (Table 1, Figs 1,
2a and 3a).

Histological findings
Fetal ovaries
At the earliest stage examined histologically (cull no. E70/95, 650 g fetus, 7.6 months’ gestation; Table 1a) the
overall picture was much as one would expect to see in the
ovaries of a late-stage fetus or newborn offspring of any
large domestic species. Namely, a dense accumulation of
large, lightly stained oocytes (Fig. 2c), most of which were
surrounded by a single layer of rather flattened follicle or
putative granulosa cells, to create primitive or primordial
follicles in the cortical region of the ovary. Cords of these
primordial follicles extended through the dense, fibrotic
ovarian stroma towards the central, medullary region of the
organ (Fig. 2c) and there was no sign of any follicular enlar-
gement or interstitial cell development at this early stage.
The next stage examined (cull no. E42/94, 7.4 kg, 11.3
months; Table 1a) showed some marked changes. Most
notably, considerable enlargement and antral formation in
many of the follicles throughout the cortex and medullary

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**Figure 1** The mean weights of 23 pairs of ovaries and 21 pairs of
testes recovered from elephant fetuses that ranged in gestational age
(calculated on the basis of fetal weight; Craig 1984) from 6.1 to 21.3
months. Note how the ovaries begin to enlarge earlier in gestation
and become appreciably bigger than the testes between 16 and 19
months.
seminiferous tubules within each lobe appeared to both multiply in number and become more tightly coiled, thereby increasing their density within the interstitial cell parenchyma (Fig. 3e; cull no. E33/95). As in the fetal ovaries, clusters of interstitial cells at the periphery of the lobes could be seen to be degenerating but, in contrast to the ovaries, this process did not appear to attract infiltrations of mononuclear cells.

Figure 2  (A) Grossly enlarged ovaries attached to the tips of the horns of a very underdeveloped uterus recovered from a fetus weighing 42 kg (cull no. E72/94, 16.7 months’ gestation; Table 1a). Note the cobbled appearance of the external surface of the ovaries (asterisks) due to the presence of enlarged antral follicles in the cortical region. Note also the cross-section of the parallel uterine horns (arrows). These extend almost down to the cervix to give a very small communicating uterine body. (B) Section of an ovary recovered from a fetus weighing 7.4 kg (cull no. E42/94, 11.3 months; Table 1a). Note the very lobular appearance of the parenchyma (asterisk) and the commencing development of antral follicles throughout the cortical and medullary regions of the organ. (C) High-power photomicrographs of a section of an ovary recovered from a fetus weighing 650 g (cull no. E70/95, 7.6 months; Table 1a). Cords of oocytes in the typically dense fibrous stroma of the outer cortex progress towards the more loosely arranged stroma of the medulla (scale bar, 90 μm). (D) Low-power photomicrograph of a section of an ovary recovered from a 7.4 kg fetus (cull no. E42/94, 11.3 months; Table 1a) showing enlarged antral follicles (asterisks), each of which is associated with a zone of pink-stained interstitial gland cells (scale bar, 30 μm). (E) Higher-power photomicrograph of (D) showing hypertrophy and transformation of the typically fibroblast-like stromal cells into the larger pink-staining interstitial cells (asterisks; scale bar, 90 μm). (F) Low-power photomicrograph of a section of an ovary recovered from a fetus weighing 60 kg (cull no. E74/94, 18.2 months; Table 1a) showing commencing degeneration and atresia of the previously enlarged follicles (asterisks; scale bar, 30 μm). (G) Medium-power photomicrograph of (F) showing an aggregation of lymphocyte-like mononuclear cells (arrowed) within and around the remnants of an atretic follicle (scale bar, 90 μm).
Discussion

This study confirmed and extended the earlier findings of Perry (1964) and Hanks (1971). Namely, in a similar manner to the horse and other equids, the gonads of both male and female fetal elephants undergo a marked growth and enlargement phase during the second half of gestation which is driven largely by the transformation and hypertrophy of slender fibrocyte-like stromal cells into much larger interstitial cells. These then become tightly packed...
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need for progestagens synthesized by the fetal gonads
the elephant fetus right up to term may reflect a biological
healthy interstitial cells in both the testes and ovaries of
be important in promoting fetal growth and maturation
unsaturated oestrogens that are present in the blood and
equilenin (Tait et al. 1983, 1985). These include androstenedione and dehydroepiandrosterone,
which are rapidly aromatized to oestrone and oestradiol
by the aromatase-containing placental tissue (Bhavani et al. 1969) and also unusual 7α-hydroxylated forms of
both these androgens which the placenta aromatizes to
the equine-specific ring B unsaturated oestrogens, equilin
and equilinen (Tait et al. 1983, 1985). In this way the equine fetal gonads perform a pivotal role in producing
the very high concentrations of both phenolic and ring B
unsaturated oestrogens that are present in the blood and
urine of the mare during the second half of pregnancy
(Cox 1975, Raeside & Liptrap 1975) and which appear to
be important in promoting fetal growth and maturation
and in preparing the myometrium and other uterine tissues
for parturition (Pashen & Allen 1979).

The persistence of sizeable accumulations of apparently
healthy interstitial cells in both the testes and ovaries of
the elephant fetus right up to term may reflect a biological
need for progestagens synthesized by the fetal gonads
(Allen et al. 2002) to be added to those coming from the
two to eight large plum-like corpora lutea which have
been observed to persist in the ovaries of the pregnant ele-
phant throughout gestation (Laws 1969, Hodges 1998,
Allen et al. 2003). In a recent elegant study, Meyer et al.
(2004) measured the profiles of progestagens, prolactin,
relaxin and cortisol in serial peripheral vein blood
samples recovered throughout pregnancy from 19 Asian
and 8 African elephants maintained in zoos across Amer-
ica. Individual progestagen profiles in both species
showed a pronounced fall and secondary rise around the
end of the first month of gestation, similar to that seen in
the pregnant mare around days 35–40 after ovulation in
conjunction with the occurrence of the first of what
becomes a series of secondary ovolutions stimulated by
the luteinizing hormone-like activity of equine chorionic
gonadotrophin secreted by the fetal endometrial cups
Thereafter in the pregnant elephant, progestagen levels
rose steadily to a peak around the fifth month of gestation,
remained relatively constant for the next 5–6 months and
then, at least in the African elephant, declined quite shar-
ply again to a lower plateau which was then maintained
until a sudden final drop 1–2 days before birth. It is inter-
esting to speculate that this late gestation decline in levels
may mirror some sort of degeneration and reduction in
secretory activity of the ovarian corpora lutea and the
replacement of the missing progestagens by those secreted
from the fetal gonads in the last months of gestation. Such
an hypothesis would be supported by the original obser-
vations made by Laws (1969). When serially sectioning
the ovaries of 109 pregnant elephants culled in Uganda
and Kenya, he noted a definite decline in the total weight
of luteal tissue in both ovaries during the second half of

gestation.

Meyer et al. (2004) confirmed the earlier findings of
McNeilly et al. (1983) that a pronounced increase in
serum prolactin concentrations occurs in pregnant ele-
phants from around the fifth month of gestation. They also
made the intriguing observation of significantly higher
mean progestagen concentrations in the blood of Indian,
but not African, elephants carrying male as compared
with female fetuses. Perhaps this reflected a lower con-
tribution of progestagens from the fetal ovaries in the later
stages of gestation due to the enlarged follicles restricting
the total volume of progestagen-secreting interstitial cells
that could develop. But why the same situation should not
pertain in the African elephant fetus, in which the present
study has confirmed the enlargement of multiple ovarian
follicles, remains a puzzle.

Another mystery in the pregnant elephant involves the
nature and source of the gonadotrophic and/or luteo-
trophic stimuli for both the development and persistence
of the multiple large corpora lutea of pregnancy and the
enlargement and steroidogenic function of the fetal
gonads. Hodges (1998) questioned whether the elephant
is monovular or polyovular and, after discounting the earlier
hypothesis of Short (1966) that the multiple corpora lutea
encountered in pregnant and non-pregnant elephants
might reflect accumulation of luteal structures from cycle
to cycle in order to achieve a critical mass of productive
luteal tissue to support pregnancy, he concluded that the
formation of multiple corpora lutea, with and without ovu-
lation stigmata, probably occurs in each oestrous cycle,
with structural – but not functional – persistence into

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subsequent cycles. Hodges and his colleagues also confirmed the original findings of Short & Buss (1965) and Smith et al. (1969) of the complete absence of native progesterone in the luteal tissue and peripheral blood of cycling and pregnant African elephants and its replacement by 5α-reduced progestagens, especially 5α-dihydroprogesterone (Heistermann et al. 1997a, 1997b, Hodges et al. 1997). Coincidentally, Meyer et al. (1997) and Greyling et al. (1998) determined that 5α-dihydroprogesterone exhibits higher affinity for the endometrial progesterone receptor in the elephant than progesterone itself. Taken together, these two findings highlight the likelihood that, as has been demonstrated in the mare during the second half of gestation (Hamon et al. 1991, Holtan et al. 1991), 5α-dihydroprogesterone, rather than progesterone, is the most biologically significant progestagen in the elephant in terms of support for the pregnancy state.

With regard to gonadotrophic and/or luteotrophic stimulation in pregnancy, McNeily et al. (1983) were unable to detect any differences in serum levels of immunoreactive follicle-stimulating hormone and luteinizing hormone between cycling and pregnant elephants, and Allen et al. (2002) could find no hint of biological or immunological gonadotrophic activity in five saline extracts of placental tissue recovered from pregnant elephants between 4 and 11 months of gestation. This was perhaps not surprising in view of the zonary endotheliocorial nature of the elephant placenta and the complete absence of binucleate cells or syncytiotrophoblast formation in the trophoblast layer (Wooding et al. 2005). Furthermore, as discussed by Maston & Ruvolo (2002), the secretion of gonadotrophic hormones by the placenta is relatively recent in evolutionary terms, even among the primates, and equids are the only other non-primate genus known to have developed this placental function. The pronounced rise in serum prolactin levels after 5 months of gestation in the elephant (McNeily et al. 1983, Meyer et al. 2004) offers the possibility that prolactin could also provide the gonadotrophic stimulus necessary to induce interstitial gland cell development, follicular growth and steroidogenesis in the fetal gonads. This being so, and in the absence of a placental gonadotrophin, premature activity of the fetal pituitary gland seems the last remaining option and the most likely source of this mid-gestation gonadotrophic drive. Clearly, much more research is needed to provide answers to these and many other fascinating questions concerning the controlling mechanisms and biological roles of gonadal enlargement in the fetal elephant.

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