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A preliminary study of the heterogeneity in endometrial morphology and glycosylation in the uterine horns of the non-pregnant impala (*Aepyceros melampus)*

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ABSTRACT

Placentation commences only in the right uterine horn in impala (*Aepyceros melampus)*. To investigate possible differences in morphology or glycosylation between the two horns, right and left uterine horns from six non-pregnant, wild impala were examined morphometrically and histochemically using a panel of 23 lectins and an avidin-biotin revealing system. The presence of ovarian 3ß hydroxysteroid dehydrogenase (3ßHSD) and aromatase was also investigated using immunocytochemistry. There were few detectable differences in morphology and glycosylation between right and left uterine horns in five of the specimens, but the sixth had deep clefts and plentiful exocrine secretions in the right horn, and not the left. Heavily glycosylated clusters of supranuclear granules were present in the epithelial cells, which had many classes of O-linked glycans. The serum progestagen was not markedly different, however, from that of the other specimens. In five of the six specimens, the height of luminal epithelium was greater on the right than that on the left, and the height of the gland epithelium was also greater on the right side in four of these. The 3ßHSD and aromatase activities were present in the ovaries and were similar in impala with or without progestagen concentrations > 1 ng/ml in peripheral blood. No *corpus haemorrhagicum* or *corpus luteum* could be discerned. These findings indicate there are morphological and biochemical differences between right and left uterine horns in the impala and further studies are needed on both impala and other species in which placentation commences only in one uterine horn, to establish the cyclical hormone changes which induce them.

1. Introduction

Placentation in ruminants is a highly complex process involving "cross talk" between the embryo and the endometrium, the latter undergoing controlled changes in gene expression regulated by the ovaries and the embryo (Spencer et al., 2008). These result in temporal and spatial alterations in a plethora of hormone receptors, cytokines, transcription/growth factors and other macromolecules as well as changes in the glycosylation of the luminal epithelium of the endometrium (Bazer et al., 2011; Raheem, 2018).

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This endometrial epithelial surface is coated with a thick glycocalyx, and in many species its composition changes during the period of foetal attachment receptivity (Surveyor et al., 1995; Jones et al., 1998; Raheem, 2018).

In the camel, the blastocyst attaches only in the left horn, and induction of gene expression for steroid hormone receptors and selected transcription factors can differ between the left and right uterine horns during the early placentation phase (Abdoon et al., 2017). In non-pregnant animals, it appears as though, of the 12 genes studied, abundance of FOXL2 transcript is less in the left uterine horn compared to the right horn, though this was evident in only two females. Impala (*Aepyceros melampus)* blastocysts also attach in only one horn of the bicornuate uterus (Mossman and Mossman, 1962; Lee et al., 1977) but, in contrast to the camel, this occurs on the right side. Factors affecting the preference of the blastocyst to attach on one side are not yet understood. As changes in glycosylation are associated with foetal attachment receptivity in many species, this lectin histochemical study was undertaken to attempt to identify alterations in the glycan expression and/or morphology of the luminal epithelium or uterine glands in that horn to account for this preference as the site of attachment and subsequent placentation.

2. Materials and methods

2.1. Animals and tissue collection

Six uteri from non-pregnant impala were obtained during a management-driven cull on a large, professional hunting conservancy in southern Zimbabwe during August 2017. Reproductive tracts were fixed in 10% formalin for transport to the UK where two 5 mm thick pieces of tissue half-way along each uterine horn and the ovaries were removed (only the left ovary was present on Impala 30, the right one having been inadvertently lost when the tract was removed at post mortem). One piece of the uterine horn tissue and the bisected ovaries were processed and embedded prior to histological and immunocytochemical staining. A cross-section 1 mm thick was taken from the second piece of tissue and subsequently post-fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2 for 4 h before being embedded in epoxy resin, which allows for precise resolution, for lectin histochemistry (see Table 1 for lectin specificities). This was performed on the tissues as described previously (Jones et al., 2007). Some sections, that were subsequently stained with AHA, ECA, SBA, SNA-1, MAA, PAA and WGA, were pre-treated with Type VI neuraminidase (Sigma, from *Clostridium perfringens*) in 0.2 M acetate buffer, pH 5.5, with 1% (w/v) calcium chloride at 37 °C for 2 h (Jones et al., 1992) to cleave off terminal sialic acid and controls were processed as previously described. Lectin binding was assessed using a semi-quantitative ranking system of analysis where the distribution of stained intracellular granules was allocated a grade from - (absent) to + + + (densely distributed). Morphometry was performed on the epithelial cells of the luminal and glandular epithelia using Image Proplus Software (Media Cybernetics, Rockville, USA), with 100 cells from each section stained with *Wisteria floribunda* agglutinin (which clearly delineates the basal lamina) being assessed in each specimen. Luminal epithelium over caruncles was used to provide a series of regular cells except for the right horn of Specimen 26 where caruncles were not evident in the sections used; in this case the flattened areas of luminal epithelium between the clefts in the tissue were used. The data were analysed using GraphMap Prism version 7.03. Data for cell height were mainly, but not always, normally distributed when assessed using the D-Agostino-Pearson omnibus normality test, therefore, the Mann Whitney *U* test for non-parametric data was selected to ascertain any significant difference in the height of the cells between the right and left horns.

2.2. Immunohistochemistry

The formalin-fixed, wax-embedded samples from the uterine horns and both ovaries were sectioned at 5 µm and three sections were stained with haematoxylin and eosin (H&E). Further sections of the ovaries from five of the six impala were mounted on positively-charged slides for immunocytochemical staining using two mouse monoclonal primary antibodies which had previously been used in assessing giraffe tissue (Wilsher et al., 2013). The first of these was raised against recombinant 3β hydroxysteroid dehydrogenase (3βHSD) of human origin (3βHSD [37-2]; sc100466; Santa Cruz, Insight Biotechnology Inc., Santa Cruz, CA, USA) and the second was raised against a synthetic peptide corresponding to amino acids 376 to 390 of human aromatase (Cytochrome P450 aromatase; MCA2077S, AbD Serotec, Raleigh, NC, USA). Staining was undertaken using a Dako Autostainer as described previously (Wilsher et al., 2013). After staining, the slides were removed from the machine, dehydrated, cleared and mounted in DPX. Negative controls were conducted by replacing each primary antibody with an unrelated mouse-monoclonal antibody. In addition, tissues from horses known to stain positively with the antibody in question were used as further controls.

2.3. Measurement of progestagens in peripheral blood

Concentrations of progestagens were measured in serum samples collected from the jugular vein immediately after each animal was killed, using an ELISA previously described by Allen and Sanderson (1987). Dilution curves generated by a pool of pregnant impala serum with a relatively greater concentration of progestagen were parallel to the curve produced using the horse progesterone standards. The assay had a limit of sensitivity of 0.15 ng progesterone/ml serum and the intra- and inter-assay coefficients of variation were, respectively, 6% and 8%. The anti-progesterone monoclonal antibody had cross reactivities of 100% with 5β-pregnane-3,20-dione (5β dihydroprogesterone; 5βDHP), 79% with 5αDHP, 56% with 5β-pregnanedione and 18% with 5α-pregnanedione.

Table 1
Lectins: source and major specificities.

Acronym	Source	Major specificity
CON A	*Canavalia ensiformis* Jackbean	α-D-glucosyl and α-D-mannosyl (terminal or 1,2 linked) in high mannose, intermediate and small complex N-linked sequences
PSA	*Pisum sativum* Garden Pea	α-D-mannose in non-bisected bi/tri-antennary, complex N- linked sequences
e-PHA	*Phaseolus vulgaris* (erythroagglutinin) Kidney Bean	Bi/tri-antennary bisected complex N-linked sequences
L-PHA	*Phaseolus vulgaris* (leukoagglutinin) Kidney Bean	Tri/tetra-antennary, non- bisected complex N-linked sequences
UEA-1	*Ulex europaeus-1* Gorse	H type 2 antigen (αL-Fuc(1,2)-Galß1,4 GlcNAcß1-) and Le^y
LTA	*Tetragonolobus purpureus* Lotus	L-fucosyl terminals (especially where clustered), Fucα1,6GlcNAc > Fucα1,2-Galß1,4(Fucα1,3)-GlcNAcß, $Le^{xy,}$
ALA	*Aleuria aurantia* Mushroom	Fucose linked α1,6- to GlcNAc
DBA	*Dolichos biflorus* Horse Gram	GalNAcα1,3(LFucα1,2)Galß1,3/4 GlcNAcß1-
VVA	*Vicia villosa* Hairy vetch	GalNAcα1-Ser/Thr and GalNAcα1,3Galß1-
MPA	*Maclura pomifera* Osage orange	Galß1,3GalNAcα1- > GalNAcα1-
BSA-$1B_4$	*Bandeiraea simplicifolia* Griffonia	Galα1,3Galß1,4 GlcNAcß1-
DSA	*Datura stramonium* Jimson Weed	ß1,4 GlcNAc, *N*-Acetyl lactosamine > chitotriose
STA	*Solanum tuberosum* Potato	ß1,4 GlcNAc oligomers
LEA	*Lycopersicon esculentum* Tomato	ß1,4 GlcNAc oligomers
HPA	*Helix pomatia* Roman snail	Terminal GalNAcα1-
AHA	*Arachis hypogaea* Peanut	Galß1,3GalNAcß1- > Galß1,4 GlcNAcß1-
ECA	*Erythrina cristagalli* Coral Tree	Galß1,4 GlcNAcß1-
SBA	*Glycine max* Soybean	Terminal GalNAcα1- > Galα1
WFA	*Wisteria floribunda* Wisteria	GalNAcα1,6 Galß1- > GalNAcα1,3Galß1-
SNA-1	*Sambucus nigra* Elderberry Bark	NeuNAcα2,6 Gal/GalNAc-
MAA	*Maackia amurensis*	NeuNAcα2,3Galß1-
PAA	*Phytolacca americana* Pokeweed	Similar to WGA
WGA	*Triticum vulgaris* Wheatgerm	Di-*N*-acetyl chitobiose, *N*-acetyl lactosamine (especially if clustered) and some sialyl residues

3. Results

3.1. Morphology

Examination of the dissected uteri confirmed that the right horn was invariably larger than the left (Fig. 1A and D). When the endometrium was examined histologically, there was little detectable difference between left and right horns in five of the six specimens. All specimens had an irregular lumen lined with columnar epithelium with numerous exocrine secretory glands emanating from this layer (Fig. 1B, C). These opened into the uterine cavity in the inter-caruncular area only but were not present in the caruncles. One specimen (Impala 26), however, had a totally different appearance both macro- and microscopically (Fig. 1E, F). Deep, branching clefts emanated outwards from the central lumen (Figs. 1F and 2 A) and these were lined with epithelial cells that had domed surfaces, in contrast to the flat surface of the cells lining the lumen and glands on the left side; because these cells in the right horn were so enlarged the cells had a pseudo-stratified appearance (Fig. 2B).

The caruncular luminal epithelia on the two horns were different in cellular height ($P < 0.0001$, Mann Whitney *U* test) with the cells of the right side being of greater height than the left in three animals (Impala 26, 27 and 30). The difference in cellular height was less marked, though still significant, in two other animals [*P*: 0.0023 (Impala 28) and 0.0061 (Impala 29) respectively; Fig. 3A]. There was no significant difference between the height of the luminal epithelium on left and right sides in Impala 25. The height of

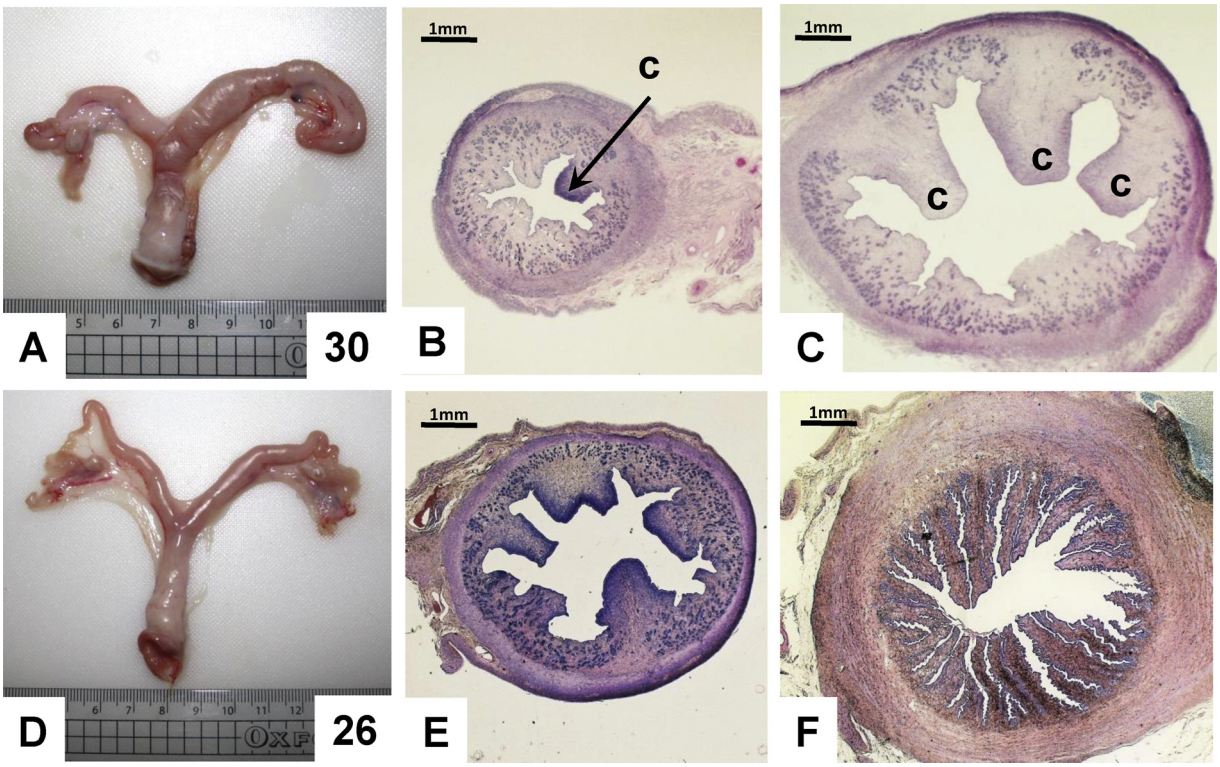

Fig. 1. Structure and histology of impala bicornuate uteri; Two examples are shown. **A:** Gross morphology of Specimen 30 with the right horn larger than the left, Scale in centimetres; **B:** Section of the smaller left horn with numerous small endometrial glands that do not penetrate the caruncular area (arrow c) (H&E); **C:** Right horn is appreciably larger than the left though the general structure is similar with several caruncules evident (c) lacking endometrial glands (H&E); **D:** Gross morphology of Specimen 26 which also had a right horn that was larger than the left; **E:** Left horn has a histological structure similar to that of Specimen 30; **F:** Right horn has deep, branching clefts extending from the lumen to the myometrium and no caruncles.

the glandular epithelium in the right horn was also markedly greater than that of the left in four of the six animals (Impala 26, 27, 28, 30, Fig. 3B) ($P < 0.0001$, Mann Whitney U test). The height of epithelial cells of Impala 25 and 29 were again not different between the two horns.

3.2. Glycosylation

The data for the primary differences in glycosylation have been summarised in Table 2. In five of the six specimens, there were only subtle changes in glycosylation between right and left horns, but in Impala 26 which, as previously described, had deep endometrial clefts, the panel of lectins showed marked differences in glycan abundance between the right and left uterine horns, with heterogeneity in the binding intensity for many lectins, especially MPA, HPA (Fig. 2B), WFA, PAA and WGA. Apical secretory granules were also very prominent, and these bound many lectins, particularly UEA-1, ALA, MPA, STA, LEA, ECA, SBA, WFA, MAA, PAA and WGA; BSA-1B$_4$ (Fig. 2C) bound to a subpopulation of granules, a sparse number of which were also stained with AHA. There was a slight increase in glycosylation that was detected after neuraminidase pre-treatment, which also resulted in a considerable increase in binding with ECA, especially in the glands of all six specimens. Neuraminidase pre-treatment had little effect on staining with AHA, SBA, SNA-1, WGA or PAA, but there was reduced staining with MAA. Binding of the lectins to the endometrium of the left horn was restricted to the apical plasma membrane and occasionally cytoplasmic granules (Fig. 2D). Glandular secretions also bound the majority of lectins, apart from LTA. Secretions and cilia bound DBA and VVA even though cytoplasmic binding was absent with these two lectins (Fig. 2E). The lectins also bound to small, apical granules in occasional cells in the luminal and glandular epithelia in the other specimens (Fig. 2G and H).

There was considerable variability among the other five animals in the binding of UEA-1, HPA and LEA to the luminal epithelium and, to a lesser extent, with the binding of BSA-1B$_4$, ECA and SBA (with and without neuraminidase). There were also differences between the luminal and glandular epithelia, especially with HPA and UEA-1 (Fig. 2I–M), where there was an abrupt change of lectin binding at the junction between the two epithelia. In general, binding also was less in the more basal parts of the glands (Fig. 2J). There was more heterogeneity of binding intensity in the endometrial glands than in the luminal epithelium and there was little difference between the specimens in the binding of CONA, PSA, ePHA and lPHA, LTA and ALA, DSA, SNA-1 and MAA. The DBA and VVA binding were similar but there were variations between specimens with respect to binding to the luminal epithelium. For

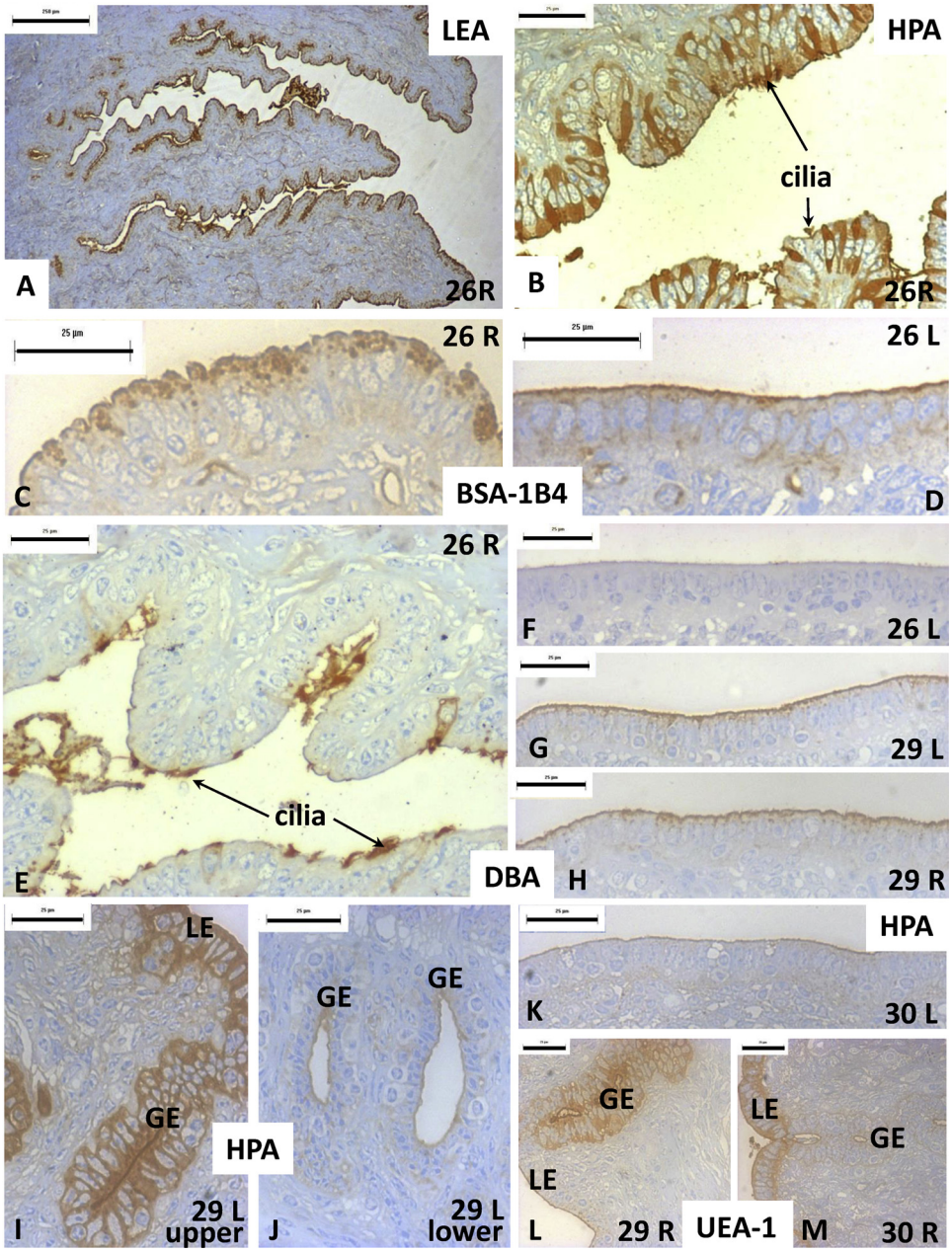

Fig. 2. Lectin histochemistry of impala uterine horns; **A:** Low power section of the right uterine horn of Specimen 26 stained with LEA with deep branching clefts extending out from the lumen, Scale bar 250 μm; **B:** High power section of an endometrial cleft from impala 26 showing its pseudo-stratified appearance and the heterogeneity of the binding of HPA to the cells. Intensity of staining was great in the cilia; **C:** Great amount of BSA-1B_4 binding occurred to the microvillous membrane and apical granules in luminal epithelium of the right uterine horn of Impala 26; **D:** Luminal epithelium of the left uterine horn of Impala 26 had very little staining with BSA-1B_4; **E:** Large amount of DBA binding to cilia and endometrial gland secretions in the right horn of the uterus in Impala 26; **F:** Very little DBA binding to the luminal epithelium is present in the left uterine horn of Impala 26; **G:** DBA binds to microvilli in Impala 29 and to apical cytoplasm in some cells of the luminal epithelium of the left uterine horn; **H:** Right uterine horn of Impala 29 had similar binding as the left with DBA; **I:** With HPA, Impala 29 had binding to the luminal epithelium (LE) and upper gland epithelium (GE); **J:** Glandular epithelia (GE) deeper in the endometrium did not bind HPA in Impala 29; **K:** Impala 30 had virtually no binding of HPA to the luminal epithelium; **L:** With UEA-1, Impala 29 had binding by the glandular (GE) but not the luminal epithelium (LE); **M:** Impala 30 had the reverse pattern for UEA-1, with binding to the luminal (LE) but not glandular epithelium (GE); B-M: Scale bars 25 μm.

example, Specimens 27 and 30 had binding to occasional cells, especially in the apical regions, and less to the microvillous surface, while Specimens 25, 28 and 29 had greater binding to microvilli and to apical granules (Fig. 2G, H). Specimens 27 and 30 had slightly more binding to the right horn endometrium than the left with these lectins.

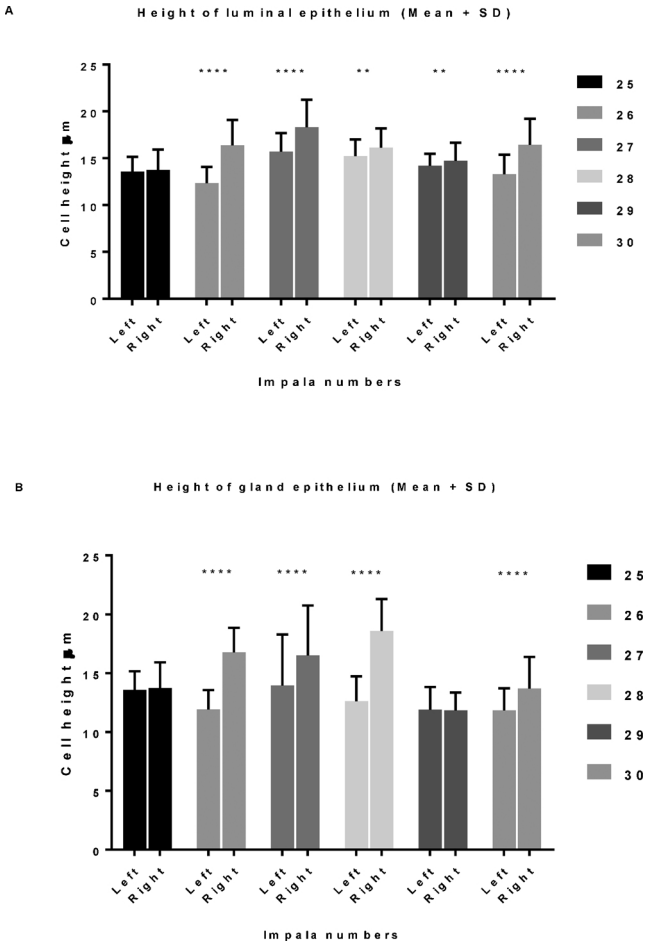

Fig. 3. A: Graph depicts heights of the caruncular* luminal epithelium in left and right uterine horns of six non-pregnant impala; There was a difference (**** $P < 0.0001$) in epithelia from Impala 26, 27 and 30 with less marked differences in Impala 28 and 29 (** P: 0.0023 and ** P: 0.0061 respectively, Mann Whitney U test); There was no significant difference between the cell heights in the left and right uterine horns of Impala 25.

*In the absence of caruncles in Specimen 26, cells in areas of luminal epithelium between the clefts were measured.

B: Graph depicting heights of the glandular epithelium measured in left and right uterine horns of six non-pregnant impala where there was a marked difference (**** $P < 0.0001$, Mann Whitney U test) in Impala 26, 27, 28 and 30; There was no difference in gland cell height between the left and right uterine horns of Impala 25 and 29.

The endometrium from right and left horns was, however, generally similar with there usually being a slight increase in glycosylation on the right side. The intensity of staining did not appear to correlate with the concentration of progesterone measured in the animals' blood (Table 3).

3.3. Progestagens in peripheral blood

Of the six impalas sampled, progestagens were present at concentrations of > 1 ng/ml in peripheral blood, albeit at low concentrations, in three of the animals (Specimens 26, 29 and 30; Table 3). The weight of the ovaries and progestogen concentrations did not appear to be associated (Table 3).

3.4. Gross morphology, histology and immunohistochemistry of ovarian tissue

Gross examination of the ovaries when bisected prior to fixation indicated there were no ovulation papillae (stigmata) and no luteal tissue (Fig. 4A and B). Examination of the H&E stained sections from the ovaries of the six impala indicated multiple antral follicles at different stages of development, with primordial follicles being present in the cortex of the ovary (Fig. 4C and D). In no section was there a *corpus haemorrhagicum* or *corpus luteum* detected. Likewise, immunohistochemical staining for 3βHSD (Fig. 4E–G) did not result in detection of luteal structures with the capacity to synthesise progesterone. Interestingly, in a subset of primordial follicles in the cortex of the ovary, 3βHSD was present (Fig. 4F) in all five non-pregnant impalas, a finding previously noted in the

Table 2
Lectin staining of the luminal epithelium (LE) and the upper epithelium of the endometrial glands (GL).

Impala No	25		26				27		28		29		30	
			Left		Right									
Lectin	LE	GL	LE	GL	LE	GL	LE	GL	LE	GL	LE	GL	LE	GL
CONA	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
PSA	++	++	++	++	+	+	++	++	++	++	++	++	++	++
ePHA	++	++	++	++	++	++	++	++	++	++	++	++	++	++
lPHA	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LTA	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UEA-1	+	++	++	+	***G***+++	***G***+++	++	++	+	++	+	++	++	–
ALA	+++	+++	+++	+++	G+++	+++	++	+++	+++	+++	+++	+++	+++	+++
DBA	+	–	–	–	-/+	-/+	-/+	-/+	+	+	+	-/+	–	–
VVA	-/+	–	–	–	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	–	–
MPA	+	+	+	-/+	G++*	G++*	+	+	+	+	+	+	+	+
BSA-1B$_4$	+	+	+	-/+	***G***+++	***G***+++	++	++	++	++	+	-/+	+	+
DSA	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	++
STA	+++	+++	+++	+++	G+++	G+++	+++	+++	+++	+++	+++	+++	+++	+++
LEA	+	+/-	+++	++	***G***+++	***G***+++	+++	++	++	++	+	-/+	+	-/+
HPA	+++	+	+	++	+++*	+++*	+	++	+++	++	+++	+++	+	-/+
AHA	–	–	–	–	-/+	G+	–	–	–	–	–	–	–	–
AHA + N	–	+	-/+	-/+	+/-	G+	-/+	-/+	-/+	–	-/+	-/+	-/+	-/+
ECA	+	+	++	++	***G***+++	***G***+++	++	-/+	+	-/+	+	+	++	-/+
ECA + N	++	++	+++	++	G+++	G+++	+++	++	++	++	++	++	++	+
SBA	+	-/+	+	+	***G***+++	***G***+++	++	+	++	+	++	-/+	++	-/+
SBA + N	+	-/+	+	+	***G***+++	***G***+++	++	+	++	+	++	+	++	-/+
WFA	++	+	++	++	G+++	G+++	++	++	++	++	+	+	++	++
SNA-1	+++	+++	+++	+++	+++*	+++*	+++	+++	+++	+++	+++	++	+++	++
SNA-1 + N	+++	++	+++	++	+++	+++	+++	++	+++	+++	++	++	+++	++
MAA	+	+	+	-/+	G+++	G++	+	+	+	+	+	+	+	+
MAA + N	-/+	-/+	-/+	-/+	G+	G+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
PAA	++	+	++	++	G+++*	G+++*	+++	++	+++	+	++	++	++	+
PAA + N	++	++	++	++	G+++	G+++	+++	++	+++	+	++	++	++	+
WGA	+++	++	+++	++	G+/-*	G++*	+++	++	+++	++	+++	++	+++	++
WGA + N	+++	++	+++	++	G+++	G++	+++	++	+++	++	+++	++	+++	+

Key: – absent, -/+ occasional, + some, ++ moderate numbers, +++ densely distributed. G: large granules in the right uterine horn of impala 26.
Rows in bold, italic type indicate differences in staining between different specimens.
*Great heterogeneity in staining between cells.

Table 3
Progestagen concentrations in peripheral blood and ovarian weights in six non-pregnant impala.

Animal ID	Progestagens in peripheral blood (ng/mL)	Ovarian weight (g)	
		Right ovary	Left ovary
25	0.5	0.3	0.2
26	1.9	0.2	0.2
27	0.5	0.3	0.1
28	0.8	0.4	0.2
29	2.8	0.3	0.2
30	1.8	*Not recorded*	0.2

ovaries of pregnant impala (S. Wilsher and W.R. Allen, personal communication). There was also 3βHSD present in oocytes visible within antral follicles (Fig. 4G), with pale staining seen in the follicular fluid (Fig. 4G). Greater 3βHSD staining was observed within the blood vessels (Fig. 4E). There was relatively lesser staining for aromatase in the follicular fluid of antral follicles, as compared with the blood vessels (Fig. 3H). Staining patterns for 3βHSD and aromatase did not differ between impala in which there were and were not concentrations of progestagen > 1 ng/ml in peripheral blood. There was very little staining in the negative control samples (inset Fig. 4F).

4. Discussion

There are no reports of histological comparisons of the endometrium between left and right uterine horns in those species in which there is initiation of blastocyst placentation on a specific side of the uterus. These include the camel and impala (Mossman and Mossman, 1962; Lee et al., 1977) and several African antelope including the Uganda kob (*Adonta kob*, Buechner, 1961), suni

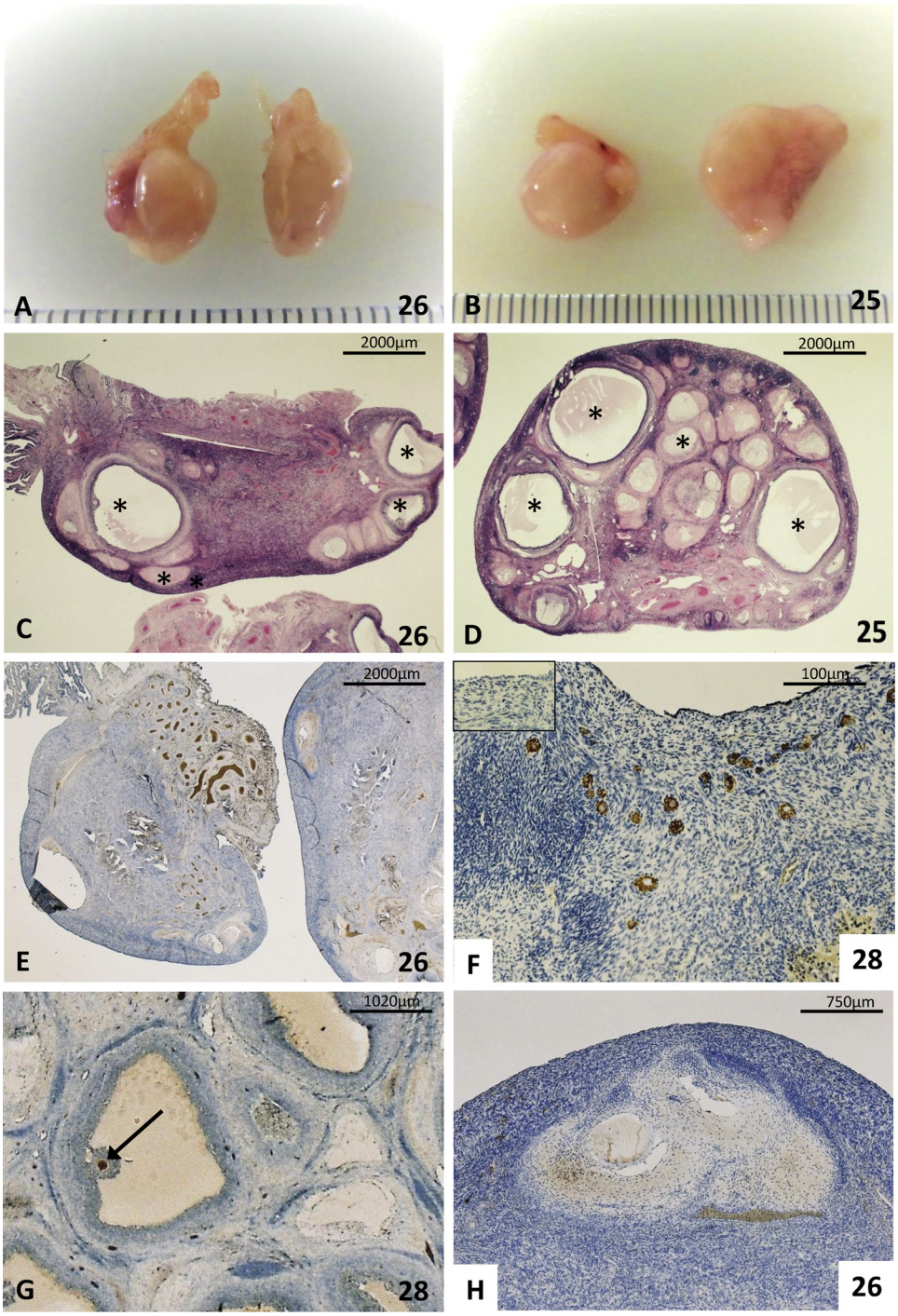

Fig. 4. Gross morphology and immunocytochemistry of impala ovaries; **A – B:** Gross morphology of the ovaries from Impala 26 with > 1 ng/ml (A) and 25 with < 1 ng/ml progestagen in peripheral blood (B); No ovulation stigmata were present on the ovaries (each division of the scale bar 1000 µm); **C – D:** Haematoxylin and eosin stained sections of the bisected ovaries shown in (A) and (B); No *corpora lutea* or *haemorrhagica* were detected; Note the multiple antral follicles present in the ovaries (asterisks; scale bar 2000 µm); **E:** Immunohistochemical staining on ovaries of Impala 26 with an antibody raised against 3β hydroxysteroid dehydrogenase (3βHSD); No luteal structures were detected using this antibody although there was binding in the blood vessels (scale bar 2000 µm); **F**: A sub-population of primordial follicles had 3β hydroxysteroid dehydrogenase (3βHSD) staining in one of the ovaries of Impala 28 (Scale bar 100 µm); Inset top left: negative control where antibody is replaced by an unrelated mouse monoclonal antibody; **G:** Very little staining of the follicular fluid within the antral follicles with an antibody raised against 3β hydroxysteroid dehydrogenase (3βHSD), and greater staining of the oocyte (arrowed) in the ovary of Impala 28 (Scale bar 1020 µm); **H:** Very little staining of an antral follicle and a blood vessel with an antibody raised against cytochrome P450 aromatase in the ovary of Impala 26 (scale bar 750 µm).

(*Neotragus moschatus,* Loskutoff et al., 1990), dik-dik (*Madoqua kirkii*) and several others (Hayssen and Orr, 2017). The mechanism controlling this unilateral initiation of placentation is unknown and results from previous studies indicate ovulation occurs equally from both ovaries (Mossman and Mossman, 1962).

Unfortunately, it was not possible in the present study to determine the age of the animals examined, nor to accurately gauge the stage of the ovarian cycles at the time of tissue collection. Because 25 (74%) of the total of 34 impala that were culled were pregnant with fetuses weighing 1.3 to 535 g, and the non-pregnant animals were marginally smaller in stature, it was assumed that the non-pregnant animals were likely to be either pre-pubertal or had only recently become pubertal because annual pregnancy rates approach 100% in impala, and breeding out-of-season has been reported to be uncommon (Cowley, 1975).Three of the six non-pregnant impala in the present study had serum progesterone concentrations of > 1 ng/ml including Impala 26 which had the marked differences in endometrial morphology between right and left horns. Progesterone concentrations increase during a 1 to 4 day period prior to puberty in ewe lambs (Fitzgerald and Butler, 1978; Ryan and Foster, 1978) which is thought to be caused either by premature ovulation or follicular luteinisation within an ovary In pre-pubertal beef heifers, there are usually two transient increases in serum progesterone concentrations before a normal luteal phase (Gonzalez-Padilla et al., 1975) and these again may be produced by some transient luteal development in the ovary (Berardinelli et al., 1979). In goats, an ungulate to which the impala is more closely related, the serum progesterone concentrations at oestrus are about 0.2 ng/ml (Thorburn and Schneider, 1972). These concentrations increase to about 4 ng/ml from days 10 to 15 after ovulation before decreasing on Day 19 preceding the subsequent oestrus. During pregnancy, concentrations are consistent at 2.5 to 3.5 ng/ml from 8 to 60 days of gestation after which concentrations further increase to 4.5 to 5.5 ng/ml, before decreasing just prior to parturition.

The immunohistochemical staining of the impala ovary in the present study indicated there were no luteal structures in the ovary that had the capacity to produce progesterone and the tissue source for the progestagens in three of the six impala could not be determined. Furthermore, staining for aromatase in the ovaries indicated there was no difference in oestrogen producing capacity between those impala with relatively greater and lesser progesterone. Hence, it is not clear why the Impala that had the profound changes in endometrial morphology between right and left uterine horns had a serum progesterone concentration of 1.9 ng/ml which was very similar to that of another Impala (1.8 ng/ml) that had no such morphological contrasts in endometrial histology.

There were distinct differences in endometrial glycosylation between the different animals examined but the differences between the two uterine horns was more subtle and was only conspicuous in one animal (Specimen 26). With this specimen, the most distinct marker was the development of aggregates of apical granules staining intensely with various lectins to indicate the presence of simple fucosyl residues such as H_2 antigen and Le^y (UEA-1), α1,6-linked fucosyl residues (ALA), *N*-acetyl glucosamine oligomers (STA, LEA, PAA and WGA), unsubstituted *N*-acetyl lactosamine (ECA), *N*-acetyl galactosamine (MPA, SBA, WFA), α2,3-linked sialic acid (MAA), with subpopulations having terminal α-galactosyl residues (BSA-$1B_4$) and terminal β-galactose (AHA). Neuraminidase pre-treatment had little effect on the staining of AHA, ECA and SBA thereby indicating there were few terminal sialic acid residues. Cilia also had fucosylation staining in the presence of *N*-acetyl galactosamine (DBA, VVA) though cytoplasmic staining was usually absent. There was little evidence of *N*-linked glycans in the large apical granules. There was also heterogeneity in abundance of some cytoplasmic glycans, including *N*-acetyl galactosamine (MPA, WFA) which was sometimes terminal (HPA), *N*-acetyl glucosamine (PAA and WGA) and α2,6-linked sialic acid (SNA-1). This finding is likely a reflection of cell death due to high cell turnover, as has been reported previously when there was use of lectin histochemistry assessments (Jones et al., 1997).

Glycosylation sometimes varied between the luminal and glandular epithelia and this was particularly striking with fucose (UEA-1) which, in three specimens (numbers 25, 28, 29), was greater in the glandular epithelium than in that of the lumen. There was also greater staining for terminal *N*-acetyl galactosamine (HPA) in the luminal epithelium than that of the glands in the same three specimens, which also had greater staining of the luminal epithelial surface with DBA (fucosylated *N*-acetyl galactosamine). It would be interesting to establish if these changes are associated with the stage of the oestrous cycle or reflect individual differences among animals.

The DBA binding was of special interest because it reflects changes in blastocyst binding receptivity in human endometrium (Jones et al., 1998, 2009), which probably relates to the presence of fucosylated molecules similar to Blood Group A substance (Etzler and Kabat, 1970). In the impala endometrium, there was variation in these molecules among the animals, probably as a reflection of changes in the presence of fucosylated molecules during the oestrous cycle. These changes were also detected by VVA which binds to a similar molecule (Grubhoffer et al., 1981).

There was a difference in the height of the luminal and gland epithelial cells between the right and left uterine horns in most of the six specimens examined which presumably related to the amount of biosynthetic organelles present, in preparation for the production of prominent secretory granules such as those detected in Specimen 26. Differences in the height of both luminal and glandular epithelia when there is a predominance of either oestrogen or progesterone during oestrous cyclicity have been reported in other species such as the horse (Ricketts, 1975), so it was surprising that there did not appear to be any relationship between the height of epithelial cells and the concentration of progestagens in the peripheral blood of impala. The animals with the least difference in epithelial height between left and right sides of the uterus (Specimens 25, 28, 29) were, however, the animals with most DBA-positive material in the luminal epithelium.

Ideally, examination of impala uteri from mature animals at known stages of either the oestrous cycle or pregnancy would enable for more conclusions than what could be ascertained in the present study as to the significance of any differences between the two uterine horns. Nevertheless, these preliminary findings indicate that the uterine horns of the non-pregnant impala, a species that commences placentation only in the right horn, warrant further study of this phenomenon.

Conflict of interest

The authors declare no conflicts of interest.

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