

GROSS AND MICROSCOPIC OBSERVATIONS OF OVARIAN ABNORMALITIES FROM FIVE BURCHELL'S ZEBRA, *EQUUS BURCHELLI ANTIQUORUM*, SMITH 1841

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ABSTRACT

IMES, G. D. & SMUTS, G. L., 1975. Gross and microscopical observations on ovarian abnormalities in five Burchell's zebra, *Equus burchelli antiquorum*, Smith 1841. *Onderstepoort J. vet. Res.* 42, (4), 109-116 (1975).

Five pair of zebra ovaries were submitted to the Onderstepoort Veterinary Research Institute for examination because of unusual uni- and/or bilateral gross appearances. Microscopic findings revealed an accessory spleen encapsulated by the ovarian peritoneum, a case of tubo-ovarian cysts, one example of ovarian aplasia, an example of germinal inclusion cysts and an abnormal follicular haemorrhage related to pressure necrosis of a nearby *corpus luteum*. Similarities to the ovary of the domestic mare were also seen and some are briefly described.

Résumé

IMES, G. D. & SMUTS, G. L., 1975. *Observations macroscopiques et microscopiques d'ovaires anormales provenant de cinq zebres Burchell, Equus burchelli antiquorum, Smith 1841. Onderstepoort J. vet. Res.* 42, (4), 109-116 (1975).

Cinq paires d'ovaires de zebre ont été amenées à l'Institut de Recherche Vétérinaire d'Onderstepoort en raison de leur apparence exceptionnelle. Des coups histologiques ont mis en évidence une rate accessoire renfermée dans le péritoine ovarien, un cas de kystes tubo-ovariens, un cas d'aplasie ovarienne, un cas d'inclusions kystiques germinales et une hémorragie ovulaire anormale associée à une nécrose à pression d'un corpus luteum à proximité de celui-ci. Des similitudes à l'ovaire de la jument domestique ont été aperçues, dont certaines sont brièvement décrites.

INTRODUCTION

The increasing importance of all aspects of wildlife management and ecology has resulted in detailed investigations of several animal species which have been culled for population control measures. The opportunity to grossly examine zebra reproductive organs occurred recently as part of a study concerning the growth, reproduction and population characteristics of Burchell's zebra in the Kruger National Park (Smuts, 1974). In that investigation abnormal appearing ovaries were forwarded to the Onderstepoort Veterinary Research Institute for further studies. Findings are described in this paper.

MATERIALS AND METHODS

Two hundred and sixty-two pairs of ovaries were grossly examined by Smuts (1974). Of these, 5 pairs from mares over 3 years of age had what appeared to be uni- or bilateral abnormalities. These ovaries were fixed in 10% formalin, incised, examined and photographed. Portions appearing abnormal were sectioned at 4 μ m and stained with haematoxylin-eosin (HE) for routine histological examination. Special staining techniques were used in a few cases. These were the periodic acid Schiff (PAS) reaction, Mayer's mucicarmine stain, a modification of Mowry's 1958 colloidal iron stain (Anon, 1968) and alcian blue stain. Frozen sections were stained with the oil red O (ORO) stain.

RESULTS AND DISCUSSION

Zebra 394

This mare was approximately 4½ years old and pregnant. The conceptus was estimated as being 129 days old using the method of Hugget & Widdas

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(Smuts, 1974). The right ovary was normal in appearance, it measured 2,4 cm wide by 5,1 cm long and its mass was 21,8 g. The left ovary was much larger, measuring 5,0 cm wide by 8,4 cm long and its mass was 162,3 g. On incising the left ovary it was found to contain a large mass of clotted blood which was visible on the ovarian surface about midway along its length where the wall was thin (Fig. 1). The distortion of the ovary was such that the ovulation fossa was nearly obliterated. The wall surrounding the blood mass appeared to consist of connective tissue, but embedded in it at one pole of the ovary was a *corpus luteum* which seemed to be invaded by the blood mass (Fig. 2). None of the other ovaries examined contained such a large follicular haemorrhage and this was concluded to be an excessive post-ovulation haemorrhage.

Histological examination revealed that the outer portion of the wall surrounding the blood mass consisted of normal ovarian stroma. The inner portion varied in appearance. The greater part was composed of follicular wall components. The cells of the *membrana granulosa* were arranged in a loose to lacy pattern and thin strands of basophilic material extended between them. They were separated from the *theca interna* by an intact limiting membrane. The *theca interna* was composed of large polyhedral cells with profuse granular vacuolated cytoplasm and large vesicular nuclei. The cells were interspersed by capillaries and larger vessels and were arranged in a layer 3-4 cells thick (Fig. 3). The segment of the ovary, which grossly appeared to be an existing *corpus luteum* invaded by the blood mass, had an entirely different appearance. Congestion and haemorrhage were only present in the portion of the *corpus luteum* directly adjacent to the blood mass and there was a connection between the 2 haemorrhages. Coagulation necrosis in which the reticulum and occasional luteal cells could be recognized was also observed (Fig. 4). Further necrotic foci were composed primarily of deeply basophilic cellular debris or dead and degenerating neutrophils (Fig. 5). Very active fibroblast and neocapillary proliferation interspersed with large luteinized cells, which were identical to those in the unaffected parts of the *corpus luteum*, was also observed (Fig. 6).

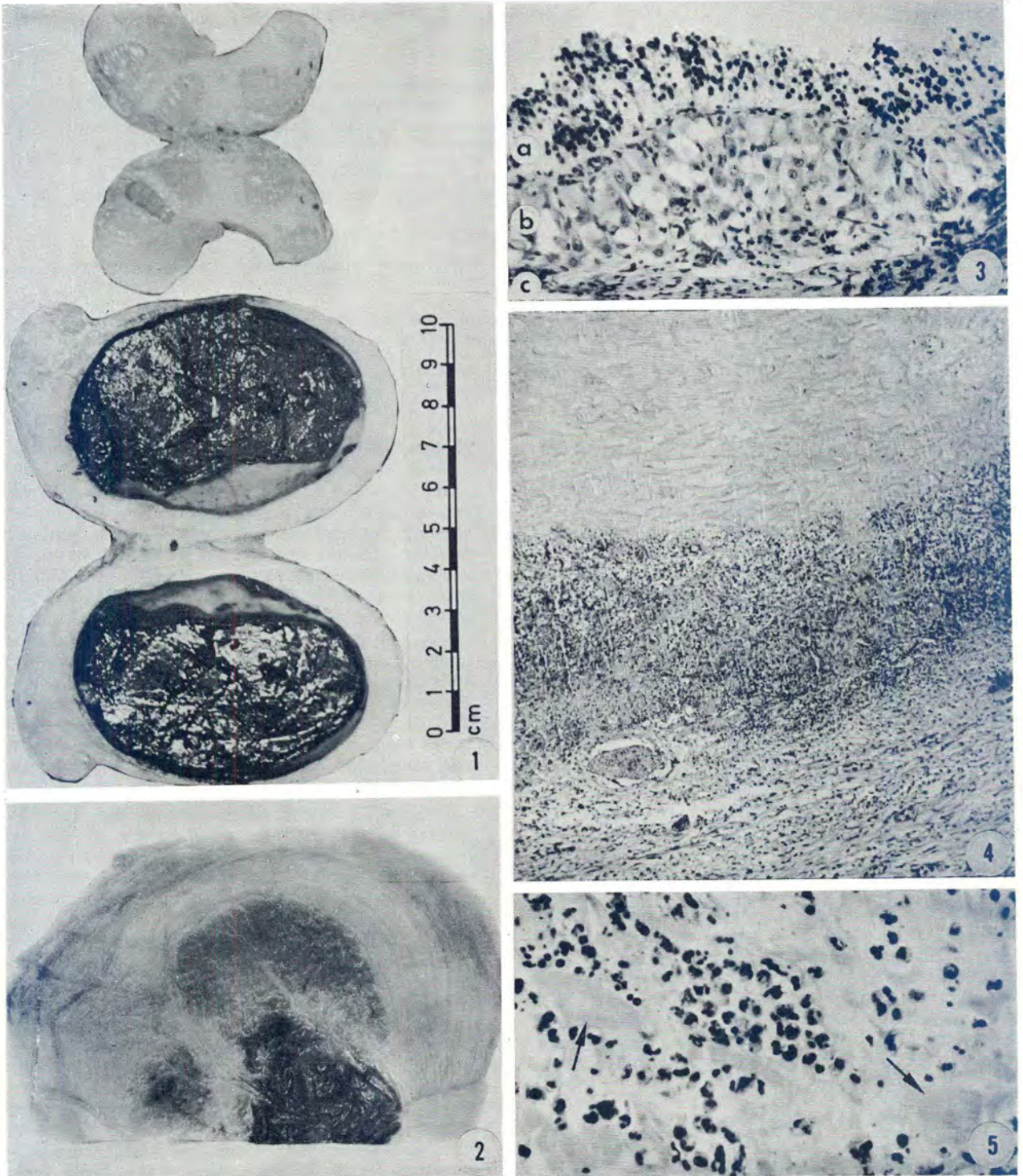


FIG. 1 Zebra 394. Abnormal post-ovulation haemorrhage in left ovary. Normal right ovary at top
 FIG. 2 Zebra 394. Haemorrhage from pre-existing *corpus luteum* contributing to abnormal post-ovulation haemorrhage
 FIG. 3 Zebra 394. Portion of wall surrounding haemorrhage: Mature *membrana granulosa* (a), *theca interna* (b) and *externa* (c). HE $\times 200$
 FIG. 4 Zebra 394. Pre-existing *corpus luteum* with coagulation necrosis (top) and basophilic cellular debris. HE $\times 75$
 FIG. 5 Zebra 394. Lutein cells (arrows) which have undergone coagulation necrosis and are interspersed with accumulations of neutrophils. HE $\times 1\ 200$

These findings strongly suggest that abnormal haemorrhage occurred both prior to and following ovulation. Since two literature concerning the histology of the development and morphology of follicles and *corpora lutea* of zebra is available, data on these structures as seen in the domestic mare (Harrison, 1946; Van Niekerk, 1973) are used in an attempt to interpret the microscopic changes seen. The stage of development of the *theca interna* cells in this ovary is similar to that described in mature follicles of the mare. Following ovulation the follicle collapses, resulting in folding of the thecal layers and *membrana granulosa*. The granulosa cells lose their compact arrangement and develop a lace-like appearance. They are covered by a basophilic mucoid substance which appears to originate from them. According to Van Niekerk (1973) haemorrhage commences about 14 h following ovulation and by 24 h the cavity is filled with blood, but the folding remains. On the basis of their gross observations Hamilton & Day (1945) reported that haemorrhage occurred as early as 8 h after ovulation and Küpfer (1928) found haemorrhage immediately after ovulation in the donkey. About 10 h post-ovulation degeneration of the *theca interna* cells commences and by 24 h they are in the final stages of degeneration. In addition, fibroplasia and neocapillary proliferation are in evidence at 24 h, forming trabeculae for the developing *corpus luteum*.

In the case of the zebra ovary, there was no folding of the layers of the follicle, as expected after ovulation, but the granulosa cells had lost their compact arrangement. Only in the tissues of the pre-existing *corpus luteum* were active haemorrhage, congestion, fibroplasia and neocapillary proliferation found. The following interpretation was therefore made: The space occupied by the blood mass was that of a large follicle. Because of its size and the consequent pressure exerted, necrosis occurred between the follicle and the periphery of an adjacent *corpus luteum* resulting in communication between the two. Congestion and haemorrhage occurred in the necrotic portion of the *corpus luteum* which increased the intrafollicular pressure and probably assisted in promoting slightly premature ovulation. The antrum was immediately filled by haemorrhage from the injured *corpus luteum* thereby preventing folding of the follicular wall. The fibroplasia and neocapillary proliferation seen in the *corpus luteum* which mimicked the reaction expected at approximately 24 h following ovulation in the domestic mare, was in fact a healing response of the mature *corpus luteum*. The presence of mature lutein cells within the fibroplasia and neocapillary proliferation helps to verify this interpretation.

Zebra 528

This mare was 4½ years old and not pregnant. Her ovaries were found to be much larger than any of the others examined (Fig. 7). The left ovary measured 4,3 cm wide by 6,5 cm long and its mass was 48,3 g. The right ovary measured 4,1 cm wide by 5,6 cm long and its mass was 52,8 g. They both contained large numbers of follicles, a total of 97 being recognizable, but none larger than 1,5 cm. Because of their size and the number of follicles, they were thought to be either hyperactive or cystic.

Microscopically there was no evidence of cyst formation or other abnormalities and the ovaries were therefore considered normal. Their size was attributed to the large numbers of developing and atretic follicles, which occur in the domestic mare when the ovaries go from an inactive to active stage and also

during a normal oestrus cycle (Van Niekerk, 1965). Ovaries are also at their largest in young domestic mares, 3–4 years of age (Sisson & Grossman, 1953; Hammond & Wodzicki, 1941; Roberts, 1956).

Zebra 556

This mare was 15 years old. She was pregnant and the age of the foetus was 347 days. The left ovary was normal in appearance and measured 2,0 cm wide by 3,5 cm long; its mass was 10,2 g. The right ovary measured 3,0 cm wide by 4,2 cm long and its mass was 18,9 g. At one pole 10–20 small cysts were present, of which the contents had not coagulated as in the normal follicles of the other fixed ovaries. This ovary was considered to be cystic (Fig. 8).

The histological examination revealed that the cystic structures were distinct from, but adherent to, the ovary. Individual cysts were separated from each other by septa consisting of varying thicknesses of well vascularized smooth muscle and connective tissue (Fig. 9). The cysts were lined by a single layer of ciliated columnar epithelium (Fig. 10) with a basement membrane as indicated by the PAS reaction. A few papillary projections were also present.

Re-examination of the ovary, in the light of the microscopical findings, revealed that it was really spherical in shape, measuring 2,0 cm in diameter. The cystic structures were adhered in a capsule about 0,1 cm in thickness and tightly adherent to the ovary.

The cystic structure was identified as a cluster of tubo-ovarian cysts. It was differentiated from an ovarian cystadenoma, also a strong possibility, on the following grounds: (1) It was not part of the ovary but adhered to it; (2) the cysts were lined with typical oviduct epithelium; (3) a significant amount of smooth muscle and vascular development accompanied the cysts and the vasculature resembled the *stratum vasculare* of the oviduct.

Tubo-ovarian cysts develop as a result of adhesions of the fimbriae to the ovary and even though no inflammation was present the age of the mare was such that cellular evidence of the inflammation could long since have disappeared. Another possibility is that adherence may have occurred during embryonic development.

Other cystic conditions considered were:

- (1) Cystic derivation of the paramesonephric (Müllerian) duct, which is frequent in the mare. These cysts are lined by typical oviduct epithelium but lack a basement membrane (Jubb & Kennedy, 1970).
- (2) Germinal inclusion cysts which are also frequent in the mare, but are an integral part of the ovary, occurring in the ovulation fossa, and usually have cuboidal epithelium (Jubb & Kennedy, 1970).
- (3) Cystic derivation of the mesonephric (Wolffian) duct, but these cysts have unciliated epithelium (Hertig & Gore, 1960).

Zebra 660

This mare was estimated at 9 years of age; she was not pregnant. The left ovary measured 3,0 cm wide by 6,1 cm long, its mass was 28,3 g. A dark mass constituting about ¼ of the ovary was present at one pole. It was covered by peritoneum and conformed to the general shape of the ovary and was suspected to be a tumour on gross examination (Fig. 11). The incised surface of the dark mass revealed the presence of several randomly distributed small white foci (Fig. 12).

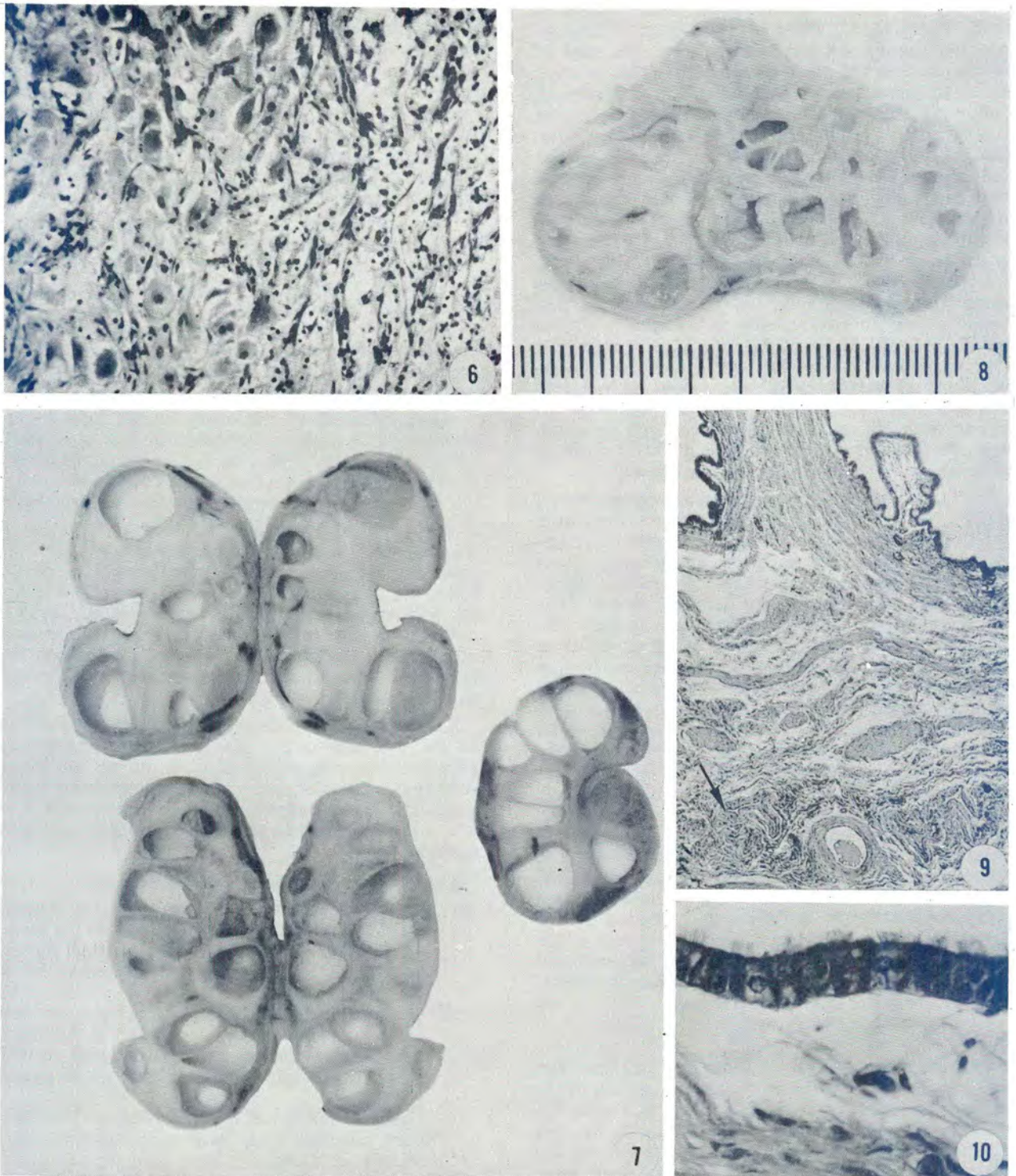


FIG. 6 Zebra 394. Pre-existing *corpus luteum* showing fibroplasia and cellular infiltrate. HE \times 500
 FIG. 7 Zebra 528. Section of large ovaries compared with an average size normal ovary from another zebra mare
 FIG. 8 Zebra 556. Tubo-ovarian cysts. Normal ovarian tissue on left and adhered cystic Fallopian tube on right
 FIG. 9 Zebra 556. Septum of cyst (with papillary projections) extending from tissue containing smooth muscle and a well-defined vascular layer (arrow). HE \times 75
 FIG. 10 Zebra 556. Columnar ciliated epithelium lining cystic structure. HE \times 500

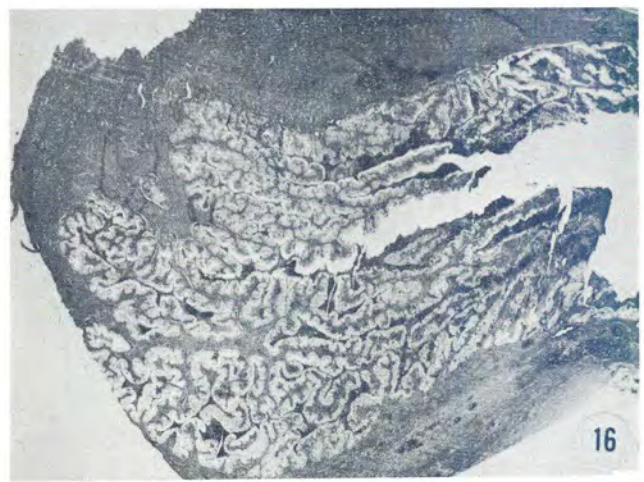
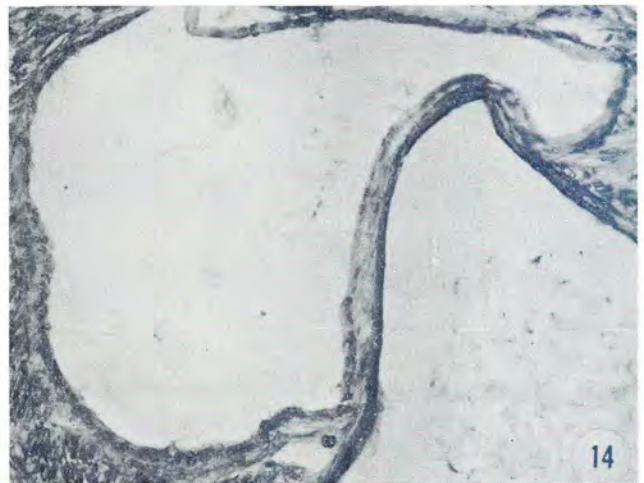


FIG. 11 Zebra 660. Left ovary with an accessory spleen, the dark encapsulated structure on the right
 FIG. 12 Zebra 660. Section of ovary and accessory spleen. Note germinal centres (small white foci)
 FIG. 13 Zebra 660. Accessory spleen encapsulated by ovarian peritoneum. HE \times 75
 FIG. 14 Zebra 660. Left ovary. Germinal inclusion cysts lined by cuboidal epithelium. HE \times 200
 FIG. 15 Zebra 660. Right ovary. Normal *corpus haemorrhagicum* (early corpus luteum)
 FIG. 16 Zebra 660. Right ovary. Low magnification of same structure. HE \times 5.5

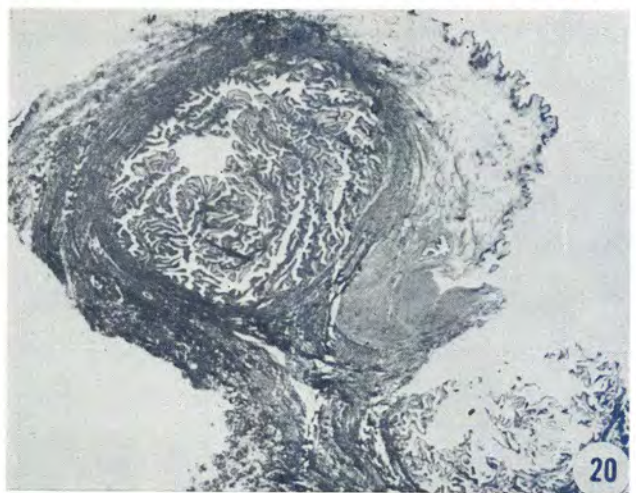
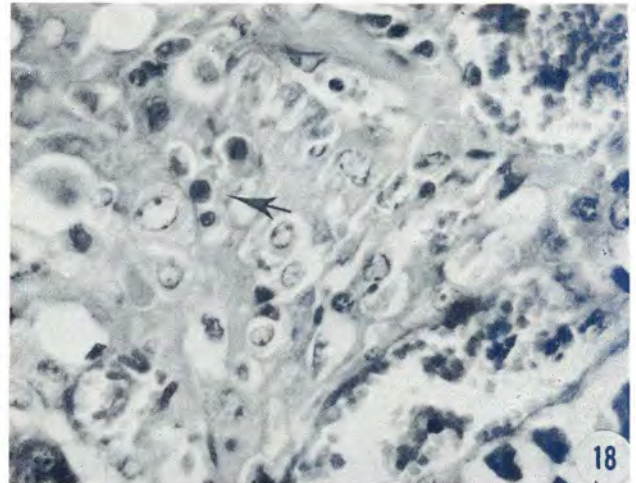
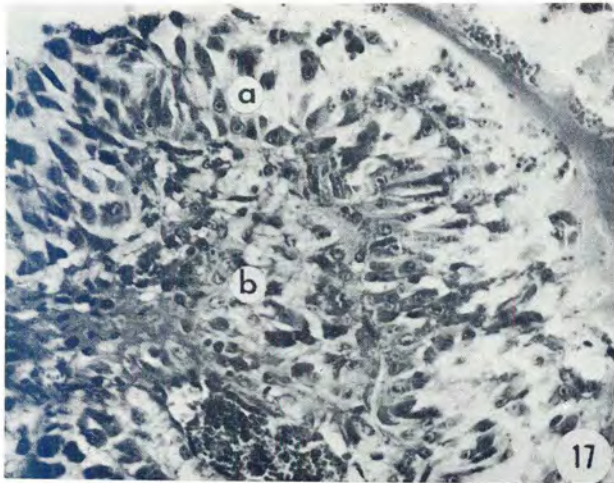


FIG. 17 Zebra 660. Tip of a trabecula composed of *theca interna* cells (b), some of which are starting to degenerate (dark nuclei). The tip is covered by granulosa cells (a) which will become the lutein cells of the mature *corpus luteum*. HE \times 200

FIG. 18 Zebra 660. Normal early *corpus luteum* demonstrating individual degeneration of *theca interna* cells (arrows) in a trabecula (GC). HE \times 500

FIG. 19 Zebra 719. Normal, left ovary at top. Structure at bottom was thought to be an atrophic right ovary

FIG. 20 Zebra 719. Low magnification of small structure in Fig. 19 demonstrating the Fallopian tube architecture with rudimentary fimbriae on the surface. HE \times 8

FIG. 21 Zebra 719. Typical oviduct epithelium lining the numerous folds. HE \times 500

Microscopically the mass was readily identified as normal splenic tissue. It was encapsulated and separated from adjacent ovarian stroma by a splenic capsule. The splenic capsule was covered by a continuation of the ovarian peritoneal capsule. The white foci seen grossly were active splenic corpuscles (Fig. 13). A small amount of phagocytized haemosiderin was seen in the red pulp. The ovary itself was essentially normal except for a few microscopic cysts lined by cuboidal epithelium in the ovulation fossa (Fig. 14).

The splenic tissue was classified as an accessory spleen. These develop either as a continuation of the very early mesenchymal hillocks in the dorsal mesogastrium or as exaggerated temporary incisures in the spleen (Arey, 1965). The latter, however, occur at about the end of the first trimester and the accessory organ would be expected to remain in close proximity to the spleen. It is therefore more likely that this accessory spleen was of the multiple hillock type and formed next to the developing ovary, to be enclosed with it by the covering peritoneum.

The microscopic cysts in the ovulation fossa were typical germinal inclusion cysts and, in domestic animals, are most often seen in the mare (Jubb & Kennedy, 1970).

The right ovary of this mare measured 2,7 cm wide by 4,2 cm long and its mass was 18,2 g. It contained what was grossly identified as a *corpus haemorrhagicum* and was considered normal (Fig. 15). This observation was verified on histological examination (Fig. 16) and although it was normal it warrants description from a comparative anatomical point of view. There was folding of the follicular wall and nearly complete obliteration of the antrum. The folding resulted in the trabecular formation characteristic of *corpora lutea* of the domestic mare (Harrison, 1946). These trabeculae were formed from the *theca externa* and contained the vasculature required for the *corpus luteum*. Secondary and tertiary trabeculae had also formed and *theca interna* cells could be demonstrated in the trabeculae, but they were degenerating (Fig. 17 & 18). The granulosa cells had acquired a lacy pattern, their nuclei were larger and more vesicular, and they had increased cytoplasm when compared to granulosa cells observed in developing follicles. This change is clearly demonstrated by comparing Fig. 3 and 17 both taken at the same magnification. Stretching between the granulosa cells and extending into the antrum was a basophilic substance which stained positive for acid mucopolysaccharides by a modification of Mowry's 1958 colloidal iron stain and alcian blue stain. It was not stained by Mayer's mucicarmine stain or the ORO stain and was negative to the PAS reaction.

There was only a small amount of haemorrhage in the antrum. The gross appearance (Fig. 15) suggests there is much haemorrhage, pointing out how inaccurate estimates of the time when haemorrhage commences are on a macroscopic basis. Many eosinophils were present in the trabeculae and among the remaining *theca interna* cells. According to Van Niekerk's (1973) findings in the domestic mare, this early *corpus luteum* was approximately 14 h old.

Zebra 719

This mare was about 5 years of age and in heavy lactation. She had a 1-2 month old foal. The left ovary appeared normal and measured 4,3 cm wide by 4,8 cm long, with a mass of 41,9 g. The right ovary was classified as being atrophied. It measured 0,9 cm wide by 1,4 cm long and its mass was 3,0 g. Additional gross examination revealed a small

smooth-surfaced oblong structure constricted near the centre. A piece of roughened villous tissue was attached to one end whereas a small tubular opening was present at the other, where it had been excised. Incision revealed a cavity with a 0,1 cm thick limiting wall lined by tissue which nearly filled it. The stricture in the centre gave a false impression that 2 cavities were present (Fig. 19).

No ovarian tissue could be found by histological examination. The cavity was lined by epithelium consisting of ciliated columnar cells arranged in many primary and secondary folds (Fig. 20 & 21). This epithelial layer as surrounded by circularly arranged smooth muscle and connective tissue which contained numerous vessels in the periphery. Portions of the serosal surface consisted of villous projections covered by ciliated columnar epithelium.

This abnormality was identified as ovarian aplasia. The object concerned had the typical microscopical anatomy of an oviduct ampulla and the villous epithelium on portions of the surface was interpreted as being rudimentary fimbriae of the oviduct. Why the oviduct developed the oblong ovarian-like appendage at its ovarian end is unknown.

GENERAL DISCUSSION

The histopathological examination of the ovaries from 5 zebra was primarily intended to determine the changes responsible for their gross abnormal appearance. While some of the abnormalities are interesting, they have little practical significance considering that 2 zebra were pregnant, one had a foal and, from a histologic viewpoint, the other 2 were fertile. What appears to be more important are the similarities between these ovaries and those of the domestic mare. Although exact information on the time of ovulation was not available and the chronological sequential development of follicles and *corpora lutea* could therefore not be studied, the histological picture was very similar to that of the domestic mare, as described by Harrison (1946) and Van Niekerk (1973). Their findings were therefore used in this investigation as an aid to distinguish normal from abnormal.

The only abnormality which might be of practical clinical significance was the abnormal follicular haemorrhage seen in Zebra 394. Normal follicles may reach a size of 5-7 cm in the domestic mare (Van Rensburg & Van Heerden, 1953; Van Niekerk, 1973) and consideration of the fact that the central space fills with blood following normal ovulation until the *corpus haemorrhagicum* reaches a size of about 70% of the original follicle (Van Niekerk, 1973) makes it clear that this allows for accumulation of a large volume of blood. When first examined the ovary concerned was thought to contain such a post-ovulation haemorrhage and it was only after a detailed study that it was recognized as being abnormal. Rarely, post-ovulation haemorrhage continues in the domestic mare until death results (A. P. Schutte, Research Institute, Onderstepoort, personal communication, 1974) and the possibility exists that such haemorrhages are associated with damage to other ovarian structures as seen in this instance. Ovulation occurs during pregnancy in domestic mares (Van Rensburg & Van Heerden, 1953); however, none have been recorded with a conceptus age of ± 129 days and therefore this ovulation may also have been abnormal in that respect. Possibly it would not have occurred without the added pressure increase produced by blood seeping into the follicle from the damaged *corpus luteum*.

The zebra has the following histological features in common with the domestic mare:

(1) The large polygonal *theca interna* cells in the mature follicle (Zebra 394) correspond to the "thecal gland" of the mare as described by Harrison (1946); (2) the presence of mucoid material in the early *corpus luteum* (Zebra 660). This acid mucopolysaccharide is produced by the granulosa cells and most of it accompanies the ovum into the oviduct but a little is retained in the early *corpus luteum* (Van Niekerk, 1965; Van Niekerk & Gerneke, 1966; Van Niekerk, 1973); (3) degeneration of the *theca interna* cells leaving the granulosa cells as the sole source of lutein cells (Zebra 660), a feature described by Harrison (1946) and Van Niekerk (1973) in the domestic mare.

This brief study has substantiated the assumption that members of a given genus should have very similar gross anatomical and histological features, and similar anomalies. Since the ovary of this genus is anatomically distinct, the study of ovaries from animals suspected of being related to the genus *Equus* could be of assistance in their classification.

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