



Differentiation and development of buffalo uterus

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Studies of laboratory animals (Mori and Nagasawa 1988), sheep (Bartol *et al.* 1988) and cattle (King *et al.* 1995) indicated that exposure of the developing uterine tissues to agents that disrupt critical organizational events can have long lasting effects on reproductive health. In literature histogenesis of uterus in cattle (Bazhenova, 1975, Atkinson *et al.* 1984), sheep (Wiley *et al.* 1987) and equines (Ginther 1979) was described. The detailed data regarding the histogenesis of buffalo uterus is scanty so the present research was proposed.

The present study was conducted on 27 female buffalo foetii of different gestational age collected from abattoir and Veterinary clinics. The crown vertebral rump length of the foetii was measured by using the formula given by Soliman (1975).

$$Y = 28.66 + 4.496X[\text{CVR} < 20 \text{ cm}]$$

$$Y = 73.544 + 2.256X[\text{CVR} \geq 20 \text{ cm}]$$

where, Y is the age in days and X is the CVR length in centimeters.

Based on the CVR length the samples were divided into three groups;

Group 1: Foetii of CVR length up to 20 cm

Group 2: foetii of CVR length above 20–40 cm

Group 3: foetii of CVR length above 40 cm.

The tissues from left and right horns and body of uterus were fixed in Bouin's fixative, processed and paraffin sections of 5–6 mm were stained with hematoxylin and eosin, Masson's trichrome for collagen fibres, Gridley's method for reticular fibres, Verhoeff's method for elastic fibres and Holme's for neuronal elements (Luna 1968). The micrometrical observations were recorded on the hematoxylin and eosin stained sections with the help of Filar and ocular micrometer duly calibrated with stage micrometer. The values of all the parameters were subjected to statistical analysis.

The morphogenetic events associated with the development of uterus in buffalo may be characterized in a

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similar way as described in ovine fetal uterine development by Wiley *et al.* (1987).

1. Stratification and reorientation of the subepithelial mesenchymal layers
2. Differentiation of an eosinophilic presumptive myometrial layer
3. Endometrial restructuring and formation of fixed mucosal surface structures i.e caruncles and folds
4. Development and proliferation of the uterine glands

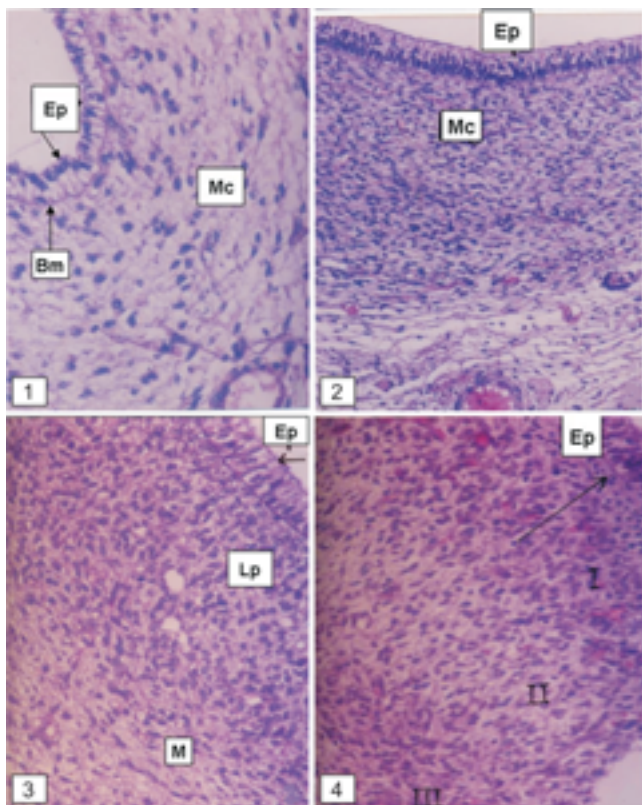
Endometrium

Lamina epithelialis mucosae: At 10.0 cm CVRL (73 days) the uterine wall was lined by simple columnar epithelium with a well defined basement membrane (Fig. 1). The nuclei of the cells were located towards the apical border of the cells. At 14.0 cm CVRL (91 days) stratified columnar or pseudostratified columnar epithelium with cilia could also be observed at some places (Fig. 2) as reported in fetal uteri by Atkinson *et al.* (1984) in bovines and Wiley *et al.* (1987) in ovines. In group 2 at 25.0 cm CVRL (130 days) the epithelium was pseudostratified columnar ciliated with inter- and intra-epithelial vacuoles. At 35.0 cm CVRL (152 days) some light and dark cells could also be observed. In group 3, the epithelium was stratified columnar, simple columnar or pseudostratified columnar at certain places as reported earlier by Uppal and Roy (2002) in neonatal buffalo uteri.

The average height of the epithelium of left, right horn and body of uterus was 26.97±2.40 mm, 28.84±3.09 mm, 29.22±3.20 mm in group 1; 29.24±1.03 mm, 29.01±1.03 mm, 26.88±1.52 mm in group 2; and 20.70±0.33 mm, 22.14±0.37 mm, 21.45±0.37 mm, respectively.

Lamina propria: At 10.0 cm CVRL (73 days) the epithelium was surrounded by the mesenchymal cells. The cells were under the process of differentiation and mitosis. At 14.0 cm CVRL (91 days) the subepithelial mesenchymal cells were spindle shaped with little cytoplasm and prominent nuclei as observed by Wiley *et al.* (1987) in 55 to 60 day fetal sheep uterus.

In group 2 at 25.0 cm CVRL (130 days) the underneath mesenchymal tissue of the epithelium was organized into 2



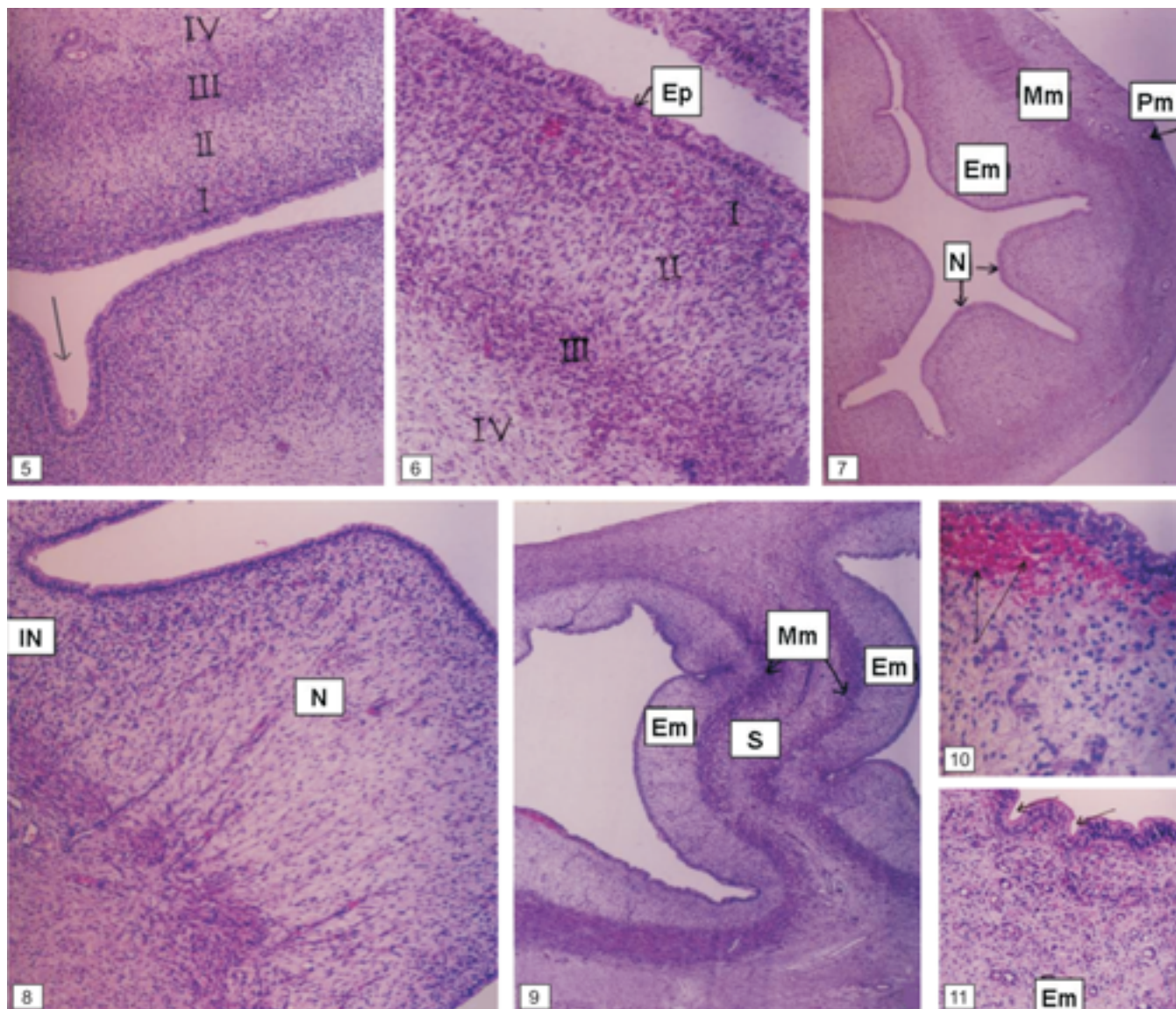
Figs 1–4. **1.** 10.0 cm CVRL (73 days) foetus showing epithelium (Ep) and differentiating mesenchymal cells (Mc). H & E×350; **2.** 14.0 cm CVRL (92 days) foetus showing epithelium (Ep), differentiating mesenchymal cells (Mc). H & E×175; **3.** 25.0 cm CVRL (130 days) foetus showing ciliated epithelium (Ep) and differentiating mesenchymal cells into lamina propria (Lp) and smooth muscle cells (M). H & E×350; **4.** 35.0 cm CVRL (152 days) foetus showing epithelium (Ep), a layer of densely packed cells (arrow) and subepithelial tissue arranged into a layer of densely packed cells (I), loosely arranged intermediate layer (II) and circularly arranged densely packed cells (III). H & E×350.

layers; inner and outer (Fig. 3). Inner layer consisted of densely packed cells having rounded to elongated nuclei and below this layer was a layer of loosely packed cells with elongated nuclei as reported by Wiley *et al.* (1987) in 60-day-old fetal sheep uterus. Blood capillaries could also be seen in between the cells at this stage. Further at 35.0 cm CVRL (152 days) the inner mesenchymal cell layer was stratified into 3 layers (Figs 4,6) as described by Wiley *et al.* (1987) in 100-day ovine fetal uterus. Blood vessels could also be seen in this layer. With the basal aspect of the epithelium, a band of cells with intensely stained nuclei was seen. These cells were migrating into epithelium at some places. Wiley *et al.* (1987) has suggested these cells to be lymphocytes. Uterine mesenchymal differentiation was also initiated during fetal life in other species including cow (Atkinson *et al.* 1984). Later on in group 3, the connective tissue was better differentiated and at 42.0 cm CVRL (168 days) the connective tissue component was organized into 2

distinct regions (Figs 7, 8) as observed by Atkinson *et al.* (1984) in bovine fetal uterus differentiation. There was a densely stained internodular region that was extending up on the sides of each nodule. In the center of the nodule, under the epithelium the connective tissue was less intensely stained. Large number of blood vessels could be seen radiating into the stroma. Besides the vessels, red blood cells were also seen at the mesenchymal epithelial interface (Fig. 10). The increased blood supply to the growing endometrium was suggestive of the faster development of the caruncles at this stage. A well defined septum between 2 horns could also be observed at 43.0 cm CVRL (Fig. 9). Further at 79.0 cm CVRL (252 days) large number of blood vessels could be observed in the propria, actually this is the stage when adenogenesis was going to initiate, as large number of epithelial invaginations could be observed (Fig. 11). During the last days of gestation the internodular connective tissue showed progressive changes. These findings were in agreement with those observed by Atkinson *et al.* (1984) in bovine fetal uterus just before and after birth when glandular development was initiated.

The total thickness of wall in left, right and body of uterus was 211.81 ± 17.15 mm, 217.64 ± 12.43 mm and 236.34 ± 17.01 mm in group 1, 404.64 ± 33.01 mm, 401.77 ± 77 mm and 395.61 ± 27.97 mm in group 2 whereas in group 3 average thickness of wall at nodular area of left, right and body of uterus was 1447.88 ± 154.21 mm, 1555.36 ± 168.96 mm, 1522.30 ± 174.07 mm and at internodular area was 766.16 ± 156.39 mm, 777.39 ± 167.50 mm, 761.20 ± 162.44 mm respectively.

Endometrial glands: Uterine adenogenesis is a complex process governed by a variety of endocrine, cellular and molecular mechanisms but many of the details remain to be defined (Gray *et al.* 2001) In the present study at 48.0 cm CVRL some of the epithelial invaginations could be seen but no evidence of adenogenesis was observed. The propria was also richly supplied with blood vessels. At 79.0 cm CVRL (252 days) the epithelium was highly folded and lots of epithelial invaginations could be observed but endometrial glands could not be observed till 100.00 CVRL. So it may be concluded that gland formation started either at the end of gestation period or just after the birth. Atkinson *et al.* (1984) also observed short invaginations from surface by 250 days of gestation in bovine fetus and occasional very short glands were distinguished at 265 days and extensive glandular development occurs between birth and 3 months of age. Uppal and Roy (2002) observed the initiation of adenogenesis in the newborn buffalo calf. Wiley *et al.* (1987) reported shallow slightly coiled tubular glands in 9 day old ovine neonatal uterus and extensive glandular development and growth occurred between day 9–26. The unconjugated estrogens in the maternal plasma increased dramatically during the final 2 weeks of pregnancy (Robertson and King, 1979).



Figs-11. **5.** Posterior part of the uterine horn of 35.0 cm CVRL (152 days) foetus showing invagination of the mucosa to form nodule (arrow) and four layers of subepithelial tissue, layer of densely packed cells (I), loosely arranged intermediate layer (II), circularly arranged densely packed cells (III) and a layer of loosely packed cells (IV). H & E×87.5; **6.** 35.0 cm CVRL (152 days) foetus showing epithelium (Ep) and subepithelial tissue arranged into a layer of densely packed cells (I), loosely arranged intermediate layer (II), circularly arranged densely packed cells (III) and a layer of loosely packed cells (IV). H & E×175; **7.** 42.0 cm CVRL (168 days) foetus showing nodule formation (N), endometrium (Em), myometrium (Mm) and perimetrium (Pm). H & E×35; **8.** 42.0 cm CVRL (168 days) foetus showing densely stained internodular region (IN) and less stained area in the center of nodule (N). H & E×175; **9.** Body of uterus of 43.0 cm CVRL (171 days) foetus showing septum (S) between 2 horns, endometrium (Em) and myometrium (Mm). H & E×35; **10.** 43.0 cm CVRL (171 days) foetus showing blood vessels and RBCs in endometrium at mesenchymal epithelial interface (arrow). H & E×35; **11.** 79.0 cm CVRL (252 days) foetus showing invagination of the epithelium (arrow) and large number of blood vessels in the endometrium (Em). H & E×175

Caruncles: Caruncles, the raised aglandular structures that are macroscopic features of the adult endometrium in sheep and cattle, emerge during fetal life as precaruncular nodules. In the present study the caruncles first made their appearance at 35.0 cm CVRL (152 days) more prominent in the posterior part of the uterine horn (Fig. 5). Whereas Baishya and Vyas (1993) reported that in surti buffalo the endometrial caruncle first appeared at 130 days of gestation and Wiley *et al.* (1987)

observed slight symmetric luminal clefts at 90–100 days in uterus of ovine fetus which deepened in day 100 uteri establishing clearly defined nodular and internodular areas. In the middle of gestation the nodules were very close to each other and as observed by Atkinson *et al.* (1984) in bovine fetal uterus and they totally obscured the lumen of uterus. The base of the nodule was narrow and only very little cytoplasm was present between the epithelium and

myometrium in the internodular area. Later on this internodular area is recognized as intercaruncular area as described by Atkinson *et al.* (1984) who observed many gland openings on the undulating surface of internodular area at 3 months. They also claimed that entire nodule does not form the caruncle but only the apical portion of the nodule develops into caruncle because they observed the gland openings not only on the internodular area but also over the sides of the nodules during the first 6 months of age after birth. Logically the glands should only be present in the internodular region so they hypothesized that only the apical portion of the nodule forms the caruncle. The average height and width of the nodules in left, right and body was recorded as 963.95 ± 178.68 mm, 1036.86 ± 137.16 mm, 1142.75 ± 115.59 mm and 1557.40 ± 184.17 mm, 1533.91 ± 147.41 , 1487.87 ± 194.47 mm respectively in group 3. There seems to be a reverse relationship between the height and width of the nodules.

Myometrium: Smooth muscle cells of the mature organs are derived from mesenchyme, which is also the progenitor of fibroblastic cells (Konishi *et al.* 1984). Though the uterus is an estrogen target organ, several lines of evidence indicate that the ability of uterine epithelium to induce myometrial development is probably not an estrogen dependent process (Ogasawara *et al.* 1983).

In the present study in group 1 at 14.0 cm CVRL (90 days) the mesenchymal cells were circularly arranged but no indication of muscularis was evident. Further at 23.0 cm CVRL (125 days) the differentiation was better and at 35.0 cm CVRL (152 days) a distinct eosinophilic layer of densely packed cells which were arranged in circular manner parallel to uterine lumen was observed. This layer represented differentiating smooth muscle cells which are destined to develop into the inner circular layer of myometrium as described by Wiley *et al.* (1987) in fetal ovine uterus. Below this layer was a layer of loosely packed cells with elongated nuclei. Blood vessels could also be seen in this layer.

Baishya and Vyas (1997) observed the basic myometrium in 166-day-old buffalo fetus observed the formation of the myometrium. In group 3 at 41.0 cm CVRL the circular muscle cell layer was established with large number of blood vessels nearby. It was observed that the development of the muscle layer was caudo cranial i.e. the development was more in the body of uterus than the horns at a given time. Near the completion of gestation at about 100.0 cm CVRL the inner circularly arranged muscle layer was completely established, a layer of blood vessels i.e stratum vasculare was also seen. The muscle cells in different orientation could also be observed but the well organized outer layer of longitudinally arranged muscle cells was not observed. Wiley *et al.* (1987) also reported well developed both inner circular and outer longitudinal myometrial layers in neonatal ovine uteri. The average thickness of myometrium in left, right and body of uterus was 104.48 ± 6.12 mm, 108.75 ± 7.07 mm, 112.40 ± 6.72 mm in group 2 and 337.96 ± 63.07 mm, 350.52 ± 62.95 mm,

358.47 ± 80.24 mm in group 3 respectively. These values are in accordance with those observed by Baishya and Vyas (1997) in fetal uterus of Surti buffalo.

Perimetrium: In group 1 at 14.0 (91 days) cm the serosa consisted of loosely arranged mesenchymal cells, blood vessels and nerve fibers. In group 2 a mesothelial lining could be observed at 21.0 cm CVRL (121 days) and the differentiation was better at 32.5 cm CVRL. In group 3 at 43.0 cm CVRL (170 days) the perimetrium consisted of densely packed cells in a meshwork of collagen and reticular fibers. A large number of blood vessels and nerve fibers were also observed in the future broad ligament just outside of the perimetrium. Wiley *et al.* (1987) observed a serosal layer in the uterus of 90–100 day-old fetal uteri. The average thickness of the perimetrium of left, right horn and body of uterus in group 2 was 122.77 ± 2.11 mm, 132.23 ± 1.59 mm, 128.27 ± 4.22 mm and in group 3 was 202.03 ± 7.64 mm, 214.51 ± 5.88 mm, 202.90 ± 8.50 mm respectively. It was also concluded from the present study that the development of uterus proceeds caudocranially because the development was better in the body than in the horns at a given time.

SUMMARY

The present study was conducted on 27 female buffalo foetii of different gestational age ranging from 10.0 to 100.0 cm CVRL. The tissues were collected from both the horns and body of uterus and processed for paraffin block preparation. In the initial stage the uterine mucosa was lined by simple columnar epithelium which later on transformed into stratified columnar. At 25.0 cm CVRL the underneath mesenchymal tissue of the epithelium was organized into 2 layers and at 35.0 cm CVRL the inner mesenchymal cell layer was stratified into 3 layers. Uterine caruncles first made their appearance at 35.0 cm CVRL and were more prominent in the posterior part of the uterine horn. In the present study the uterine glands could not be observed till 100.0 cm CVRL. It was also concluded from the study that the development of uterus proceeds caudocranial i.e. from body of uterus to uterine horns.

REFERENCES

- Atkinson B A, King G J and Amoroso E C 1984. Development of caruncular and intercaruncular regions in the bovine endometrium. *Biology of Reproduction* **30**: 763–74.
- Baishya G and Vyas K N 1993. Prenatal development of the uterus in surti buffalo (*Bubalus bubalis*). Proc 8th Annual convention of Indian Association of Veterinary Anatomists. pp Bidhan Chandra Krishi Viswa Vidyalaya, Mohanpur, Nadia, West Bengal.
- Bartol F F, Wiley A A, Coleman D A, Wolfe D F and Riddell M G 1988. Ovine uterine morphogenesis: effects of age and progestin administration and withdrawal on neonatal endometrial development and DNA synthesis. *Journal of Animal Science* **66**: 3000–09.
- Bartol F F, Wiley A A, Spencer T E, Vallet J L and Christenson R K

1993. Early uterine development in pigs. *Journal of Reproduction and Fertility* (suppl) **48**: 99–116.
- Bazhenova N B 1975. Morphology of the endometrium in the fetal calf. *Sbornik Rabot Leningradsk2 Veterinaryi Institut* **41**: 195–202.
- Ginther O J 1979. Placentation and embryology. *Reproductive Biology of the Mare*. pp 255–320. (Ed.) Ginther O J. Ann Arbor, MI: McNaughton and Gunn.
- Gray C A, Bartol F F, Tarleton B J, Wiley A A, Johnson G A, Bazer F W and Spencer T E 2001. Developmental biology of uterine glands. *Biology of Reproduction* **65**: 1311–23.
- Konishi I, Fuj2 S, Okamura H and Mori T 1984. Development of smooth muscle in the human fetal uterus: an ultrastructural study. *Journal of Anatomy* **139**: 239–52.
- Luna L G 1968. *Manual of Histologic Staining: Methods of the Armed Forces Institute of Pathology*. 3rd edn. McGraw Hill Book Co, New York.
- Mori T and Nagasawa H 1988. *Toxicity of Hormones in Perinatal Life*. CRC Press, Boca Raton, FL.
- Ogasawara Y, Okamoto S, Kitamura Y and Matsumoto K 1983. Proliferative pattern of uterine cells from the birth to adulthood in intact, neonatally castrated and /or adrenalectomized mice, assayed by incorporation of I125 iododeoxyruridine. *Endocrinology* **113**: 582–87.
- Robertson H A and King G J 1979. Conjugated and unconjugated oestrogens in fetal and maternal fluids of the cow throughout the pregnancy. *Journal of Reproduction and Fertility* **55**: 463–70.
- Soliman M K 1975. Studies on the physiological chemistry of the allantoic and amniotic fluid of buffaloes at various periods of pregnancy. *Indian Veterinary Journal* **52**: 111–17.
- Uppal V and Roy K S 2002. Age correlated histomorphological changes in the endometrium of buffalo (*Bubalus bubalis*). *Indian Journal of Animal Sciences* **72**: 730–34.
- Wiley A A, Bartol F F and Barron D H 1987. Histogenesis of the ovine uterus. *Journal of Animal Science* **64**: 1262–69.