CONGENITAL ANOMALIES OF THE UTERUS IN RIVERINE BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

The developmental defects of the tubular genital tract including ovaries, oviduct, uterus, cervix and vagina have been reported in cattle as well as buffalo. These defects cause infertility and even complete sterility. As such less information on these aspects in buffalo is available. The present study reported the morphological and histological diagnosis of two rare congenital abnormalities of the uterus i.e. uterus unicornis and uterus didelphys in riverine buffalo.

Keywords: buffalo, *Bubalus bubalis*, terus, congenital anomalies, histopathology

INTRODUCTION

Congenital defects are the structural or functional abnormalities present at birth (Noden and De Lahunta, 1985). These defects are caused by either genetic or environmental factors or interaction of both the factors, and broadly classified into definite anatomical defects of reproductive organs and hereditary forms which are obscure in nature (Roberts, 1986). It is difficult to assess the origin of condition due to multi-factorial aetiology. Several developmental defects of the tubular genital tract including ovaries, oviduct, uterus, cervix and vagina as observed in cattle have also been reported in buffalo (Sharma *et al*., 1993; Hatipoglu *et al*., 2002; Saxena *et al*., 2006; Azawi and Ali, 2011; 2015). These defects cause infertility and even complete sterility (Agarwal *et al*., 2005). It is of utmost importance to diagnose and eliminate such defects in order to prevent their dissemination in the future generation through artificial insemination (AI). Congenital anomalies of the uterus reported in cattle include segmental aplasia of the paramesonephric duct, uterus unicornis, uterus didelphys and congenital lack of endometrial glands (Roberts, 1986). Meagre reports on these aspects are available in buffalo (Azawi *et al*., 2009; Azawi and Ali, 2011). Normally such defects could be viewed when slaughtered or diagnosed morphologically at the time of post-mortem examination. Histology based diagnosis of these defects has not been reported so far in farm animals. Histopathological examination reveals the exact changes that occur at tissue level which also provides additional information on

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diagnosis of such cases. The present study reports morphological and histopathological diagnosis of uterus unicornis and uterus didelphys in riverine buffalo.

MATERIALS AND METHODS

Buffalo genital tracts with observed congenital anomalies such as uterus unicornis and uterus didelphys were selectively collected immediately after exsanguination, during the period January 2014 to February 2015 along with routine sample collection (n = 750) for research purpose from local slaughter house, Bareilly, U.P., India. Genitalia were transported to laboratory on ice in plastic bags. Information regarding breed, identity and history of the animals were not available, though most of the samples were appeared to be of graded Murrah breed, which is predominantly observed in Rohilkhand region. Genitalia were examined grossly in the laboratory and observations were recorded. Morphologically, the case of uterus unicornis was diagnosed based on the presence of right uterine horn and a band like tissue instead of normal left horn. Similarly, genital tract with the presence of two external os of the cervix was diagnosed as uterus didelphys. The uterine tissues from right uterine horn and band like tissue from uterus unicornis case were preserved in 10% neutral buffered formalin for histopathological examination. Tissues were processed, embedded in paraffin and sections of 5 µm thickness were prepared. These sections were stained with hematoxylin and eosin (H and E) stain (Luna, 1968) and examined under light microscope for evaluation.

RESULTS AND DISCUSSION

The uterus unicornis condition observed in the present study was characterised by complete aplasia of left uterine horn which was completely

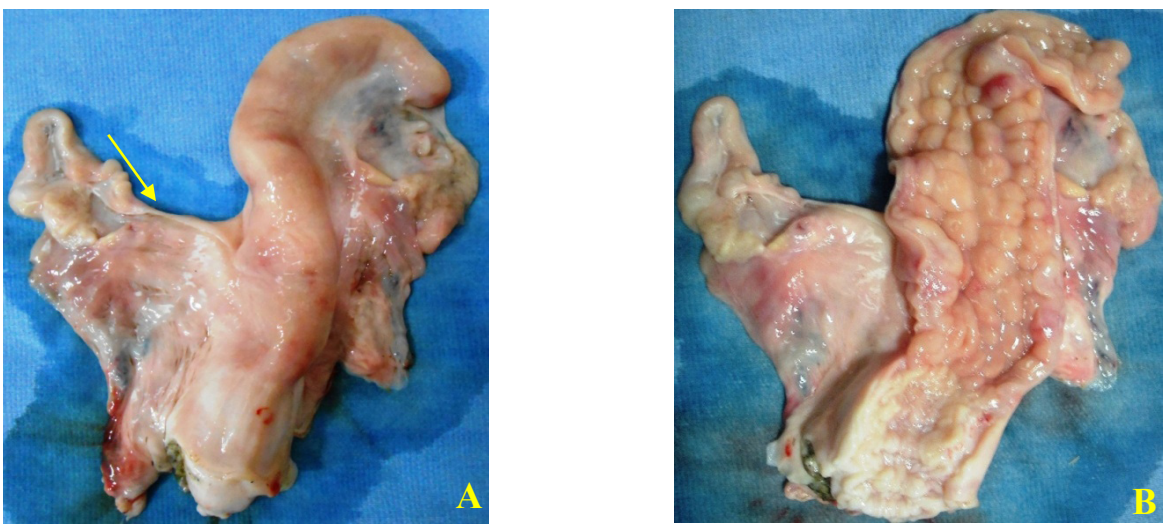

Figure 1. Uterus unicornis; (A) Aplasia of left uterine horn (yellow arrow), (B) Normal right uterine horn and cervix (black arrows).

replaced by band like tissue (Figure 1A). Right uterine horn development was normal and four cervical rings were noticed (Figure 1B). Ovaries and oviducts development were apparently normal on either side. Histopathology of band like tissue of aplastic left uterine horn revealed absence of normal architecture of the uterus, failure of the development of endometrial lumen, lining epithelium and endometrial glands. However, irregularly dispersed smooth muscle bundles were present in between the primitive loose connective tissue stroma along with numerous blood vessels (Figure 2). On the other hand, right uterine horn tissue section revealed normal endometrial lining epithelium and the stromal layer with endometrial glands. The myometrium was consisting of inner circular and outer longitudinal smooth muscle fibres with outer perimetrium (Figure 3).

Uterus unicornis is a congenital anomaly results from developmental failure of paramesonephric duct/ Mullerian duct also known as "white heifer disease", if complicated with persistent hymen. When segmental aplasia involves both the horns it is called as 'aplasia uteri totalis'; however, if only one horn is involved, then it is a case of 'uterus unicornis' and in some cases only a part of the horn involves resulting into cystic dilation of the uterine horn cranial to aplastic area (Agarwal *et al*., 2005; Vince *et al*., 2011). In the present case, the aplasia involves only left uterine horn while the development of right uterine horn, both oviduct and ovary were appeared normal. The condition has been reported in cattle (Roberts, 1986; Hatipoglu *et al*., 2002), buffalo (Kozicki *et al*., 2001), horses (Brown *et al*., 2007), dogs (Vince *et al*., 2011) and cats (Robinson, 1965). During embryonic development, paramesonephric ducts arise in pairs as denser cords in the mesodermal tissue of the nephric ridge and their caudal portions take part in the development of uterus and upper

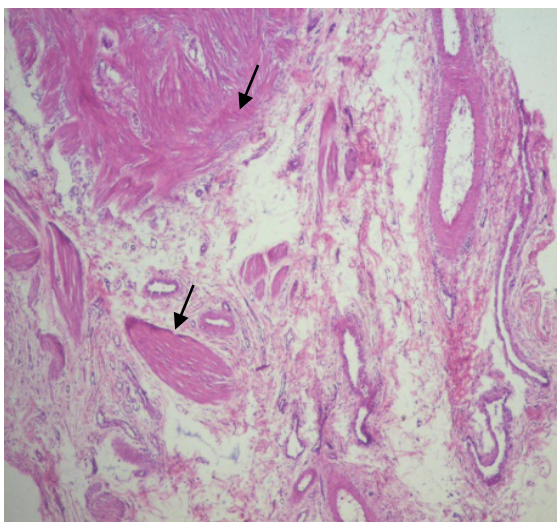

Figure 2. Absence of endometrial layer and irregularly dispersed muscle bundles within the loose connective tissue stroma, H and E, 10X.

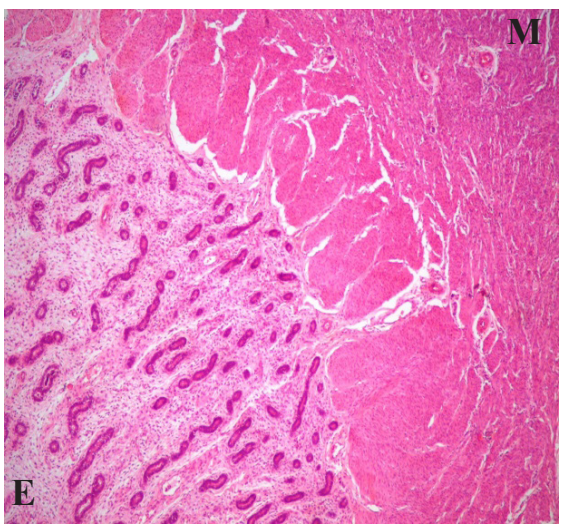

Figure 3. Normal endometrial (E) and myometrial (M) layer in right uterine horn, H and E, 4X.

end of vagina (Bullmer, 1956). These cords are solid in early development but tend to develop lumen about the time of median fusion of their caudal portions, when the cords become closely associated with dorsal wall of urogenital sinus. Because of common embryonic origin of uterus and kidneys, uterus unicornis is often associated with unilateral renal agenesis (Robinson, 1965).

In most cases, development of ovaries was normal as it develop from gonadal ridge (McEntee, 1990). Histologically, Vince *et al.* (2011) reported presence of irregular thick smooth muscle bundles separated with a small amount of collagen fibers, along with numerous blood vessels in canines. However, histological findings of the present case revealed characteristic changes like absence of normal architecture of uterus and endometrial lumen formation in the aplastic left uterine horn. Presence of irregularly dispersed smooth muscle bundles in between the primitive loose connective tissue indicated the formation of myometrial layer but its development might have arrested later on. To the best of our knowledge, no report is available on histopathology of uterus unicornis in bovine. Animals with such congenital defect suffer from infertility or sterility and should not be used for breeding purpose and must be culled.

Uterus didelphys condition as observed in present study was characterised by presence of two completely separated cervices with its own external and internal os leading to either left or right uterine horn (Figure 4A and Figure 4B). To confirm further, two AI gun were passed through the both external os, the gun passed freely and opened into respective uterine horn. Further, both horns were incised staring from cervix to uterotubal junction. Morphologically, there was complete separation of both uterine horns. The uterine body and intercornucal ligaments normally present in the bicornuate uterus, were absent in the present case. Both the ovaries were morphologically normal and functionally intact as evidenced by the presence of

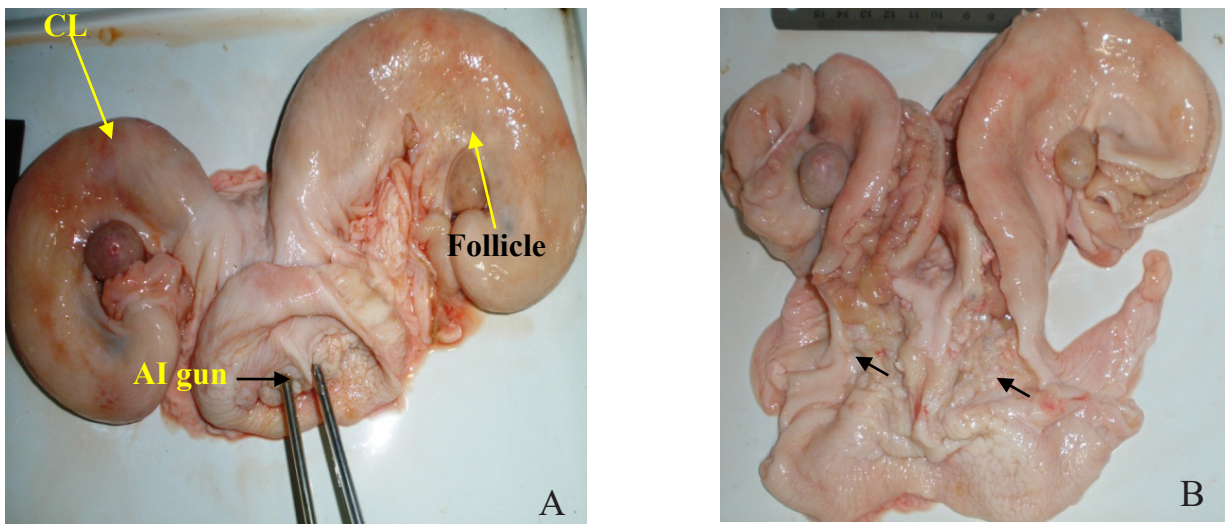

Figure 4. (A, B). Uterus didelphys- Completely separate cervices (small black arrows) and presence of CL (LO) and follicle (RO) (yellow arrows).

a >8mm diameter follicle in the right ovary (RO) and a well developed completely embedded corpus luteum (CL) in left ovary (LO) (Figure 4A).

Uterus didelphys or true double cervix is a rare congenital anatomical defect of the female genital tract reported in different species including cattle (Raggio *et al*., 2006), buffalo (Azawi *et al*., 2008; Azawi *et al*., 2009), sheep (Smith *et al*., 1995), goat (Timurkaan and Ozer, 2002) and horse (Volkmann and Gilbert, 1989). The findings of the present study are in accordance as reported in different farm animals. The anomaly is characterised by completely separate cervices each leading to separate uterine horn. This results from a complete failure of fusion of the caudal portions of Mullerian ducts during embryonic development resulting into double cervix and divided uterine body (Roberts, 1986). Uterus didelphys is a normal anatomical feature of the reproductive tract in rabbits (Hafez, 1970) and some marsupials (Eckstein and Zuckermann, 1956). The condition is thought to be hereditary in nature and associated with recessive gene of unknown etiology (Roberts, 1986). Based on the abattoir studies in Sweden (Alam, 1984) and Finland (Roine, 1977), the frequency of uterus didelphys was very low in the bovine population. However, 0.2% incidence has been reported in sheep (Smith *et al*., 1995). Similarly, only one case has been reported so far in buffalo (Azawi *et al*., 2009). Raggio *et al.* (2006) clinically diagnosed uterus didelphys in Ayrshire heifer by vaginal examination along with ultrasonography by infusing saline to one of the uterine horn and confirmed the unicornual fluid distribution on repeated massage.

Further, ovary and oviduct was normal with >12 mm follicle on left ovary on necropsy examination. The presence of CL in left ovary and follicle in the right ovary in present case might have indicated normal cyclic activity of the animal. Animals with such defect may have normal conception rate after natural service, however, conception rate may be reduced during artificial insemination when semen is deposited in the uterus contralateral to the ovary where ovulation has occurred. Animals having uterus didelphys defects reported to have normal pregnancy and calving (Roberts, 1986), but some animals may suffer from dystocia due to fetal limbs entering in each cervical canal (Noakes, 2009). Because of the possible genetic nature of the defect, such animals should not be used for breeding after diagnosis and must be culled.

In conclusion, the present study reports the gross and histopathology of uterus unicornis and uterus didelphys in the riverine buffalo.

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