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Histological and histochemical studies on the genitalia of a bovine freemartin

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Abstract

Study of structure of genital system of the animal is important for the diagnosis of genital disorders which in turn help in the enhancement of production. The majority of female calves born as hetero-sexual twins are sterile. The present study was conducted to evaluate anatomical, histological and histochemical alterations of the reproductive tract of a freemartin cow obtained from the Livestock Research Station, Thiruvazhamkundu. The external genitalia of the animal was normal and there was no tuft of hairs in the vulvar region. After recording the gross observations, histological and histochemical studies were conducted using sections obtained from different regions of the tract. Gross studies revealed that ovaries, oviduct, uterus and cervix were absent. Instead of that there was an elongated tube of length 60 cm and diameter 4 cm with a dilated posterior portion. Sections were stained using Haematoxylin and Eosin, Masson's trichrome and van Gieson's stains. The wall of the tube was not organized into the three tunics. Instead, the wall was made up of bundles of smooth muscles interspersed with dense connective tissues. The glands were absent. The histochemical studies showed that the luminal cells were negative for alcian blue (pH 2.5) indicating the absence of sulfated acidic and neutral mucopolysaccharides and the secretory activity. The findings indicate that the abnormal tissue architecture affects the uterine environment and it results in the failure of reproduction. So it is important to be diagnosed at early age to replace the stock population and to avoid the economic loss to the farmers.

Keywords: Genitalia, freemartin, histology, Histochemistry

Introduction

Freemartinism is a particular form of genital disorder in cattle. The freemartin phenotype appears in a dizygotic twin pregnancy where calves born as hetero-sexual twins characterized by gonadal transformation towards male reproductive structure and the presence of reproductive organs of both sexes. According to Ayalavaldivinos *et al.*, 2014, the incidence is higher in cattle than in other species. This syndrome develops in the female foetus because of vascular connections formed between the placenta of developing heterosexual twin foetus [2]. The degree of masculinization and developmental alterations of reproductive organs in freemartins vary from animal to animal [3]. A significant feature of the freemartin heifer is that the external genitalia are usually female in type whereas the internal genitalia show varying degrees of masculinisation, impairing reproduction and fertility. Absence of anatomical continuity in the genital system with hypoplastic or elongated gonads is common findings in freemartin heifers. Comparing the changes observed in genital system of freemartin heifer, the male co-twin shows minimal defects, with a history of subfertility. Congenital, freemartinism is not a heritable defect. Freemartins can also occur in single female births, as a result of the early fetal death of the male twin within the uterus after the development of vascular anastomoses [4]. Freemartinism can be diagnosed by physical examination and karyotyping. Study of structure of genital system of the animal is important for the diagnosis of these types of genital disorders which in turn help in the enhancement of production.

Materials and Methods

The present study was conducted on the reproductive tract of a freemartin heifer of one and half year age obtained from the Livestock Research Station, Thiruvazhamkundu. The reproductive tract was collected from the Department of Livestock Products Technology, Mannuthy.

Gross parameters were recorded with the help of Vernier Calipers and non-stretchable thread. After recording the gross observations, the tissues from different regions of the tract were

fixed at 10 percent Neutral Buffered Formalin (NBF) for 24-48 hours and subsequently tissues were processed for routine paraffin block preparation by alcohol-xylene schedule. Tissue sections of 5µm thick were stained using Haematoxylin and Eosin, Masson's trichrome and van Gieson's stains [5]. In order to evaluate the secretory activity, the tissue sections were stained using Alcian blue (pH 2.5) stain [5].

Results and Discussion

The external genitalia of the animal was normal and there was no tuft of hairs in the vulval region (Fig 1 and 2). The internal genitalia was a hollow, elongated, musculo-membranous tube of 60 cm in length and 4 cm in diameter with a dilated posterior portion of diameter 10 cm (Fig 3). The weight of the reproductive tract was measured to be 600 g. According to Esteves *et al.* (2012), anti-Mullerian hormone and androgens were responsible for disrupted differentiation of the female embryonic gonads and abnormal development of genital tract. In some animals the uterus was rudimentary or reduced to a thin tubular structure with an enlarged clitoris, small vulva and a prominent tuft of hairs. Most frequently, oviducts were absent on gross examination.

Histologically the wall of the female genital tract shows the layers *viz.*, tunica mucosa, tunica submucosa, tunica muscularis (myometrium) and tunica serosa (perimetrium) from interior to exterior [7]. In the present study, the wall of the tube was not organized into the different tunics. Instead, the wall was made up of bundles of smooth muscles interspersed with dense connective tissues (Fig. 4). The connective tissue stroma consisted of mainly collagen fibres as demonstrated using Masson's trichrome and van Gieson's stains (Fig. 5 and 6). The endometrial glands were absent. Usually the lining epithelium got invaginated into propria submucosa forming endometrial glands. Endometrial glands are present in all mammalian uterus and their secretion is required for implantation, survival of the conceptus and development of the foetus. Khan *et al.* (1994) stated that because of the failure of differentiation, normal organogenesis would be disturbed with disorganization of myometrial layers, reduction of glandular elements and reduced thickness of endometrium.

The histochemical studies showed that the luminal cells were negative for alcian blue (pH 2.5) indicating the absence of sulfated acidic and neutral mucopolysaccharides and the secretory activity (Fig. 7). In the functional uterus, uterine glands secrete a variety of molecules like enzymes, cytokines, growth factors, ions, hormones, glucose, transport proteins, and adhesion molecules which are essential for maternal support of the conceptus⁹. The findings indicate that the abnormal tissue architecture and secretory activity affect the uterine environment and it results in the failure of reproduction.



Fig 1: Freemartin heifer



Fig 2: External genitalia



Fig 3: Internal genitalia of Freemartin heifer

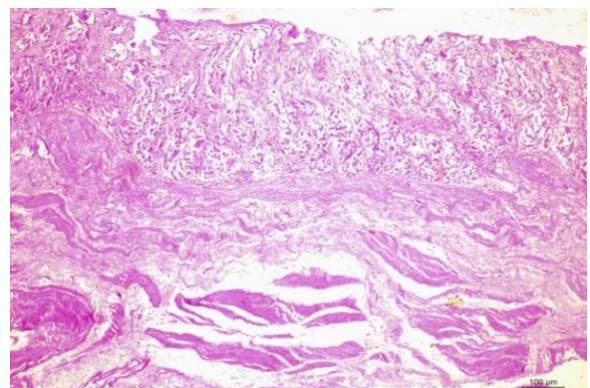


Fig 4: Wall of genitalia. H&E x10

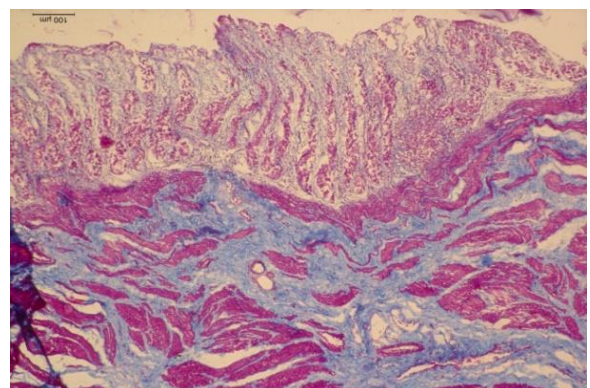


Fig 5: Wall of genitalia Masson's trichrome x10

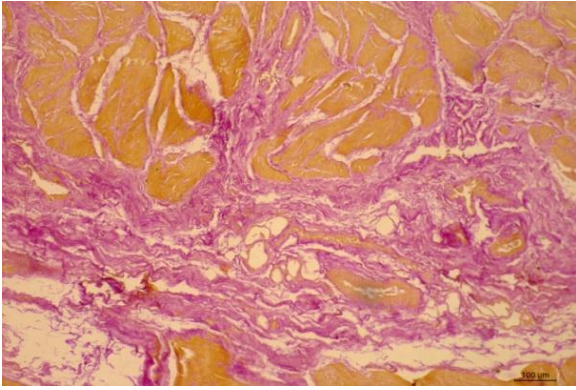


Fig 6: Wall of genitalia van Gieson's x10

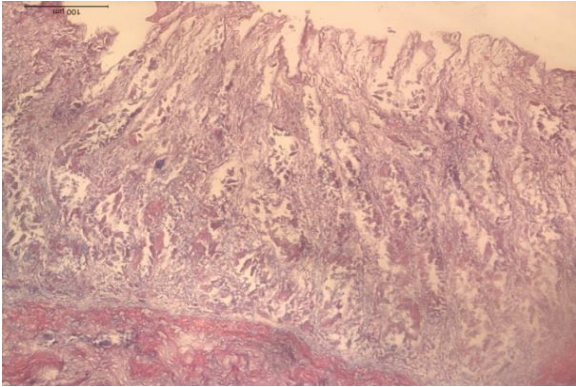


Fig 7: Wall of genitalia Alcian blue (pH 2.5) x10

Conclusion

Freemartinism is a congenital syndrome that is associated with the loss of calf and milk production. Sterility is related to the abnormal anastomoses between the placental vessels of embryos of distinct gender, which predispose the female embryo to abnormal masculinizing substances. The degree of differentiation of the gonads influences the development of the remaining genitalia. The results of the study showed that the wall was made up of bundles of smooth muscles interspersed with dense connective tissues. The endometrial glands were absent. The findings of the present study indicate that the abnormal tissue architecture affects the uterine environment and it results in the failure of reproduction. So it is important to be diagnosed at an early age to replace the stock population and to avoid the economic loss to the farmers.

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