

Short Communication

Macroscopic and Microscopic Findings in a Set of Congenital Anomalies in Two Calves Produced Through *in Vitro* Production

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Abstract

In vitro production and somatic cell nuclear transfer are biotechnologies widely used for breeding cattle, although may result in congenital anomalies. This paper aims to report a set of congenital anomalies in two Nelore calves, a male and a female, produced through *in vitro* fertilization. The major anomalies revealed at necropsy were hypospadias, bifid scrotum, atresia ani and rectum ending in blind pouch in the male calf. In the female calf accessory spleen, atresia ani, underdevelopment of extern genitalia and urethral orifice, and rectum ending in blind pouch forming a uterus-rectum fistula were observed.

Key Words: congenital anomalies, hypospadias, accessory spleen, atresia ani, *in vitro* production, bovine.

Description

It is well-known that the use of biotechnologies for breeding cattle, such as *in vitro* production (IVP) and somatic cell nuclear transfer (SCNT), may result in congenital anomalies (4). Despite a not completely understood process, it is possible that anomalies occur due to errors at critical periods of *in vitro* embryo preimplantation, resulting in wrong epigenetic patterns, which affect the imprinted gene expression. The main causes may be inadequate *in vitro* culture conditions and improper genetic reprogramming during SCNT procedures (4). Nevertheless, few studies focus on correlation among these techniques and cattle congenital defects, as Birgel et al (2011) (2), that characterizes various clinical features, such as fetal hypoxia and umbilical cord defects in animals produced by SCNT. Santos et al (2010) (12) describe IVP animals with cardiorespiratory alterations and changes of oversized animals. Rodrigues et al (2011) (11) correlate the umbilical cord defects in IVP and SCNT calves, and a review by Farin et al (2006) (4), which includes a proposal for classification according to the degree of abnormal

development of these animals. Thus this paper aims to report a set of congenital abnormalities in two Nelore calves produced by the technique of *in vitro* production.

Two Nelore calves were referred to the Veterinary Hospital from the same farm both bred through IVP: a male (animal 1) and a female (animal 2) one and two-day-old, respectively, both with obvious congenital abnormalities. Both of them were born naturally, without complications; no further technical information about IVP was supplied. However, because of their poor prognosis, the two animals were euthanized and sent to the veterinary pathology service.

Animal 1, at postmortem examination, had a wide hypospadias, from perianal up to the preputial region, characterized by urethral externalization at the abdominal midline with ventral incomplete closure and leading to a bifid scrotum (Figure 1), lack of anal orifice that characterizes atresia ani, moderate fibrinous omphalophlebitis, and oblong shaped bladder with severe mucosal edema and hemorrhage. It also had absence of

the distal segment of the rectum that ended in a blind pouch.

The female calf (animal 2), at postmortem examination showed underdevelopment of extern genitalia and urethral orifice, both grossly similar, measuring one centimeter in diameter, moderate fibrinous omphalophlebitis, atresia ani (Figure 2), oblong shape bladder with severe mucosal edema and hemorrhage, patent urachus and accessory spleen (Figure 3a), characterized by an irregular, shrunk and darker parenchyma, attached near to a normal sized spleen. The accessory spleen had a parenchyma composed only by erythrocytes and absence of lymphoid follicles (Figure 3b). Distal rectum aplasia, which ended in a blind pouch, with an obvious communication canal to the uterus (uterus-rectum fistula) (Figure 4), was also observed with lumen filled with a large amount of meconium, and ovaries with multifocal cystic parenchyma. Microscopically, both genitals and urethral structures were similar, characterized by a rudimentary lumen surrounded by a circle of rudimentary mesenchymal tissue, with an external halo of rudimentary epithelial tissue and externally covered by skin (Figure 5). It was visualized in both ovaries multiple dilated follicles, irregular in size and randomly distributed.



Figure 1. A) Animal 1, Bifid scrotum. Notice a lack of scrotum fusion (white arrows). Hypospadias: abnormal and externally position with incomplete closure of the urethra, notice a wide extension through scrotal, anal and perianal regions (black arrows). B) Animal 2, Atresia ani, characterized by absence of anal orifice (black arrow)

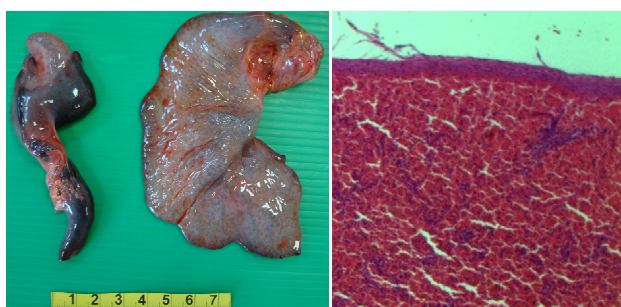


Figure 2. a) Accessory spleen, Animal 2, on the right side the normal spleen and the left side, accessory spleen (black arrow), characterized by irregular dark parenchyma. b) Histological section of accessory spleen. Notice an absence of lymphoid follicles. HE Stain. 20x.

A proposal made by Farin et al, (2006) (4) aims to facilitate the studies of congenital anomalies following biotechnologies such as IVP and SCNT, then called

“abnormal offspring syndrome (AOS)”, and consists in division of abnormalities into four types, according to the lifetime and severity of the alterations, and includes embryos, fetuses and newborns. Type I is characterized by embryonic death. In type II it occurs fetal death followed by abortion. Type III includes severe developmental abnormalities and death in the perinatal and type IV, animals alive at birth, normal-sized or not, and with malformations of variable severity. The two animals in our report fall into AOS type IV, with variable degrees of abnormalities, but alive at birth.



Figure 3. Animal 2, uterus-rectum fistula, notice distal rectum aplasia, with blind pouch formation (red arrow), and uterus filled with a large amount of meconium (asterisk)..

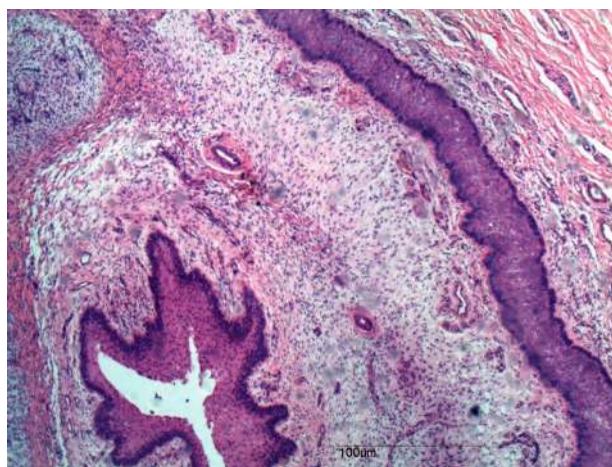


Figure 4. Animal 2, genital structure. Notice an epithelium covered lumen (asterisk) surrounded by a circle of rudimentary mesenchymal tissue (#), adjacent to a halo of epithelial cells (black arrows). HE stain, bar scale = 100µm.

Atresia ani is the most common congenital abnormality of lower digestive tract (3). On veterinary medicine, atresia ani is divided into four grades. Grade I is characterized by stricture formation in the anus and normal rectum. In grade II, anus aplasia and distal rectum ends in a blind pouch, or with imperforate anus membrane and normal rectum. Grade III is characterized by absence of anus and rectum aplasia, which ends in a distal blind pouch and in grade IV, there is normal anus with rectum in proximal blind pouch (7, 10). The animals 1 and 2 with atresia ani and rectum absence in this report fall within grade II, and are similar to the case described by Rocha et al (2010) (10) and three cases described by Macedo et al (2011) (8), although these last authors did not use degrees.

Hypospadias is a rare congenital alteration, characterized by abnormal and external position with incomplete closure of the urethra. Hypospadias may be classified according to the affected local as: glandular, penile, scrotal, perineal, or anal (1, 9). In our case, animal 1 hypospadias could be classified as scrotal, anal and perianal due to its extension. Bifid scrotum occurs by a failure in fusion of the scrotum, and accompanies other defects as hypospadias and atresia ani (5). In this case, animal 1 bifid scrotum was similar to the reported by Alam et al (2005) (1) and Loynachan et al (2006) (7). These authors also describe other associated anomalies to bifid scrotum like atresia ani and hypospadias, comparable to our case.

Generally spleen defects are relatively rare, from those accessory spleen is the most common anomaly. The accessory spleen differs macroscopically from the normal organ by lack of white pulp, and microscopically, by absence of lymphoid tissue (6). In this report, histopathological analysis of accessory spleen, from animal 2, showed no lymphoid follicles, being visible only the red pulp with large amounts of erythrocytes. For the best we know, there are no other case report that describes an accessory spleen as result of IVP congenital anomalies.

Formations of fistulas in the digestive tract may be caused by atresia of any segment and consequent adherence to neighboring organs (8). Among cases from a retrospective study of congenital defects written by Macedo et al (2011) (8), there is only one animal with a fistula similar to our report, though the authors describe it as a diverticulum in the final portion of the colon with a fistula into a structure similar to the uterus, with subsequent filling by meconium. In our case, from animal 2, the fistula was initiated by rectum aplasia and connected to a certainly well developed uterus, and also filled with meconium.

Patent urachus is defined as the no-closure of the conduit between bladder and umbilical cord, which results in an abnormal connection with high infection risk due to urine dripping (Macedo et al, 2011) (8). Rodrigues et al (2010) (11) described several omphalus disorders that occur in animals produced by different biotechnologies, and report a significant correlation between patent urachus

and animals produced by IVP. In our case, animal 2 showed this condition, but fibrinous omphalophlebitis was detected in both animals, 1 and 2.

Rocha et al (2010) (10) report a bladder with oblong shape and mucosal hemorrhage among several development abnormalities in calves. This is the only change shared with the calves from our report. Multiple ovarian follicles, to the best of our knowledge, were not included in any former descriptions, as well as underdevelopment of external genitalia and urethral orifice, both in animal 2.

Thus, we conclude that our two cases were similar to those described in other reports with congenital abnormalities as: hypospadias, bifid scrotum and atresia ani. However, we found alterations that were not described until now, such as: accessory spleen, uterus-rectum fistula, rudimentary genital and urethral structures and multiple dilated follicles in the ovary. Some of these changes had presented life-threatening degrees.

Despite the well-known possibility of developing congenital abnormalities caused by *in vitro* production technique, few reports are made on veterinary medicine. It is possible that an inadequate *in vitro* culture condition is the main factor of a downstream effect which ends in abnormal development of IVP breed cattle, and there is a clear trend of reducing this type of error due to improvements made in the field of biotechnology reproduction.

References

1. ALAM, MR., SHIN, SH., LEE, HB., KIM, CNS. Hypospadias in three calves: a case report. **Vet. Med. – Czech.**, 2005, 50, 506-9.
2. BIRGEL JUNIOR EH., MEIRELLES, FV., MAIORKA, PC., KUBRUSLY, FS., OLHOFF, RD. Medicina interna de bezerras clonados – distúrbios observados nos primeiros 30 dias de vida. **Rev. Educ. Cont. Med. Vet. Zoot. CRMV-SP.**, 2011, 9, 24-31.
3. BROWN, CC., BAKER, DC., BARKER, I. Alimentary System. In: JUBB, KVF., KENNEDY, PC., PALMER, N., GRANT, M. Eds. **Pathology of Domestic Animals** v.2., 5th ed, Academic Press: San Diego, 2007: 1-296.
4. FARIN, PW., PIEDRAHITA, JA., FARIN, CE. Development of fetuses and placentas from *in vitro*-produced bovine embryos. **Theriogenology.**, 2006, 65, 178-91.
5. FOSTER, RA., LADDS, PW. Male Genital System. In: JUBB, KVF., KENNEDY, PC., PALMER, N., GRANT, M. Ed.s **Pathology of Domestic Animals** v.3., 5th ed, San Diego: Academic Press, 2007: 566-619.
6. FRY, MM., McGavin, MD. Bone marrow, blood cells, and the lymphatic system. In: ZACHARY, JF., McGavin, MD. Eds. **Pathologic Basis of Veterinary Disease.**, 5th ed. St Louis: Elsevier, 2012: 698-770.
7. LOYNACHAN, AT., JACKSON, CB., HARRISON, LR. Complete diphallia, imperforate ani (type 2 atresia ani), and

- an accessory scrotum in a 5-day-old calf. **J. Vet. Diagn. Invest.**, 2006, 18, 408-12.
8. MACEDO, JTSA., LUCENA, RB., GIARETTA, PR., KOMMERS, GD., FIGHERA, RA., IRIGOYEN, LF., BARROS, CSL. Defeitos congênitos em bovinos da região central do Rio Grande do Sul. **Pesq. Vet. Bras.**, 2011, 31, 297-306.
 9. NASCIMENTO, EF., SANTOS, RL., EDWARDS, JF. Sistema Reprodutor Masculino. In: SANTOS, RL., ALESSI, AC. Eds. **Patologia Veterinária**. São Paulo: Roca, 2011: 856-80.
 10. ROCHA, GR., LASKOSKI, LM., LOPES, MCS., BERLINGIERI, MA., MAGALHÃES, GM., ALESSI, AC. Anal atresia, congenital urethrorectal fistula, accessory scrotum and pseudohermafroditism in a crossbred calf. **Ciênc. Rural.**, 2010, 40, 1231-34.
 11. RODRIGUES, CA., SANTOS, PSP., PERRI, SHV., TEODORO, PHM., ANHESINI, CR., ARAÚJO, MA., FILHO, MNV. Correlação entre os métodos de concepção, ocorrência e formas de tratamento das onfalopatias em bovinos: Estudo Retrospectivo. **Pesq. Vet. Bras.**, 2010, 30, 618-22.
 12. SANTOS, CR., GRANDI, F., MIGLINO, MA., MEIRELLES, FV., MAIORKA, PC. Patologia de neonatos bovinos originados por meio da técnica de transferência nuclear de células somáticas – Clonagem. **Braz. J. Vet. Res. Anim. Sci.**, 2010, 47, 447-53