HISTOPATHOLOGIC CHARACTERIZATION OF "IVP" CALVES THAT DURING THE PERINATAL PERIOD - CASES REPORT

CARACTERIZAÇÃO HISTOPATOLÓGICA DE BEZERROS GERADOS POR "PIV" VINDOS A ÓBITO EM FASE PERINATAL – RELATO DE CASOS

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SUMMARY

The aim of the study was to report histopathological alterations in different organs and to describe the possible cause of death in IVP (*in vitro* production) calves that die during the perinatal period, with the intention to characterize the main lesions related to the death of these animals. Five hundred and twenty nine labors of IVP embryo recipient cows were monitored. Animals that did not deliver until the 290th gestational day and had labor induced as used by farms: Flucortan® (10mL i.v.); if an animal did not respond to this injection, Flucortan® (10mL i.v.) was administrated again at the 295th gestational day. Only if the above mentioned animals did not show any characteristics of parturition, there was administrated Azium® (10mL i.v.) at the 300th day. The assessment for the developmental pattern of the calves in the perinatal period was accomplished. Necropsy was performed in the fatal cases (n=42). Samples of several organs were collected and sent for histopathological examination, with the release of result reports. The main organs affected were kidneys, lungs and liver. The definitive post-mortem diagnosis of the death cause was only possible for 17 animals out of the 42 studied. The septicemia (n=5) and endotoxemia (n=5) were the major cause of the death answerable in the report of necropsy.

KEY-WORDS: *In vitro* production. Calves. Histopathological characterization. Perinatal period.

RESUMO

O objetivo do presente estudo foi descrever as alterações histopatológicas em diversos órgãos e a possível causa da morte de bezerros oriundos de produção *in vitro* que vieram a óbito durante o período perinatal. Foram monitorados 529 partos de receptoras de embriões produzidos *in vitro*. Os animais que não pariram até o 290º dia de gestação tiveram o parto induzido com protocolo empregado pelo veterinário responsável pelas propriedades: Flucortan® (10mL i.v.), se o animal não respondesse a essa primeira indução, no dia 295 recebia uma segunda aplicação de Flucortan® (10mL i.v.). Se após a segunda aplicação o animal não entrasse em trabalho de parto, era administrado Azium® (10mL i.v.) no 300º dia. A avaliação do desenvolvimento dos bezerros foi realizada no período pós-parto. Nos recém-nascidos que morreram (n=42) realizou-se necropsia com colheita de material de diversos órgãos e essas amostras encaminhadas ao serviço de patologia para o exame histopatológico com emissão de laudo. Os principais órgãos afetados foram rins, pulmões e fígado. O diagnóstico de post-mortem foi possível em 17 dos 42 animais estudados. A septicemia e a endotoxemia foram as principais causas de morte descritas nos laudos histopatológicos.

PALAVRAS-CHAVE: Produção *in vitro*. Bezerros. Caracterização histopatológicas. Período perinatal.

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CASES REPORT

Over the past 50 years, biotechnology improved continuously to meet production needs for the consume demand. Progress in animal reproduction: as semen cryopreservation, artificial insemination, folicullar superstimulation, embryo transfer, ovum pick up, *in vitro* production of embryos, sperm and embryo sexing, transgenesis and cloning. Has speeded up reproductive efficacy and genetic gain, specially for cattle. Other promising standpoint is genetic engineering, selecting genes linked to specific traits, varying the germ cells population in animals used for traditional breeding.

Precise genetic manipulation has economic impact for animal production, mainly for cattle, and takes into account the possibilities of positive influence on growth rates, improvement of body condition, disease resistance and creation of bioreactors for the pharmaceutical industry, a revolution for the human therapeutic arsenal (WOLF, 2001, GONÇALVES et al., 2002a).

The main problems detected in bovine breeding systems do not allow the complete use of advanced bio-techniques. Brazilian low average production rates are caused by basic problems such as nutritional deficiency, lack of prevention for virus and bacterial diseases, parasitic diseases, and inept reproductive management (GONÇALVES et al., 2002b).

Prestes (2005) affirms that when noticed problems of gestation and parturition of *in vitro* production (IVP), offspring deserves careful reflection due to scarcity of statistically reliable data on the national herd in the literature.

Several problems during gestation, calving, and postnatal life of IVP calves have been reported, such as increased birth weight (WALKER et al., 1996), prolonged gestation period (KRUIP & DEN DAAS, 1997), dystocia (HASLER, 2000), a greater incidence of hydroallantois (VAN WAGTENDONK-DE LEEUW et al., 1998), increased incidence of abortions (HASLER et al., 1995), congenital abnormalities (SCHMIDT et al., 1996), and enhanced peri- and postnatal mortality (BEHBOODI et al., 1995). The "large offspring syndrome" (LOS) is of particular interest. Causes of LOS are thought to be factors present in culture systems used for preimplanted stages of embryos, such as high amounts of ammonia (McEVOY et al., 1997), enhanced activity of growth factors present in fetal serum used in culture media, and paracrine growth factor influences associated with embryonic cell interactions in the co-culture (VAN WAGTENDONK-DE LEEUW et al., 2000). Possibly contributing to the development of LOS, embryonic manipulations and the surrounding environment before implantation may result in inappropriate epigenetic modifications of imprinted genes and affect gene expression during later fetal development (YOUNG & FAIRBURN, 2000; YOUNG et al., 2001). To date, most studies on IVP calves have focused on the perinatal period, characterizing problems such as the viability of IVP bovine embryos (TAVERNE et al., 2002), abnormally high birth weight (YANG et al., 2001), calving, and metabolic and endocrine status (JACOBSEN et al., 2002, SANGILD et al., 2000).

Having command over IVP technology, which is being implemented in the country on an extensive commercial scale; breeders, technicians, researchers and businessmen should invest assets and effort for the improvement of the technique, correcting distortions, know biochemical and physiologic mechanisms: capacitate involved personnel, follow bioethical precepts guaranteeing creation of employment, and consolidating the procedure in a save full-credibility manner (PRESTES, 2005, RUMPF & MELO 2005).

The present study has the objective to report histopathological characterization and to describe the possible cause of death for IVP calves that die in the prenatal period, with the intention of characterizing the main lesions related to the deaths of these animals.

This study was performed at the Department of Animal Reproduction and Veterinary Radiology in collaboration with the Veterinary Pathology Service of the College of Veterinary Medicine and Animal Sciences, Botucatu, Brazil ($22^{o}51'S/48^{o}26'W$) in the period from January of 2005 to December of 2006.

Five hundred and twenty nine IVP embryo recipient cows of mixed breeding, aged 3 to 8 years were used in this study. Herd health and vaccination programs were performed according to the farm management schedule. The animals belonged to farms located in the Avaré region, São Paulo, Brazil ($23^{o}03'S/48^{o}55'W$). The IVP embryos and the inovulations were performed by a private company.

The recipient cows had confirmed soundness and were maintained in pasture with silage and concentrate supplementation, in addition to water *ad libitum*. Close to the moment of parturition, recipients were transferred to appropriate maternity paddocks so that parturitions could be monitored and assisted if necessary.

Cows that did not deliver until the 290^{th} gestational day (normal duration of the gestation) had labor induced by protocol used on the farms: Flucortan® (10mL i.v.); if an animal did not respond to this injection, Flucortan[2]® (10mL i.v.) was administrated again at the 295^{th} gestational day. Only if the above mentioned cows showed no results, there was administrated Azium[3]® (10mL i.v.) at the 300^{th} day. This protocol for labor induced was implemented because the effect of corticosteroids as dexametaxone and flumetaxone in the end of pregnancy induce the labor, by increasing the production of $PGF_{2\alpha}$ in the endometrium and, consequently, the luteolysis. Furthemore accelerates fetal lung maturation and improves respiratory function after birth because the production of surfactant is enhanced by the effect of corticosteroids (PRESTES &LANDIM-ALVARENGA, 2006).

The assessment of the developmental pattern of the calves in the perinatal period was accomplished. All

[2] Flucortan® - Flumetasona 0,5mg/mL – Fort Dodge, Brazil
[3] Azium® - Dexametasona 0,5mg/mL – Schering, Brazil

calves sucked the colostrum within 6 hours after the delivery. Necropsy was performed on the fatal cases. Samples of liver, heart, kidneys, spleen, lungs, encephalo and gastrointestinal tract were collected and sent for histopathological examination, with the release of result reports.

All recipient cows had induced parturition on day 290, 15% were treated on day 295 and just 5% they had needed treated on day 300. Beginning with the 529 cows attended labors, 406 (76.75%) were parturitions without necessity to assist, 92 (17.39%) had to be assisted using forced traction, and on 31 (5.86%) were made cesarean section. The cesarean section was carried through when the forced traction did not succeed or when there was absence of response of the recipient cow to the protocol used for parturition induction.

A total of 42 necropsies were executed, what means that 7.93% (42 in 529) of the calves died within a week after delivery. The macroscopic examination weren't made during the necropsy. The main organs affected were lungs, kidneys and liver. The major lesions found were congestion and hemorrhage (n=35 in the lungs, n=34 in the kidney), edema, emphysema and atelectasis (n=21, n=15, n=14 in the lungs, respectively).

The definitive diagnosis of the death cause was only possible for 17 animals out of the 42 studied. The main organs affected were kidneys, liver and lungs despite the fact that parturition induction with the use of corticosteroids is important to accelerate appropriate pre-partum fetal lung maturity (ZAREMBA et al., 1997, PTAK et al., 2002, PRESTES & LANDIM-ALVARENGA, 2006).

The mortality of calves born from IVP embryos was 7.93%, being superior to the index verified with conventional embryo transfer, AI or natural mating. Similar findings were described by Peixer et al. (2000), Farin et al. (2001), PTAK et al. (2002) and Farin et al. (2004), tough the rate is below the 15.6% reported by Hasler et al. (1995).

High incidence of dystocia are evident at labors with the need for forced traction (17.39%) or caesarean (5.86%), reported by Prestes (2005), FARIN et al. (2001) and Mcevoy et al. (2006). The indication for cesarean section was according to the size of calves and the absence of response of the recipient cow to the protocol used for parturition induction. An exaggerated offspring size was moreover described by HASLER et al. (1995), Peixer et al. (2000), Farin et al. (2001), Farin et al. (2004), Betteridge (2006) and Mcevoy et al. (2006).

Description of necropsy findings was jeopardized due to little availability of comparison bibliography. Congestion combined with hemorrhage cause hypoxia in affected tissue. Hydropic degeneration is usually associated with epithelial lesions and considered the first indication of tissue insults, in this case derived from hypoxia. Lipid degeneration occurred mainly in liver, kidneys and myocardium, originated from toxic or hypoxic dietetic factors (THOMSON, 1983). Regarding the data presented herein, the factor that most likely contributed to the development of this degeneration was the tissue hypoxia caused by congestion and hemorrhage.

Atelectasis during the prenatal period is related to deficiency in surfactant production by the fetus and thus leads to development of compensatory pulmonary emphysema. The lung problems observed in calves born from IVP embryos were the second harms answerable cause of death. Lymphoid rarefaction (hypoplasia) verified in the thymus is related to abnormal embryological development where the tissue did not develop satisfactorily, being characterized as congenital malformation. FARIN et al. (2001) also described the presence of this type of deviation. Calves examined were born with depressed immune system due to hypoplasia of the thymus. The septicemia (n=5) and endotoxemia (n=5) were the major cause of the death. Additional research is needed further to describe the origins of the development of those pathologies that occurs more frequently in the IVP embryos.

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