Hydroallantois and Hydramnios in Bovine Cloned Pregnancies

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Abstract

A two year old, female crossbred Angus influenced heifer, was seen on a bovine exclusive externship. The heifer was 223 days pregnant with a cloned pregnancy. On presentation, the patient was bright, alert, and responsive. On physical exam, notable symmetrical bilateral abdominal distension was observed. Vital signs were within normal limits with no evidence of respiratory distress. On rectal palpation a firm, fluid-filled uterus was palpable. A calf was not palpable. Due to the comfort level of the heifer, she was closely monitored in the hospital pen.

Hydrops (hydroallantois and hydramnios) is a very important syndrome in bovine assisted reproductive techniques including somatic cell nuclear transfer (SCNT). Hydroallantois is a more common condition than hydramnios and is a result of dysfunction of the placenta, resulting in an increased production of chorioallantoic fluid (1). Hydramnios is less common and usually results from an abnormality of the fetus (1).

This presentation will focus on the diagnosis of uterine hydrops, treatment, and prognosis. The differences between the two syndromes will be highlighted. A brief history and introduction to SCNT cloning and its importance in agriculture will also be presented.

Introduction:

The commercial use somatic cell nuclear transfer (SCNT) has changed the genetic face of livestock industry. Genetics have progressed and production has been amplified. However, pitfalls have arisen; technique inefficiencies occur, abnormal pregnancies and fetuses are

common, and a fear that genetic diversity will be lost. Abnormal pregnancies, in particular are a focus of the veterinary world. An increase in the number of cattle that experience uterine hydrops with clone pregnancies can be as high as 25% of the dams that establish a pregnancy beyond 60 days carried calf. Proper management of these individuals is very important to ensure quality care of the dam and development of the fetus.

Case History and Signalment:

The patient was a 2 year old, female crossbred Angus influenced heifer. She was seen at a bovine exclusive externship focusing on advanced reproductive technologies such as embryo transfer, in vitro fertilization, and ovum pick up. She was 223 days pregnant carrying a cloned pregnancy. This was her first pregnancy and she was a recipient dam for a donor cell line that was commercially cloned. She was obtained from a ranch in Montana and upon her arrival at the facility, she was BVD and Johne's disease tested. She was vaccinated for BVD, upper respiratory complex, IBR, BRSV, PI3, and several abortive agents.

Clinical Findings:

On physical exam, the heifer exhibited bilateral symmetrical distension of her abdomen, her vital signs were within normal limits, and she exhibited no evidence of respiratory distress. She showed no signs of discomfort getting up and down in her paddock. On rectal palpation, a firm turgid uterus was noted. There was no palpable fetus or placentomes. On trans abdominal ultrasound, a live, but small fetus was identified.

Problem List:

The problem list for this heifer includes bilateral symmetrical abdominal distension, a firm, turgid uterus, and a live fetus. A live fetus is not necessarily a problem, but it determined the direction of treatment for the patient.

Differential Diagnosis:

At the top of the differential diagnosis list for this heifer are the uterine hydropsy syndromes, hydroallantois and hydramnios (1). A large fetus or multiple fetuses can produce bilateral abdominal distension, but usually at least one fetus and placentomes are palpable through the uterine wall. Lower on the differential diagnosis list is ruminal bloat, displaced abomasum, and vagal indigestion. All three of these diseases can cause some form of abdominal distension, however a firm, turgid uterus is not associated with any of the disease processes.

Therefore, the diagnosis of hydroallantois was made. Hydroallantois is the excessive, rapid accumulation of chorioallantoic fluid (1). Usually the condition exhibits progressive signs after mid gestation. There is a 10 fold increase in the volume of chorioallantoic fluid that the dam has. The normal volume of chorioallantoic fluid is 8 to 15 liters, while an effected dam can carry upwards of 200 liters of chorioallantoic fluid (1). This adds an additional 440 pounds for the dam to carry around.

The etiology of hydroallantois is placental dysfunction often characterized by adventitious placentation. Adventitious placentation is multiple areas of adhesion between the endometrium of the dam and chorioallantois of the fetus (1). These affected areas may appear as mini placentomes. To better understand the relationship between the maternal and fetal component of the placenta and the location of the pathology during placental dysfunction, a quick review a ruminant placentation is important. The fetal component of the placenta is the

extra embryonic membranes which consist of the amnion and the chorioallantois (7). During embryogenesis, the chorion and the allantois fuse to form the chorioallantois. The interface between maternal and fetal component of the placenta is called the placentome (7). The maternal component is the carunclar region of the uterus and the fetal component is the chorioallantois (7). At the placentome, nutrients and metabolic wastes are exchanged between the fetus and dam. During placental dysfunction, areas of adhesion between the carunclar region of the uterus and the chorioallantois outside of the placentome occur resulting in adventitious placentation (7).

Clinical signs of hydroallantois include bilateral abdominal distension, discomfort, decreased appetite, and reluctance to move (1). However, there are many sequeale to the clinical signs of hydroallantois. First, dehydration is common because a very large uterus is pressing and occupying the space of the rumen (1). This decreases water intake of the dam leading to dehydration. Constipation is a result of decreased water intake and tachycardia can be seen as a compensatory mechanism for dehydration. Recumbency, prepubic tendon rupture, and musculoskeletal complications are common in these dams due to the excessive weight in chorioallantotic fluid that they must carry around. Dystocia is a common sequela found in hydroallantoitic females (1). Due to the distension of the uterus with fluid, the uterine myocytes are stretched very tautly and are unable to contract during patritution. If these dams successfully carry a calf to term, they will need assistance during delivery, usually caesarean section. Dams with hydroallantois are traditionally in a state of negative energy balance prior to calving. This predisposes them to a host of fresh cow diseases including ketosis, retained placenta, and metritis which lead to decreased milk production (1). In particular, retained placenta and metritis are particularly worrisome because of the formation of adhesions made between the caruncluar

region of the uterus and the chorioallantois (1). This has an effect on the dam's fertility and overall longevity.

Risk factors for hydroallantois include pregnancies from modern in vitro reproductive technologies, especially SCNT, the type of cloned pregnancy our patient was carrying (10). A second but far less common cause is twin pregnancies due to increase placentation, and finally nutritional deficiencies are reported in the literature as cause of hydroallantois (10). The gold standard to diagnose hydroallantois is rectal palpation. A firm, turgid uterine wall will be palpated that is too taut to distinguish a fetus or placentomes.

The second type of uterine hydropsy syndrome is hydramnois, hydrops of the amnion, which is a gradual accumulation of amniotic fluid during pregnancy (1). It is caused by fetal anomalies of the calf, in particular fetal monsters, pituitary hypoplasia/aplasia calves & hydrocephalic calves. These abnormal claves have impaired deglutition of amniotic fluid. Also, these calves have impaired kidney function from renal dysgenesis or agenesis (1). This results in the accumulation of amniotic fluid around the fetus. Clinical signs include a pear shaped abdomen of the dam when viewed from rear. Diagnosis of hydramnios is made based on a palpable placentomes and\or a fetus and 20L or greater of amniotic fluid at birth (1). The normal volume of amniotic fluid is 5 liters (1).

There are distinct differences between hydroallantois and hydramnios. First, hydroallantois is a disease of maternal placental dysfunction while hydramnios is a disease of fetal anomalies. The prevalence of hydroallantois is much greater at 85 to 95% while the prevalence of hydramnios is very low at 5 to 15% (1). The shape of the abdomen is helpful in differentiating the two diseases. The abdomen is tense and round in hydroallantois, while it is piriform and not

tense in hydramnios. Placentation with hydroallantois is usually adventitious while placentation with hydramnios is normal. Complications are common for hydroallantois with abortion and maternal death common, while there are few complications for hydramnios, however the calf is usually not viable.

Prognosis

Prognosis differs for the dam and fetus in hydroallantois and hydroamnios. In hydroallantois, the prognosis for the dam is guarded to poor due to the sequelae of adventitious placentation in particular retained placenta and metritis (1). The prognosis for the calf is favorable if the calf is delivered near term. Hydramnios has different outcomes for the dam and fetus. The prognosis for the dam is excellent, however the same mating should be avoided in the future to prevent fetal anomaly. If the dam experiences a dystoica due to a fetal contracture monster, she should be assisted. The calf is non-viable.

Treatment

There are three options to pursue when a heifer or a cow is diagnosed with hydroallantois. If the cow is recumbent or has suffered from severe muscoskeletal injuries, she should be euthanatized (1). Based on the poor prognosis, salvaging the cow by culling is not an unreasonable option. However, if the cow or fetus is valuable or if the dam is within 2 weeks of calving, treatment can be pursued (1). There are two treatment options to pursue. Treatment option 1 is to induce parturition. This is usually a very unsuccessful option because the uterus is so distended that the uterine myocytes can't contract. The more successful option is delivery of the calf via cesarean section. Twenty four hours prior to surgery the dam should be induced with dexamethasone to encourage fetal lung maturation (1). Also, draining the chorioallantois before

surgery allows manipulation of the uterus during surgery. The drainage should occur slowly over 30min with Foley catheter/trochar in the right lower flank of the dam. Trocharization should occur within 48 hours of surgery to avoid refilling of the chorioallantois (1). The dam must be supported by rapid IV fluid therapy to prevent onset of shock, which may result from venous fluid shifting after decompression (10. A course of Procaine Penicillin G (PPG) is recommended to limit the risk of metritis (1). Dams diagnosed with hydramnios usually don't require treatment and calve near term.

Outcome:

Since the patient was very comfortable and not exhibiting signs of distress, she was placed in the hospital pen. She would be monitored closely over the next 2 months and a planned induction and Cesarean section would occur at 280 days carried calf.

Discussion

The use of modern in vitro reproductive technologies in livestock, especially somatic cell nuclear transfer, at a commercial level has led to much advancement in the agricultural industry and scientific community. However, challenges with this technology are still faced.

Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer, SCNT, is a method used to clone animals from an adult somatic cell. First, a fibroblast cell is collected by skin biopsy from an animal to be cloned (5). This animal is called the donor animal. The nucleus is removed from the fibroblast forming the donor nucleus. Next, an oocyte is collected from a female of the same species of the donor. The nucleus is removed from the oocyte forming an enucleated oocyte (6). The second step is to

insert the donor nucleus into the zona pellicudia of the enucleated oocyte. The two cells are joined by electrofusion. The embryo is allowed to grow in culture media that mimics the environment of the uterine tube for 7 days until it reaches the blastocyst stage (6). Once the blastocyst stage is reached, the embryo is transferred into a synchronous female recipient of the same species of the donor. The embryo is gestated for the appropriate amount of time and a clone of the donor is produced.

History of Cloning

The history of cloning dates back to 19th century. In 1885, Hans Dreisch separated cells from a two-celled sea urchin mechanically (5). Each cell grew independently and formed a separate, whole sea urchin clone. The greatest accomplishment in cloning history thus far, occurred in 1996 when the first mammal was cloned from an adult somatic cell. Ian Wilmut at the Roslin Institute created Dolly the sheep (5). Since SCNT was utilized to produce Dolly, the technology of cloning exponentially increased in popularity. Among many advancements in 1997, the first Holstein calf was cloned by ABS Global, Inc. In 2001, the first domestic pet was cloned in Texas, Copy Cat, a kitten (5). In 2005, the first dog was cloned by the South Koreans (5). His name was Snuppy named after the **S**eoul **N**ational **U**niversity pu**ppy.** The latest advancement in cloning occurring in 2011 involved a group of South Korean scientists cloning coyotes, which are an endangered species in South Korea (5).

Challenges of SCNT

Although the history of cloning is vast and exciting it does have its challenges as a technology. SCNT is currently a very inefficient technology. A success rate of approximately 64% is reached during the fusion of the donor nucleus and the enucleated oocyte (10). The

second issue contributing to the poor success of SCNT focuses on culture media for donor cell lines. Without culture media conditions that support the growth of the fibroblast cell, it is difficult to obtain a healthy donor nucleus (10). A third issue contributing to the poor success of SCNT involves the growth of the blastocyst. There is only a 33% success rate of growing a blastocyst prior to implantation into a synchronous recipient female (10). This greatly decreased the odds that a viable embryo with be transferred to a synchronous recipient.

A very important reason for the limited success of SCNT is a high early embryonic loss rate. In recipient females carrying a SCNT clone, the early embryonic loss rate can be as high as 80% (3). The majority of these losses occur during the first 30-60 days of pregnancy (2). A hypothesized reason for very high rates of early embryonic loss includes inappropriate placental major histocompatibility complex (MHC) I expression in bovine cloned pregnancies during the first trimester (4). During the first trimester of a bovine natural mating pregnancy, MHC I is not expressed. This MHC I expression in the cloned bovine pregnancy placenta leads to an increased number of T lymphocytes in the endometrium of the dam (4). A maternal lymphocytic response is induced resulting in an immune mediated abortion of the cloned pregnancy (4). The expression of MHC I in bovine clone placentas is also hypothesized to be responsible for abnormal fetuses and placentation experienced in cloned pregnancies.

For further perspective of the high early embryonic loss rate experienced, only 10% of cloned embryos are carried to term (10). Of the 10% of cloned embryos carried to term, only 1% of calves born are viable (10). There is also a high rate of abnormal pregnancies and offspring produced from SCNT cloned pregnancies. The focus for the remainder of this paper will be on uterine hydropsy conditions seen in 25% of bovine cloned pregnancies carried to near term.

Hydroallantois, hydrops of the chorioallantois, is commonly seen with adventitious placentation (2). A hypothesis causing adventitious placentation during bovine cloned pregnancies is inappropriate genetic imprinting.

Genetic imprinting, known as an epigenetic process, involves methylation and histone modification to allow expression of the maternal and paternal allele in a manner in which they can be combined and not disturb the DNA sequence (8). In short, the DNA of the nucleus somatic cell donor is asked to behave like an embryo when it is fused with the enucleated oocyte. The nucleus of the somatic cell donor is an adult cell that must act as an embryo to allow appropriate cell division and embryo growth. A hypothesis for placental dysfunction leading to macroscopic lesions in the placenta is impaired expression of IGF-2 receptor (8). This is a very important receptor in cell signaling pathways. The impaired expression of IGF-2 receptor may potentially disrupt maternal-chorionic interaction. The impaired expression of IGF-2 receptor is thought to occur due to inappropriate DNA methylation existing in adult somatic cell nuclear donors (8).

Loss of genetic diversity is a concern with somatic cell nuclear transfer (10). By cloning elite animals for their genetic or phenotypic merits, genetic improvement is eliminated. However, using this technology appropriately and also continuing to produce novel individuals, genetic diversity will be preserved.

Benefits of Somatic Cell Nuclear Transfer

There are several downfalls to SCNT which required improvement of the current technology. But the benefits that have been experienced now and potentially in the future are tremendous. The ability to generate multiple copies of genetically elite farm animals is a great

accomplishment in the fight against world hunger (10). Productively elite animals produce more meat and milk than average animals with fewer inputs. The ability to produce transgenic animals using SCNT is a vital. These animals are used for pharmaceutical protein production and xeno-transplantation (10). SCNT can also be used to preserve endangered species. SCNT can be used for therapeutic cloning and allo-transplantation in the medical field. There are also practical applications to SCNT that can be applied on an academic and scientific level. The technique of SCNT has helped researchers understand the study of gene function, genomic imprinting, genomic re-programming, and regulation of development, genetic diseases, and gene therapy (10).

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