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# Induced parturition in recipient cattle carrying nuclear transfer calves

## Summary

Historically there has been a lack of spontaneous parturition in nuclear transfer animals produced from somatic cells. A significant number of nuclear transfer calves are born with health problems and died within a week of birth. To a lesser degree in-vitro produced (IVP) calves have had the same abnormalities at birth. There are numerous reports of nuclear transfer calves having placental dysfunction such as hydroallantois or hydroamnion, and placental pathology such as edema or extremely large and/or abnormal placentomes. In an attempt to circumvent some of these maladies, a program was devised to induce recipients carrying nuclear transfer fetuses to calve 1 week earlier than expected full term gestation. All recipient cows were induced toward parturition at day 274 (+/- 2 days) of gestation. The cows were injected IM with 20 mg of dexamethasone (Pro Labs Ltd., St. Joseph, MO, U.S.A.) and 25 mg dinoprost (Pharmacia & Upjohn, Kalamazoo, MI, U.S.A.) 24 hours before an elective caesarian section. Of the 31 nuclear transfer calves born at Trans-Ova Genetics utilizing this procedure, 23 (74%) survived the immediate postnatal period with minimal medical care. Eight calves (26%) died within 36 hours of birth. Placental edema, large birth weights, pulmonary edema and pathological right side heart problems were common findings in the calves that died. Two calves (6%) died later in life from other unrelated health problems. 21 calves (68%) remain for productive ventures. Birth weights on all calves ranged from 38 to 89 kg.

Key Words: nuclear transfer, placental insufficiency, placental edema, induced parturition, dinoprost, dexamethasone

## Zusammenfassung

Titel der Arbeit: **Geburtsinduktion in Empfängerkühen mit Kerntransferkälbern**

Eine signifikante Anzahl von Kerntransferkälbern wurde mit gesundheitlichen Problemen geboren und starb innerhalb einer Woche nach der Geburt. In-vitro produzierte Kälber (IVP) wiesen die gleichen Anomalien bei der Geburt auf, jedoch in geringerem Anteil. Es gibt eine Reihe von Berichten darüber, dass Kälber aus Kerntransfer plazentale Funktionsstörungen wie Hydroallantois oder Hydroamnion, oder pathologische Erscheinungen der Plazenta wie Ödeme oder extra große und/oder abnorme Plazentome aufweisen. Um diese Todesfälle zu umgehen, wurde ein Programm erarbeitet, in dem die Trächtigkeit von Rezipienten, die ein Kerntransferkalb austrugen, eine Woche vor Ablauf der normalen Trächtigkeit beendet wurde. Bei allen Empfängerkühen wurde die Geburt am 274 (+/- 2 Tage) eingeleitet. Den Kühen wurden 20 mg Dexamethason i.m. (Pro Labs Ltd., St. Joseph, MO, USA) und 25 mg Dinoprost (Pharmacia & Upjohn, Kalamazoo, MI, USA) 24 h vor einem Kaiserschnitt injiziert. Von 31 auf diese Weise bei Trans-Ova geborenen Kerntransferkälbern überlebten 23 (74%) die unmittelbare postnatale Periode mit minimaler medizinischen Versorgung. Acht Kälber (26%) starben innerhalb von 36 h nach der Geburt. Plazentaödeme, hohes Geburtsgewicht, Lungenödeme und pathologische, rechtsseitige Herzprobleme waren die Hauptursachen für den Tod der Kälber. Zwei Kälber (6%) starben später an anderen gesundheitlichen Problemen. 21 Kälber (68%) standen für die Reproduktion zur Verfügung. Das Geburtsgewicht aller Kälber lag zwischen 38 und 89 kg.

Schlüsselwörter: Kerntransfer, Placentainsuffizienz, Platentaödem, induzierte Geburt, Dinoprost, Dexamethason

## Introduction

In 1998 the Genetic Advancement Center, a division of Trans-Ova Genetics, became involved with the process of nuclear transfer in domestic cattle. The first calves from this project were due to be born in April of 1999. Large start up expenses had been incurred during the nuclear transfer and gestation phases of this process. This resulted

in a great desire to maximize survival rates at parturition. The goal at the outset, was to keep the nuclear transfer conceptuses alive if at all medically possible. The medical problems anticipated were large size, placental insufficiency, and late gestation metabolic disturbances that compromise adaptation to extrauterine life.

An intense review of the literature was very enlightening. The first cloning in sheep was reported in 1986 (WESTHUSIN et al., 2001; WILLADSEN, 1986). In the realm of mammalian nuclear transfer, no event has achieved more acclaim than the first adult cell cloned sheep (Dolly) in 1997 (WESTHUSIN et al., 2001; WILMUT et al., 1997). This one success has triggered a veritable explosion in research efforts targeted at nuclear transfer in many mammalian species (WESTHUSIN et al., 2001).

The lack of spontaneous parturition in nuclear transfer animals produced from somatic cells has been reported consistently (HILL et al., 1999). This single piece of information set a course toward elective caesarian sections. Nuclear transfer calves are often larger than normal (FARIN et al., 1995; GARRY, 1999; KEEFER et al., 1994; PACE et al., 2001; SINCLAIR et al., 1998; THOMPSON et al., 1995; WALKER et al., 1996; WILLADSEN et al., 1991; WILSON et al., 1995). Occasionally some are small. This unexplained variation in size could happen within the same batch of full siblings, grown in the same media, at the same time (CAROLAN et al., 1998; GARRY et al., 1996; GARRY, 1999; PACE et al., 2001; WILSON et al., 1995). Abnormal size, whether large or small, has been implicated as a serious risk factor for postpartum health in newborn mammals (GARRY et al., 1996; HOLLAND et al., 1992; KOTERBA, 1990). Many other factors in addition to culture media have been implicated in the size issue (GARRY, 1999; MERTON et al., 1998). While the results are highly variable, they show that a significant number of nuclear transfer offspring are born with health problems and die within a week of birth (BEHBOODI et al., 1995; CIBELLI et al., 1998; GARRY et al., 1996; GARRY, 1999; HILL et al., 1999; KATO et al., 1998; KEEFER et al., 1994; KRUIP et al., 1997; SHIGA et al., 1999; TANEJA et al., 2001; WALKER et al., 1996; WELLS et al., 1998; WILLADSEN et al., 1991). To a lesser degree in-vitro produced (IVP) calves have the same abnormalities at birth (BEHBOODI et al., 1995; HASLER et al., 1995; KRUIP et al., 1997; VAN WAGTENDONK-DE LEEUW et al., 2000). Most of these postnatal health problems fall into one or more of these four physiological conditions: hypoglycemia, metabolic acidosis, hypothermia and hypoxemia (GARRY et al., 1996; GARRY, 1999). The latter abnormality, most likely resulting from pulmonary hypertension or elevated systemic venous pressure, coupled with pulmonary surfactant deficiency, can cause what is known as respiratory distress syndrome (HILL et al., 1999; OWENS et al., 1987).

Placental insufficiency plays a large part in causing many of these health problems. Personal experience with IVP calves has demonstrated many with large umbilical vessels. The literature bears this out as well (HASSLER et al., 1995). There are numerous reports of nuclear transfer calves having placental dysfunction such as hydroallantois, hydroamnion, and placental pathology such as edema or extremely large and/or abnormal placentomes (DE SOUSA et al., 2000; FARIN et al., 2000; GARRY, 1999; HILL et al., 1999; HILL et al., 2000; KRUIP et al., 1997; OWENS et al., 1987; SINCLAIR et al., 1998; WALKER et al., 1996; WELLS et al., 1998). Experimental work has recently shown that as early as 75 days of gestation, umbilical vessels are enlarged and placentomes are either enlarged and/or poorly developed (DE

LILLE et al., 2001; FARIN et al., 2000; STICE et al., 1996). These placental problems could easily be one of the main reasons for the large amount of embryo loss throughout pregnancy (BONDIOLI et al., 1990; GARRY, 1999; HEYMAN et al., 1994; LEWIS et al., 1998; SEIDEL et al., 1997).

Other groundbreaking research has looked at metabolic and hormonal profiles of nuclear transfer calves during the perinatal period. This research was trying to ascertain the origin of the observed physiological and behavioral abnormalities (ADAMS et al., 1998; GARRY, 1999). Naturally derived and nuclear transfer late gestation fetuses were cannulated and blood samples collected between days 255-265 of gestation. The cloned fetuses had significantly lower levels of IGF-1, IGF-2, and arterial oxygen as compared to their non-nuclear transfer counterparts (ADAMS et al., 1998; GARRY et al., 1998; GARRY, 1999). The surprising conclusion is that these measurements match what would be expected in intrauterine growth retardation (IUGR) (GARRY, 1999; OWENS et al., 1987). Apparently early growth can be variable leading to the enlarged or small calf situation. Due to placental insufficiency, late term nuclear transfer, and to a lesser extent IVP fetuses suffer from IUGR (GARRY, 1999; OWENS et al., 1987).

In an attempt to circumvent some of these maladies, a program was devised to induce recipients carrying nuclear transfer fetuses to calve 1 week earlier than expected full term gestation. This would allow for sterile delivery, with no dystocia, immediate oxygen supplementation and other medical intervention as needed.

## Materials and Methods

To prevent dystocia problems exacerbated by the lack of spontaneous parturition, caesarian sections would be performed on all recipients carrying nuclear transfer fetuses. In order to keep birth weight as low as possible and to remove the calf before placental insufficiency had produced irreparable harm, the caesarian would need to occur as early in gestation as possible hopefully without compromising calf viability. Also a medical protocol needed to be developed that dealt with the expected immediate postnatal problems (GARRY, 1999).

The experimental procedure used involved inducing the recipient cows toward parturition starting on day 274 (+/- 2 days) of gestation. 20 mg of dexamethasone IM (Pro Labs Ltd., St. Joseph, M.O., U.S.A.) (WELLS et al., 1998; ZAREMBA et al., 1997) and 25 mg of dinoprost tromethamine IM (Pharmacia & Upjohn, Kalamazoo, M.I. U.S.A.) (ZAREMBA et al., 1997) were administered to the recipient cows. It was highly desirable that the recipients be at least in stage one and preferably in stage two of parturition within one day of treatment. Twenty-four hours later, day 275 (+/- 2 days) of gestation, an elective caesarian section was performed. During surgery the placenta was monitored for the presence of edema and the relative size of the placentomes. For the future health of the recipient, the majority of the intrauterine fluid was expelled from the uterus. The newborn calf was dried off with towels, rubbing vigorously to stimulate activity and general circulation. An arterial blood sample, drawn from the brachial artery, was submitted to determine $_{P}O_2$ concentration. This was repeated at 1 hour, 3 hours, 6 hours, 12 hours and at 24 hours after birth. The calf's birth weight was measured and recorded. High flow intranasal oxygen supplementation was begun within 10 minutes of birth and continued until the calf was

stabilized. On many calves this lasted for 12 hours some took 24 hours before $_PO_2$ concentrations stabilized at an acceptable level. The umbilicus was either clamped or sutured and dipped in iodine. The calf was offered via nipple bottle, 2 liters of pasteurized colostrum, from an outside source, as soon after birth as possible. If it had not consumed that amount within an hour, the colostrum was force fed with an esophageal feeder. As soon as practically possible the calf was moved to a well-bedded nursery stall, situated under heat lamps and body temperature was monitored every hour for 8 hours. The recipient cow was immediately removed from the surgery ward after being sutured and never had contact with the calf.

A calf that lived through the first 36 hours and was removed from all supplemental medical intervention was considered to have successfully survived the critical postnatal period and had adapted to extrauterine life.

## Results

Of the 31 nuclear transfer calves born at Trans-Ova Genetics, 23 (74%) survived the immediate postnatal period with minimal medical care. Minimal medical care would include 12 to 24 hours of intranasal oxygen supplementation and several hours of supplemental heat in the form of heat lamps suspended over the nursery pen.

Eight calves (26%) died within 36 hours of birth. The most usual cause of death was from complications of placental insufficiency. Placental edema, large birth weights and pulmonary edema were common findings in the calves that died. Occasionally postmortem results would show some mild right side cardiac pathology. Usually several abnormalities were present at the same time. More intense medical treatments tried on these problem calves included: fluid therapy, trying to correct metabolic acidosis and hypoglycemia, diuretics, prophylactic antibiotics, anti-inflammatories, and extended periods of supplemental heat. There was very little response to any of these treatments.

Two calves (6%) died later in life from other health problems. 21 calves (68%) are living and available for productive ventures. Birth weights on all calves ranged from 38 to 89 kg with a mean of 50.7 kg. The average birth weight of the 8 calves that expired within 36 hours of birth was 59.6 kg with a range of 40 to 89 kg. Of the 23 calves that survived the initial 36-hour postnatal period, birth weights ranged from 38 to 61 kg with a mean of 45.5 kg.

## Discussion

The majority of these calves were of Holstein descent and 282 days was used as the normal gestation length. Two hundred seventy-five days was picked arbitrarily as the desired day to do an elective caesarian. By doing a caesarian a full 7 days before due date, it was our opinion that we would circumvent most attempts at a natural delivery. All caesarians were done using a standing left flank approach, and sterile technique. It was anticipated that the administration of dexamethasone and dinoprost 24 hours before elective caesarian section would stimulate surfactant production (HILL et al., 1999; ZAREMBA et al., 1997), as well as initiate the beginning stages of parturition (TANEJA et al., 2001; WELLS et al., 1998). An attempt was made to produce a crude surfactant extract to administer intratrachealy to problem calves (HILL et al., 1999). In our hands this crude extract seemed to have little effect. Many of the nuclear transfer

calves born at our facility have been slow to stand and have had a reduced suckling response compared to what would be expected with A.I. or E.T. calves (KING et al., 1985). Colostrum production and even mammary development was not seen on some of the beef cows induced into parturition one week early. Since an outside source of health screened, pasteurized colostrum was being utilized, no ill effects were noted in this project. A supplemental source of good quality colostrum should be secured prior to birth for calves whose dams are induced early.

Placental edema, noticed during the caesarian, is a possible indicator of the presence of congenital pulmonary hypertension (HILL et al., 1999). This early warning sign, noticed soon after the start of the caesarian, often is an indicator that this calf is very likely to have a difficult time adjusting to the extrauterine environment. Several of the calves that did not survive the first 36-hour postnatal period had placental edema.

Arterial blood gas analysis was a very helpful tool to assess the respiratory status of the newborn nuclear transfer calf. It was a good sign to have the $_{P}O_2$ higher than the $_{P}CO_2$ at the first sampling taken 10-15 minutes after birth, with $_{P}O_2$ levels reading at 40-60 mmHg. At one to two hours a good reading would be a $_{P}O_2$ of 100+ mmHg with $_{P}CO_2$ in a declining pattern (HILL et al., 1999).

In the final analysis birth weight still seems to be the biggest survival factor. The number of calves in this project is probably not high enough for a good statistical evaluation. However, when the average birth weight of the calves that survived the initial 36 hour postnatal period, 45.5 kg, is compared to the average birth weight of those that expired during the fist 36 hours, 59.6 kg, the 14.1 kg difference appears to be significant.

In conclusion, the goal of this project was to remove the conceptus from the recipient as soon as feasibly possible, before placental insufficiency had damaged the calf beyond the possibility of rescuing it medically. The 74% live calf at 36 hours postnatal success rate is modestly satisfying. However, for somatic cell nuclear transfer to have universal success, the live calf success rate must improve further as well as the efficiency in getting the cloned embryos developed in this process to full term.

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