Nellore cloned calf viability during the postnatal period

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ABSTRACT: *This study evaluated the viability of Nellore cloned calves derived from somatic cell nuclear transfer (SCNT) and compare their viability with animals of the same breed derived from in vitro fertilization (IVF). Thus, two groups were formed. Group I (GI) consisted of 10 calves derived from SCNT and group II (GII) consisted of 10 calves derived from IVF. The differences detected between the groups were in the physical examination of the respiratory tract in GI, which represented the most common clinical-pathological disturbances. The Apgar index score indicated that 80% of GI animals were depressed and all had pale mucous membranes. Thus, anemia was reported in GI. In GII, this started at 12 h of life and was probably caused by an iron deficiency. Moreover, total calcium and ionized calcium levels were higher in GI immediately after birth. These alterations probably resulted in a high incidence of mortality in GI, reaching 90% of the calves, whereas mortality was only 20% for the calves in GII. In conclusion, cloned calves, which were derived from SCNT, had physiological and metabolic alterations after delivery, leading to a higher mortality rate during the perinatal period.*

Key words: *calves, cloned, clinical, pathology, laboratorial assessment.*

Viabilidade de bezerros nelores clonados durante o período pós natal imediato

RESUMO: *O objetivo deste trabalho foi avaliar a viabilidade de bezerros da raça Nelore oriundos da técnica da transferência nuclear de células somáticas (TNCS), no período pós natal imediato, comparando-a com animais desta mesma raça, oriundos de fertilização in vitro (FIV). Para tanto, os animais foram alocados em dois grupos, a saber: Grupo I (GI) - 10 animais frutos de TNCS; e, Grupo II (GII) - 10 animais oriundos de FIV. Nos respectivos bezerros, todos obtidos por cesariana, foram realizadas as avaliações físicas, escore de APGAR, bem como coleta de amostras de sangue nos momentos 0 (ao nascimento), às 2, 4, 6 e 12 horas de vida, a fim de avaliar os resultados de eritrograma, análises bioquímicas e hormonais, comparando-os entre os grupos e momentos. Nos animais que vieram a óbito foi realizada a necropsia para investigar a causa mortis. As diferenças observadas foram em relação aos achados clínico-patológicos, envolvendo, principalmente, o sistema respiratório caracterizado por bradpneia associada à dispneia, e a presença de edema e atelectasia pulmonar observadas no GI. Ademais, após a colostragem notou-se que 80% dos animais avaliados não foram capazes de manter a glicemia sendo mais evidentes nos animais do GII, possivelmente devido à hiperinsulinemia que se manifestou neste grupo ao longo de todo o período experimental. A anemia após o nascimento foi evidente no grupo de bezerros clonados ao longo de todo período de avaliação ao contrário dos bezerros oriundos de GII, a qual foi observada somente às 12 horas de vida, sendo, possivelmente, de origem ferropriva. Casos de hipercalcemia foram denotados nos animais do GI ao nascimento, sendo possivelmente associados à asfixia perinatal. Estas alterações, em conjunto, levaram a uma taxa de mortalidade de 90% dos animais do GI e de 20% dos bezerros do GII. Conclui-se que os animais oriundos da TNCS apresentam alterações fisiológicas e metabólicas após o nascimento, responsáveis, em grande parte, pela maior taxa de óbitos dentro do período perinatal.*

Palavras-chave: *bezerro, clonagem, clínica, patologia, avaliação laboratoriais.*

INTRODUCTION

Over 25 years ago, somatic cell nuclear transfer (SCNT) produced the first food animal (WILMUT et al., 1997). Bovines are the most successfully cloned species; however, the technique is still associated with neonatal problems, including respiratory failure, fetal oversize, limb flexural deformity, and various congenital defects (CHAVATTE-PALMER et al., 2012).

In Brazil, despite efforts, mortality rates following the birth of cloned calves are still high, at

approximately 50%. The main complications included respiratory problems and metabolic abnormalities (BIRGEL JÚNIOR et al., 2011).

These situations can be seen in newborns derived from other biotechnical procedures, such as *in vitro* fertilization (IVF) (FARIN, 2006). Physiological alterations during late pregnancy can lead to clinical disturbances, including increased birth weights. This is followed by reduced vigor and viability at the birth of IVF neonates (HILL, 2014).

Therefore, pregnancy and newborns from these biotechnical procedures remain a medical challenge. A standard protocol based on laboratory data is the key to ensuring neonatal health (BIRGEL JÚNIOR et al., 2011).

This study evaluated the viability of cloned calves derived from SCNT and compared them with animals from the same breed derived from IVF. Our hypothesis was that cloned and IVF calves would develop neonatal complications and require intensive management in a dedicated neonatal intensive care unit.

MATERIALS AND METHODS

Between 2014 and 2017, at a teaching veterinary hospital, Dr. Halim Atique participated in the birth and the first few days of postpartum care of newborn Nellore calves derived from somatic cloning. The gestation of 20 recipient heifer crossbreeds (*Bos taurus × Bos indicus*) was observed. Ten animals conceived Nellore calves derived from SCNT and the other 10 recipients conceived Nellore calves derived from IVF. Thus, two groups were formed. Group I (GI) consisted of the 10 calves derived from SCNT and group II (GII) consisted of the 10 calves derived from IVF. All heifers were housed and fed together throughout gestation with corn silage, mineral salts, and water *ad libitum.*

All calves were delivered via cesarean sections according to BAIRD (2013). A proximal paravertebral block was performed in the neural branches of T13, L1, and L2 with 5 mL of lidocaine 2% (Xylestesin® 2%; Cristália, Sao Paulo, Brazil) in the ventral and dorsal surfaces of the transversal processes. Briefly, parturition was induced at 285 d of gestation with an 8 mg intramuscular (i.m.) injection of triamcinolone acetonide (Retardoesteóide, Laboratorio Hertape-Calier Saude Animal, Juatuba, Brazil), and 20 mg i.m. of dexamethasone (Azium; Merck Sharp & Dohme, Kenilworth, NJ, USA) 24 h before cesarean delivery (MEIRELLES et al., 2010).

After the exteriorization of the calf, the umbilical cord was clamped to avoid hemorrhage.

The calves were suspended upside down following delivery for less than 90 s to clean their airways. The Apgar score was determined at birth and 5 min later (FEITOSA & BENESI, 2014). If the Apgar score did not improve significantly at the second evaluation, an intranasal catheter was placed for O_2 supplementation at a rate of 5–7 L/min. In the case of meconium staining, the calves were immediately cleaned with detergent and warm water to favor drying. Newborns were quickly moved to a warm room and cleaned with towels. At this time, the physical examination was conducted, and laboratory samples were taken. Birth weight was rated and animals heavier than 45 kg were characterized as having macrosomia (MEIRELLES et al., 2010).

Calves were monitored by repeated blood sampling and determination of temperature, mucous membrane color, and heart and respiratory rates (FEITOSA & BENESI, 2014) to evaluate neonatal adaptation to life *ex utero*. Physical examinations were performed immediately after birth, and at 2, 4, 6, and 12 h after delivery.

Colostrum was offered in small bottles until the animals received approximately 10% of their body weight on the first day of life. If the calves did not have a suckling reflex, colostrum at an equivalent volume to 5% of the calf weight was administered via an esophageal tube within the first 5 h of life and another 5% was given until 24 h after birth. All colostrum was previously frozen and contained 80–140 mg/mL immunoglobulin concentration as determined by specific gravity (Colostrometer; Biogenics, Mapleton, OR, USA).

Only the animals that received colostrum by esophageal tube had their blood glucose concentrations evaluated every hour from 5 h of life using a portable glucometer. If blood glucose fell below 50 mg/dL, 0.2 g/Kg of glucose was administered intravenously (i.v.) for 50% supplementation.

Blood samples were collected from the jugular vein in Vacutainer® tubes containing ethylenediaminetetraacetic acid (EDTA; Becton Dickinson, Franklin Lakes, NJ, USA) and subjected to hematological analysis. Total red blood cells (RBC) and hemoglobin were calculated using a semiautomated Urit 3000Plus (Urit Medical Electronics, Shenzhen, China). Hematocrit was determined by centrifugation of microhematocrit tubes at 13.000 g for 5 min. The RBC indices of mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated using the results from the red fraction (THRALL, 2007). Additional blood was collected in Vacutainer tubes

containing sodium fluoride to perform insulin, glucose, and lactate analysis. Another blood sample was collected in Vacutainer® tubes with no anticoagulant to access biochemical parameters. All samples were taken to the laboratory and centrifuged. The blood plasma and serum were frozen at -20 °C until analysis.

Biochemical assays were performed to evaluate serum total bilirubin, unconjugated bilirubin, conjugated bilirubin, total calcium, iron, plasma glucose, and lactate (Bioclin LTDA, Belo Horizante, Brazil), using the automatic biochemical analyzer, the prietest Touch (Robonik, Thane, India). Ionized calcium was accessed using the ion-selective electrode method (Electrolyte Analyzer; Roche Diagnostics, Indianapolis, IN, USA). Plasma insulin was quantified using radioimmuno assays (PerkinElmer, Inc., Waltham, MA, USA).

Animals that died were necropsied at the pathology veterinary service in the hospital. All macroscopic findings from the necropsy were documented, and samples of all tissues were collected for histopathological examination.

A Shapiro-Wilk test was performed to test for normality. Data that were normally distributed were analyzed using a repeated-measures analysis of variance to determine if there were significant differences among moments and groups and Tukey's test was used to compare means. For data that were not normally distributed, the Mann-Whitney test was used to compare groups and moments. The non-paired t-test was used to compare birth weights between the groups. Physical parameters after birth were subjected to Fisher's exact test. Spearman correlation between Apgar scores and lactate levels was performed. A value of $P < 0.05$ was considered significant. The statistical analyses were performed using SAS software (Statistical Analysis System; SAS Institute Inc., Cary, NC, USA) (ZAR, 1999).

RESULTS AND DISCUSSION

Despite intensive neonatal care, the mortality rates were 90% (9/10) in GI and 20% (2/10) in GII. Abnormalities of the placenta and some gene expressions are thought to occur in SCNT and IVF pregnancies. These alterations were probably responsible for the production of oversized fetuses, which could result in high perinatal mortality (HIIL, 2014). Respiratory problems, weakness, and lack of appetite were the main clinical signs in this study.

In GI, 50% (5/10) of the calves were heavier than the mean birth weight for the breed (Table 1) based on MEIRELLES (2010). In GII, only one animal exhibited this feature. The mean birth weight was 43.03 ± 14.72 kg in GI and 37.72 ± 6.33 kg in GII; however, the difference was not statistically significant. The cell lines used could also be responsible for producing oversized fetuses. In the present study, fibroblasts were used. There is a higher

Table 1 - Comparation of physical parameters from SCNT-derived calves (GI) and IVF-derived calves (GII).

PHYSICAL PARAMETER	GI (%)	GII (%)
	n=10	n=10
Pale mucous membranes[*]	8/10 (80%) [a]	2/10 (20%)
Pink mucous membranes[*]	2/10 (20%)	7/10 (70%) [b]
Cyanotic mucous membranes[*]	-	1/10 (10%)
meconium-stained at birth[*]	6/10 (60%)	2/10 (20%)
Suckling reflex (≥20 min) [*]	2/10 (20%)	6/10 (60%)
Sternal recumbency (≥3 min) [*]	-	-
Lifetime (>24 Hours) [*]	1/10 (10%) [a]	8/10(80%)
Increased birthweight (> 45Kg) [#]	5/10 (50%)	1/10 (10%)
Flexural deformities[*]	3/10 (30%)	-
Oxygen therapy (intranasal catheter) [*]	6/10 (60%)	2/10 (20%)

[*]Fisher exact test.
Non paired t-test.
[a] Clones different from IVF, $P < 0.05$.
[b] IVF different from clones, $P < 0.05$.

incidence of oversized fetuses in clones compared to that in *in vitro*-derived embryos, especially in clones produced from somatic cells compared with clones produced from embryonic cells (BERTOLINI & ANDERSON, 2002; FARIN et al., 2006). Chromosomal abnormalities resulting from aberrant cell division can cause polyploidy in a variable proportion of the embryonic cells (SLIMANE-BUREAU & KING, 2002).

Increased body weight of animals derived from reproductive techniques is caused by aberrant *in-utero* energy metabolism resulting, for example, in hyperinsulinemia after birth (GARRY et al., 1996). In GII, the plasma insulin level was eight times higher than that in GI, which contrasted with previous research when half of the animals in GI were heavier than normal (Table 2). IVF calves possibly demonstrated insulin resistance at birth.

Because of temporary hypoxia at birth, hyperlactatemia is noted as a physiological condition (LESTER et al., 2014). When this situation is prolonged, more lactate is produced, and it becomes a marker of postnatal acid-base disturbances and may be a predictor of neonatal viability (BORRUTO et al., 2006). Moreover, there is a negative correlation between neonatal mortality and blood pH, which indicates that metabolic acidosis leads to higher

plasma lactate concentrations (BLEUL & GOTZ, 2013). Hyperlactemia was noted in both groups during the experimental period. In GI, the plasma lactate level was higher than that in GII from the second hour of life (Table 2), which agrees with BORRUTO et al. (2006) because of the high mortality in GI. The animals in GII possibly had transitory physiological alterations that were compatible with *ex utero* adaptation.

In normal neonates, fetal structures of the heart, such as the ductus arteriosus and foramen ovale, close in the first few hours after birth (JAFARI & HOSEINI, 2016; COFRÉ et al., 2017). These structures can persist and have been observed in cloned calves that died within a few days (GARRY et al., 1996). After birth, prolonged acidemia and hypoxic conditions may promote the maintenance of fetal circulation (patent foramen ovale and patent ductus arteriosus) (MEIRELES et al., 2010). Therefore, these events could contribute to hyperlactatemia in cloned calves.

Plasma glucose in newborn calves is 80–120 mg/dL (SWENSON & REECE, 1996). After birth, the umbilical cord is broken and maternal glucose is interrupted, which causes plasma hypoglycemia in newborns. This situation was observed immediately after birth in both groups (Table 2). The placenta

Table 2 - Moment (M), Number of animals (n), Mean ($\overline{x}$) and Standard deviation (SD) for values of insulin, glucose, and lactate from SCNT-derived calves (GI) and IVF-derived calves (GII).

	Moment	----GI----		----GII----	
		n	$\overline{x}\pm SD$	n	$\overline{x}\pm SD$
	M_0	10	8.72±11.39Ba	10	43.38±40.35Aa
Insulin	M_2	9	7.80±5.71Ba	10	24.86±19.96Aa
(uU/mL)	M_{12}	7	38.04±28.63a	8	44.91±32.57a
	M_0	10	30.70±11.97B	10	51.02±24.64Aa
	M_2	9	95.56±68.76a	10	48.99±40.06a
Glucose	M_4	7	104.14±64.83a	10	61.79±56.56a
(mg/dL)	M_6	7	133.00±87.17a	8	84.29±70.69a
	M_{12}	7	101.43±48.92a	8	92.64±80.22a
	M_0	10	11.02±3.20a	10	8.59±3.96a
	M_2	9	14.65±6.64Aa	10	5.80±5.07Ba
Lactate	M_4	7	11.20±5.16Aa	9	4.95±1.69Ba
(mmol/L)	M_6	7	12.18±3.39Aa	8	5.21±2.23Ba
	M_{12}	7	13.02±6.33Aa	8	5.06±2.51Ba

[Aa] Mean values followed by different capital letters in the same row according to Tukey´s Test or by different lowercase letters in the same column differ according to ANOVA ($P < 0.05$).

of cloned calves uses approximately 60%–75% of the maternal glucose (CHAVATTE-PALMER et al., 2012), and this may have contributed to the lower plasma glucose in GI after birth. Moreover, the situation observed in GI could be a result of fetal distress because of the rapid consumption of glycogen stores and the establishment of anaerobic glycolysis, which increases plasma lactate (ZACONETA, 2004).

In GI, three animals died before receiving colostrum. Of the remaining seven animals, six presented a weak suckling reflex and received colostrum via an esophageal tube. Therefore, the glucose concentration of these animals was measured with a portable glucometer starting 5 h after administration of colostrum (data not available). In these animals, the blood glucose concentration was lower than 50 mg/dL. Thus, i.v. glucose was administered. Therefore, this procedure may contribute to the measurement of median values within the physiological patterns at 6 h of life (Table 2). However, at 12 h, the plasma glucose level was lower than 50 mg/dL. In GII, eight animals had a normal suckling reflex and ingested the colostrum offered normally; for this reason, blood glucose was not measured. However, at 6 h, six of these animals were hypoglycemic even after ingesting colostrum.

These results confirmed that 80% of all calves available (12/15) were unable to maintain plasma glucose within the normal range even after ingesting colostrum. Hyperinsulinemia may be responsible for massive cell glucose entry, reducing plasma glucose levels.

According to EVERTS et al. (2008), SCNT placentomes have gene alterations that result in lower oxygen supply to the fetus, especially during the last trimester of pregnancy. Therefore, this situation contributed to fetal distress *in utero* resulting in meconium staining at birth, which was observed in 60% (6/10) of the GI animals and 20% (2/10) of the GII animals (Table 1), a situation that contributed to higher plasma lactate in GI.

The respiratory rate was not significantly different between the groups, but GII had higher values than those observed by FEITOSA & BENESI (2014) (46 ± 16 breaths/minute) for newborn Nellore calves (Table 3). However, the mean values for this group exhibited a decline, but with no statistical difference, tending to normalization concerning the findings for researchers at 2 h. In GI, the respiratory rate at birth indicated bradypnea (FEITOSA & BENESI, 2014). However, the mean values increased and exhibited tachypnea until the end of the experimental period.

Table 3 - Moment (M), Number of animals (n), Mean ($\overline{x}$) and Standard deviation (SD) for values of respiratory rate, heart rate and rectal temperature from SCNT-derived calves (GI) and IVF-derived calves (GII).

Moment		----------GI----------		----------GII----------
	n	$\overline{x}\pm$SD	n	$\overline{x}\pm$SD
Respiratory Rate (breaths/minute)				
M_0	10	39±22 b	10	51±11
M_2	9	55±6 Aab	10	42±10 B
M_4	7	57±15 Aab	10	42±7 B
M_6	7	56±15 ab	8	43±14
M_{12}	7	68±16 Aa	8	42±4 B
Heart Rate (bpm)				
M_0	10	99±37	10	127±44
M_2	9	132±24	10	137±25
M_4	7	140±22	10	137±24
M_6	7	143±40	8	127±21
M_{12}	7	141±32	8	136±19
Rectal Temperature (C°)				
M_0	10	39.22±0,65 a	10	38.84±0.78 a
M_2	9	37.23±0.87 b	10	37.74±1.10 b
M_4	7	37.87±1.00 b	10	37.88±0.80 b
M_6	7	38.17±1.16 b	8	38.16±0.70 ab
M_{12}	7	38.03±1.10 b	8	38.01±0.58 ab

[Aa] Mean values followed by different capital letters in the same row according to Tukey´s Test or by different lowercase letters in the same column differ according to ANOVA (P < 0.05).

According to the literature, approximately 90% of cloned calves have respiratory disturbances during the neonatal period (BRISVILLE et al., 2011). In this study, 89% (8/9) of GI animals underwent necropsy. All animals displayed pathological changes accompanying some respiratory tract segments, especially pulmonary edema associated with congestion and sometimes atelectasis. These pulmonary alterations were identified during the macroscopic evaluation and were probably associated with fetal distress and aspiration of meconium.

Clinically, animals with meconium aspiration had intense dyspnea associated with bradypnea after birth with subsequent tachypnea that persisted as long as they were alive, with a slow decrease during the evaluation period. The meconium prevents the incorporation of surfactant phospholipids into a stable monolayer, making breathing difficult. Moreover, vasoconstriction occurs, especially in the pulmonary artery, leading to pulmonary hypertension with reduced blood flow to the alveoli, resulting in hypoxia (BLEUL, 2009). In this study, 66.7% (6/9) of the necropsied GI animals were born with meconium staining, and 83.3% (5/6) of these animals had atelectasis and pulmonary edema associated with meconium in the alveolar space that was observed upon microscopic examination. The other animals in this group, which were born with no meconium staining, did not exhibit these respiratory changes. Thus, it was probable that the clinical-pathological changes were caused by meconium aspiration.

Atelectasis could be caused by a surfactant deficiency or meconium aspiration (LESTER et al., 2014) with serious respiratory disturbances. It is important to emphasize that in previous studies, animals with surfactant deficiency or meconium aspiration had similar respiratory changes (BLEUL, 2009).

The Apgar score index can be used to assess neonatal viability immediately after birth (FEITOSA & BENESI, 2014). In this study, a comparison of this index immediately after birth and at 5 min of life between the groups did not show a statistical difference (P > 0.05). However, it was noted that low and moderate vitality affected 80% (8/10) and 90% (9/10) of GI and GII animals, respectively, after birth. At 5 min of life, the GII animals exhibited an improvement in vitality. The same situation was not observed in GI; the six animals that remained had low (1/10) and moderate (5/10) vitality and were subjected to oxygen therapy. However, this procedure did not contribute to an improvement in their respiratory status once the plasma lactate concentration remained immutable during all periods of this study for this group (Table 1). These observations in cloned calves attested to possible disturbances suffered by these animals *in utero* and more difficult adaptation to life *ex utero.*

Furthermore, the Apgar score index could be used to assess neonatal viability and predict early signs of peripartum asphyxia (LESTER et al., 2014). Thus, calves in GI possibly had early asphyxia.

A negative correlation was noted (r = -0.513; P < 0.05) between the Apgar score and plasma lactate concentration in both groups immediately after birth; thus, animals with low and moderate vitality evolved into metabolic acidosis. According to DUFTY & SLOSS (1977), when this metabolic status was prolonged, lethargy, a weak or absent suckling reflex, and difficulty in maintaining sternal recumbency were noted. All these signs were observed in the GI animals.

GII had a minor mortality rate after birth. Therefore, it is possible that because of the normalization of the respiratory rate, plasma lactate concentration, and Apgar index score, these animals did not have difficulty in adapting to life *ex utero.*

Meconium-stained calves, which died a few hours after birth, presented bradycardia, which caused tissue oxygenation (NUNES, 2009). Bradycardia occurs when the heart rate is lower than 90 bpm (HOUSE & GUNN, 2014). In this study, there was no statistical difference between groups and moments. However, 50% (5/10) of the GI animals had this feature after birth, and three were meconium-stained and died in the first 24 h of life. Bradycardia was noted in two animals in GII at birth; although, none were meconium-stained, and one died after 4 h of life.

Additionally, pale mucus in the eyes was observed in 80% (8/10) of the GI animals and probably corresponded to a large number of animals with low (2/10) and moderate (6/10) vitality in this group (P < 0.05). Moreover, this coloration is a predictor of anemia. This pathologic situation is common in newborns diagnosed by laboratory techniques. However, pale mucus is not ordinary in such animals (SILVA, 2016). This author's values for mean hemoglobin (11.36 ± 0.46 g/dL), mean RBCs ($8.27 \pm 0.25 \times 10^6$/mm^3), and mean hematocrit ($39.01 \pm 1.73\%$) for Nellore calves born delivered from the corticosteroid-induced cesarean section were higher than those observed in both groups in this study. House and Gunn (2014) showed that newborn calves with hemoglobin values less than 10 g/dL could be considered anemic. The erythrogram showed mean hemoglobin values below the aforementioned values for GI during the experimental period (Table 4).

Additionally, clinical signs of anemia were observed in eight animals in this group.

Hemolytic factors can be responsible for this disturbance in newborns (BATCHELDER et al., 2007). Hemolytic destruction of RBCs causes increases in total and indirect bilirubin concentrations, as noted for clones in this study, indicating that this was the possible cause of anemia (Table 5). Moreover, this fact was corroborated by the observation of bile pigments in the renal tubules in the microscopic analysis. Therefore, anemia also contributed to the respiratory changes and increased plasma lactate concentrations observed in GI.

Concomitantly, serum iron values for GI were within the physiological limits during the study (Table 5). Thus, these findings excluded the hypothesis that the anemia observed in GI was caused by iron deficiency, as reported by other authors (BIRGEL JÚNIOR et al.,2011). However, regarding minerals, the average calcium concentrations in GI were above the reference range for bovine species (KANEKO et al., 2008) indicating transient hypercalcemia, with values higher than those in GII during the first 6 h of life (Table 5).

In newborn foals, this situation was related to animals with perinatal asphyxia caused by placental dysfunction (McFARLANE, 2014). No similar reference was identified in the literature for newborn calves; however, the genesis of hypercalcemia in the animals in GI may be the same as those mentioned by the previous author. Under the conditions described above, the metabolic condition of the newborn

Table 4 - Moment (M), Number of animals (n), Median (Md), Mean ($\overline{x}$) and Standard deviation (SD) for values of Total Red Blood Cells (RBC), Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin concentration (MCHC) from SCNT-derived calves (GI) and IVF-derived calves (GII).

	Moment	------------GI------------		------------GII------------	
		n	$\overline{x}\pm$SD	n	$\overline{x}\pm$SD
RBC ($\times 10^6$/mm^3)	M_0	10	6.12±1.82 b	10	7.46±1.66 a
	M_2	9	6.67±1.92 ab	10	7.37±1.84 ab
	M_4	7	6.91±1.86 a	10	7.26±1.80 ab
	M_6	7	6.88±1.92 a	8	7.04±2.15 ab
	M_{12}	7	6.58±2.02 ab	8	6.91±2.06 b
Hemoglobin (g/dL)	M_0	10	8.17±2.69 b	10	10.78±2.49 a
	M_2	9	8.76±2.74 ab	10	10.60±2.36 a
	M_4	7	9.41±2.58 a	10	10.65±2.23 a
	M_6	7	9.43±2.63 a	8	10.15±2.63 ab
	M_{12}	7	9.10±2.98 ab	8	9.81±2.56b
Hematocrit (%)	M_0	10	26.63±8.55 b	10	33.88±6.60 a
	M_2	9	29.10±9.62 ab	10	33.81±7.29 a
	M_4	7	31.20±8.55 a	10	33.50±7.02 ab
	M_6	7	31.04±8.82 a	8	31.99±7.99 ab
	M_{12}	7	29.87±8.60 ab	8	31.49±7.42 b
MCV (μ^3)	M_0	10	43.65±5.39	10	45.76±3.75
	M_2	9	43.43±5.38	10	46.33±3.55
	M_4	7	45.19±3.42	10	46.54±4.04
	M_6	7	45.03±3.79	8	46.09±4.08
	M_{12}	7	45.17±4.33	8	42.97±7.73
MCHC (g/dL)	M_0	10	30.61±1.62	10	31.60±1.95
	M_2	9	30.26±1.63	10	30.65±2.25
	M_4	7	30.17±1.72	10	30.67±3.55
	M_6	7	30.43±1.45	8	31.71±2.43
	M_{12}	7	30.16±0.96	8	31.02±2,.07

[Aa] Mean values followed by different capital letters in the same row according to Tukey´s Test or by different lowercase letters in the same column differ according to ANOVA (P < 0.05).

Table 5 - Moment (M), Number of animals (n), Mean ($\overline{x}$) and Standard deviation (SD) for values of Indirect Bilirubin (I.B.), Total Bilirubin (T.B.), Direct Bilirubin (D.B.), Calcium, Iron and Ionized Calcium from SCNT-derived calves (GI) and IVF-derived calves (GII).

	Moment	----------------------GI-----------------------		----------------------GII----------------------	
		n	$\overline{x}\pm$SD	n	$\overline{x}\pm$SD
	M_0	10	0.64±0.49Aa	10	0.15±0.13Ba
	M_2	9	1.23±1.03 Aa	10	0.27±0.17 Ba
I.B.	M_4	7	1.18±0.83 Aa	10	0.23±0.21 Ba
(mg/dL)	M_6	7	1.40±0.97Aa	8	0.26±0.25Ba
	M_{12}	7	1.32±1.07Aa	8	0.16±0.11Aa
	M_0	10	1.07±0.76 Aa	10	0.38±0.16Ba
	M_2	9	1.37±0.66Aa	10	0.46±0.19Ba
T.B.	M_4	7	1.26±1.13 Aa	10	0.44±0.21 Aa
(mg/dL)	M_6	7	1.66±0.97 Aa	8	0.51±0.23 Aa
	M_{12}	7	1.56±1.30 Aa	8	0.44±0.23 Aa
	M_0	10	0.38±0.2Aa	10	0.23±0.15Ba
	M_2	9	0.47±0.26Aa	10	0.19±0.08Ba
D.B.	M_4	7	0.28±0.17Aa	10	0.21±0.08Aa
(mg/dL)	M_6	7	0.56±0.38Aa	8	0.26±0.08Aa
	M_{12}	7	0.68±0.63Aa	8	0.31±0.09Aa
	M_0	10	15.74±3.09 A	10	11.73±2.62 B
	M_2	9	15.8±3.42 A	10	12.56±3.09 B
Calcium	M_4	7	14.14±1.93 A	10	10.82±2.79 B
(mg/dL)	M_6	7	15.28±3.69 A	8	10.27±2.80 B
	M_{12}	7	13.67±4.06	8	10.44±3.11
	M_0	10	64.72±33,24	10	53.71±14.53 a
	M_2	9	77.10±45,35	10	52.60±16.24 a
Iron	M_4	7	79.66±43,92 A	10	43.52±15.00 Bab
(µg/dL)	M_6	7	66.83±28,88 A	8	38.56±15.73 Bb
	M_{12}	7	80.60±44,14 A	8	33.43±19.23 Bb
	M_0	10	10.64±1.95 A	10	8.18±1.76 B
	M_2	9	10.39±2.04 A	10	8.42±1.86 B
Ionized calcium	M_4	7	9.30±1.32	10	7.72±2.09
(mg/dL)	M_6	7	9.96±2.40 A	8	7.02±1.98 B
	M_{12}	7	8.93±2.56	8	7.39±2.20

[Aa] Mean values followed by different capital letters in the same row according to Tukey´s Test or by different lowercase letters in the same column differ according to ANOVA (P < 0.05).

resulted in acidosis. Thus, low pH caused calcium to detach from albumin, increasing serum concentrations of ionized calcium (KANEKO et al., 2008). This observation was identified in both groups; however, it was quantitatively higher in GI (Table 5).

CONCLUSION

It was concluded that IVF animals had higher survival during the evaluation period because of greater adaptation to the extra uterine environment. The high postpartum mortality in the cloned animals was related to issues associated with fetal distress.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The experimental protocol was approved by the Ethics Principles of Animal Experimentation of the Faculdade de

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DECLARATION OF CONFLICT OF INTEREST

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript.

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