CALVES BORN UNDER TROPICAL CONDITIONS AFTER DIRECT TRANSFER OF CRYOPRESERVED *in vitro* PRODUCED EMBRYOS

Becerros nacidos bajo condiciones tropicales luego de la transferencia directa de embriones producidos *in vitro* criopreservados

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ABSTRACT

In the present study, performance of 56 crossbred dual purpose heifers diagnosed as pregnant after direct transfer of *in vitro* produced embryos cultured in either media supplemented with serum (n = 23) or in a chemically defined media (n = 33) were compared. No differences were observed in the incidence of abortion (30.34% vs. 24.24%), dystocia (52.17% vs. 51.52%) and normal calving (17.39% vs. 24.24%) in pregnant heifers with embryos produced in either serum supplemented or chemically defined media respectively (P > 0.05). Sex of calves affected significantly the rate of dystocia (males, 83.33% and females, 50%; P < 0.05). The birthweight of calves was not affected (P > 0.05) neither by serum supplementation during *in vitro* culture (46.86 ± 2.04 kg for calves derived from embryos cultured in the serum supplemented media and 46.28 ± 1.42 kg, for calves derived from embryos cultured in the chemically defined media) nor by sex of calves (males, 47.20 ± 1.50 kg and females, 45.45 ± 1.84 kg). The birthweigh of calves born dead or dying soon after birth was significantly (P < 0.05) higher (51.92 ± 1.76 kg) than that of survivors calves (43.88 ± 1.22 kg). Neither serum supplementation during *in vitro* culture, sex of calves nor dystocia affected the perinatal survival of calves. In conclusion, the presence of serum during *in vitro* culture did not affect the reproductive performance of dual purpose pregnant heifers after direct transfer of *in vitro* produced embryos. Large offspring syndrome (LOS) as observed in this study was evidenced by high birthweigh of calves, high rate of abortions and dystocia.

Key words: *In vitro* embryo, *in vitro* culture, serum, defined media, large offspring syndrome.

RESUMEN

En el presente estudio se comparó el desempeño de 56 novillas doble propósito que resultaron preñadas luego de la transferencia directa de embriones producidos *in vitro* cultivados en un medio suplementado con suero o en uno químicamente definido. No se observaron diferencias en las tasas de aborto (30,43% vs. 24,24%), distocias (52,17% vs. 51,52%) y parto normal (17,39% vs. 24,24%) entre las novillas que recibieron embriones cultivados en el medio suplementado con suero y las que recibieron embriones cultivados en el medio químicamente definido. El sexo de la cría afectó significativamente el porcentaje de distocias, 83,33% para machos y 50% para hembras, (P < 0,05). El peso al nacimiento de los becerros tampoco se vio afectado (P > 0,05) por la suplementación sérica durante el cultivo (46,86 ± 2,04 kg, para los becerros derivados de los embriones cultivados en el medio suplementado con suero y 46,28 ± 1,42 kg, para los derivados de los embriones cultivados en el medio químicamente definido) ni por el sexo de la cría (machos, 47,20 ± 1,50 kg y hembras, 45,45 ± 1,84 kg). El peso de los becerros que nacieron muertos o que murieron luego del nacimiento fue significativamente (P < 0,05) mayor (51,92 ± 1,76 kg) al de los becerros que sobrevivieron (43,88 ± 1,22 kg). La sobrevivencia perinatal no se vio afectada ni la suplementación sérica durante el cultivo embrionario,

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ni por el sexo de los becerros o el nacimiento de un parto distócico. En conclusión, la presencia de suero en el medio de cultivo no afectó el desempeño de las novillas doble propósito que resultaron preñadas luego de la transferencia de embriones producidos *in vitro*. En este estudio se observó la presencia de becerros con el síndrome del recién nacido gigante evidenciado por un alto peso al nacimiento y una alta tasa de abortos y distocias.

Palabras clave: Embrión *in vitro*, cultivo *in vitro*, suero, medio definido, síndrome del recién nacido gigante.

INTRODUCTION

In vitro embryo production includes oocyte maturation (IVM), oocyte fertilization (IVF) and embryo culture (IVC) [28]. This reproductive technology has been greatly advanced since the first calf was born from an *in vitro* produced embryo in 1981 [7]. A high degree of efficiency has been reached with an 80-90% of immature oocytes reaching the metaphase II stage and undergoing a regular fertilization, and approximately 30-40% of fertilized oocytes developing to blastocysts [27]. This efficiency is low compared to that reported *in vivo*, where about 85-90% of ovulated oocytes were fertilized and 80% of cows had a blastocyst on day 16 post-service [23]. It must be considered that the oocytes used for in vitro embryo production are immature, and even though *in vitro* conditions have been improved, these conditions are not ideal for normal development of embryos.

In vitro produced embryos differs in several aspects of their *in vivo* counterparts. In vitro embryos have a darker cytoplasm, lower density [29], and higher lipid content [1], swollen blastomeres [36], a more fragile zona pellucida [9], lower density of intercellular communications [6] and higher sensitivity to cryopreservation [10]. However, *in vitro* embryos have a higher rate of aerobic glycolisis, more lactate production [25] and a different pattern of gene expression, the latter being affected by the culture system [21, 22, 30, 31], compared to *in vivo* embryos.

Fetus and calves resulting from *in vitro* produced embryos are also different from those produced *in vivo* [4]. The birthweight of calves derived from *in vitro* produced embryos is higher than those of calves resulting after artificial insemination or superovulation [4, 15]. The Large Offspring Syndrome (LOS) is a pathology frequently observed in calves derived from *in vitro* manipulated embryos (IVF, cloning). This anomaly is characterized by high birthweight, extended gestation length and high rate of dystocia, abortions and perinatal mortality [20]. Alterations in gene expression during the preimplantation period may affect the fetal development of bovine embryos and could contribute to LOS [27], specially when *in vitro* culture is performed in the presence of high levels of proteins, despite of the protein source (serum or bovine serum albumin, BSA) [20].

Other authors have concluded that the use of chemically defined culture media does not significantly reduced the birthweight of calves in contrast with calves resulting from embryos cultured in a media supplemented with serum [15, 16]. Birthweight of calves from embryos produced in chemically defined media was intermediate between the birthweight of calves derived from embryos cultured in serum supplemented media and those produced by artificial insemination [15, 16]. Addition of serum to the culture media is one of the most common methods used to improve the efficiency of *in vitro* embryo culture, due to the nutrients, vitamins, growth factors, hormones and antioxidants that it supplies [14]. Serum supplementation increases the production of bovine blastocysts [18], by shortening the interval to reach the blastocyst stage in bovine embryos [13] and so increasing the proportion of blastocysts at day 6 of culture [30]. Nonetheless, serum supplementation to the culture media, has been reported as deleterious on embryo quality due to the increase of expression of genes related with apoptosis, oxidative stress and by reducing the expression of genes related with maternal pregnancy recognition and intercellular communications as well as reduced cryotolerance [31].

Given the possibility that serum exclusion from embryo culture could increase embryo quality [30], that the use of a chemically defined media supports the development of a reasonable number of oocytes to the blastocyst stage [17], and that these embryos result in pregnancies and calves [18], the objectives of this study were: To report the birth of calves derived from *in vitro* produced embryos cultured in a chemically defined media. To compare the gestation and the calving of dual purpose pregnant heifers by direct transfer of *in vitro* produced embryos cultured in chemically defined media or cultured in a media supplemented with serum and to compare the resulting calves, with special emphasis on the birthweight and perinatal survival.

MATERIALS AND METHODS

In vitro maturation

Bovine (*Bos taurus*) ovaries collected at slaughter were transported to the laboratory (Department of Physiology and Pharmacology, College of Veterinary Sciences, The University of Georgia, Athens, GA, USA) without added medium in a thermo (28-32°C) reaching the laboratory within 3 h. Cumulus oocyte complexes (COCs) were harvested by aspiration of follicles using a 10 mL syringe and 18G needles. Oocytes surrounded by at least two compact layers of cumulus cells, with homogeneous cytoplasm, intact zona pellucida and appropriate size, were utilized. Cumulus oocyte complexes were washed twice with maturation medium and cultured in groups of 20-22 per 100 µL drops of maturation medium covered with light mineral oil (M-3516, Sigma Chemical Co., St Louis, MO, USA). The maturation medium was TCM-199 (M-3769, Sigma Chemical

Co., St Louis, MO, USA) supplemented with 50 µg/mL sodium pyruvate (Sigma Chemical Co., St Louis, MO, USA), 2.2 µg/mL $NaHC0_3$ (Sigma Chemical Co., St Louis, MO, USA), 1 mg/mL PVA (Sigma Chemical Co., St Louis, MO, USA), 0.25 mM glutamine (Sigma Chemical Co., St Louis, MO, USA), 0.1 mM cystine (Sigma Chemical Co., St Louis, MO, USA), 0.1 mM cysteine (Sigma Chemical Co., St Louis, MO, USA), 10 mM hydroxymethylpiperazine ethanesulfonic acid (HEPES, Sigma Chemical Co., St Louis, MO, USA), 50 µg/mL gentamicin sulphate (Sigma Chemical Co., St Louis, MO, USA) plus 0.1 UI/mL of recombinant human FSH (1.7 IU/µg, Ares Advanced Technology Inc., Randolph, MA, USA) and 5 ng/mL of recombinant human IGF-I (Promega, Madison, WI, USA). Incubation of COCs was performed under a moist atmosphere of 5% CO_2, 5% O_2 and 90% N_2 in a modular incubator chamber (Billups-Rothenberg Inc., Del Mar, CA, USA) at 38.5°C for 24 h. This atmosphere and these physical conditions were employed for subsequent *in vitro* embryo cultures.

In vitro fertilization

Frozen semen from Brahman bulls was used. In all replications, swim-up selected spermatozoa [11] was prepared in modified defined medium (mDM) [8]. Three straws of frozen semen were thawed at 37°C for 30 sec; then, 150 µL of semen were layered under 1.5 mL of mDM in each of several 12 mm × 75 mm tubes. The tubes were positioned in an angle of 45° for 45 min at 38.5°C in a 5% CO_2 incubator. Then, 850 µL from each tube (top "swim-up" fraction) was pooled in a 15 mL tube and centrifuged at 320 g for 10 min. The resulting pellet was resuspended in 380 µL of mDM; then 20 µL of mDM containing 80 µg of heparin (H3146, Sigma Chemical Co., St Louis, MO, USA) were added and then incubated for 15-min sperm incubation. Spermatozoa were checked for motility and counted in a hemocytometer before insemination to adjust the volume to be added into the insemination drop. The volume needed (12-14 µL) to achieve a concentration of 2×10^6 motile spermatozoa per mL was added to each 82-86 µL drop (to complete 100 µL) of mDM prepared as above, but without caffeine (IVF medium); thus containing heparin at a final concentration of 24-28 mg/mL. Matured oocytes were added to each of these drops, and the gametes were coincubated for 18 h.

In vitro culture

After 18 h post-insemination (hpi), loosely attached cumulus cells were removed by gentle pipetting of the COCs. Presumptive zygotes were washed thoroughly before they were placed in the culture drops. Presumptive zygotes were cultured in groups of 20 in 100 µL drop of three sequential media as follows. From 18 to 72 hpi, presumptive zygotes were cultured in synthetic oviductal fluid (SOF) [34] modified by addition of 0.1 mM non-essential amino acids (NEA; M-7145, Sigma Chemical Co., St Louis, MO, USA), 0.5 mM glutamine, 0.4 mM threonine (Sigma Chemical Co., St Louis, MO, USA) and 3 mg/mL PVA. At 72 hpi, embryos of a least four cells were freed from any remaining cumulus cells and were cultured in SOF containing citrate and without glutamine, i.e. c-SOF + NEA [18] plus 0.4 mM threonine. After 144 hpi, the basic media was the same as described above for the oocyte maturation medium but without FSH and IGF-I [11]. In the serum supplemented *in vitro* culture group, culture media was supplemented with 10% (v/v) fetal bovine serum (Sigma Chemical Co., St. Louis, MO, USA).

Freezing procedure

Embryos were classified by stages of development and quality standards according to the Manual of the International Embryo Transfer Society [39]. Cryoprotectant solution was prepared with phosphate buffer saline (PBS) containing 4 mg/mL of BSA (fatty acid-free; Sigma Chemical Co., St Louis, MO, USA) supplemented with 1.8 M ethylene glycol. Embryos were placed into cryoprotectant solution for 5 min at room temperature, then loaded into 0.25 mL straws using a 1 mL syringe and sealed with plastic plugs (yellow-DT, Agtech Inc., Manhattan, KS, USA). The straws were transferred into a programmable cell freezer (Cell freezer R204, Planer Products Ltd.) and the temperature was cooled from room temperature to –7°C for another 10 min. Then, freezing continued at a rate of 0.3°C/min to –30°C before plunging into liquid nitrogen. Embryos were sent to Venezuela in liquid nitrogen for embryo transfer into recipient heifers.

Estrous synchronization and embryo transfer

Crossbred (*taurus-indicus*) dual purpose heifers were located in farms at the Maracaibo Lake basin, Zulia State, Venezuela. All heifers with a body condition score ≥ 3 (scale 1-5; 1 = very thin and 5 = very fat), were synchronized with 2 injections (i.m.) of 25 mg PGF_{2a} (Dinoprost, Tromethamine, Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI, USA) given 11 days apart. Starting 36 h after the last injection, recipients were observed for signs of estrus twice daily for a minimum of 30 min each time. At the day of transfer the straws were thawed for l0 sec in air followed by immersion in water at 37°C for 10 sec. Straws were dried, plastic plugs removed, and the straws were loaded into an embryo transfer gun for direct transfer. Each recipient heifer received one or two embryos in the horn ipsilateral to the corpus luteum (CL). Pregnancies were diagnosed by rectal examination 45-60 days after the transfer. At calving heifers were placed in a special paddock, and calves were weighted within the first 24 h after delivery.

Statistical analyses

In this study gestation length and abortion and calving rate (dystocia or normal calving) of crossbreed (*taurus-indicus*) dual purpose heifers (n = 56, TABLE I) that resulted pregnant after direct transfer of *in vitro* produced embryos were analyzed in accordance with culture media and sex of calves. In addition, birthweight of calves born were compared in accordance with the culture media, sex, calving and perinatal sur-

TABLE I
INCIDENCE OF ABORTIONS AND CALVING IN PREGNANT HEIFERS AFTER DIRECT TRANSFER OF IVF EMBRYOS

In vitro culture media	Embryos transferred	Pregnant heifers	Heifers aborting	Heifers calving[1,2]	Calves survivors[3]
	1	11	2	9	6
Serum supplemented media	2	12	5	7	5
	Sub-total	23	7	16	11
	1	11	4	7	6
Chemically defined media	2	22	4	18	10
	Sub-total	33	8	25	16
	Total	56	15	41	27

[1] Heifers with dystocia and normal calving. [2] Each heifer to term calving only one calf. [3] Calves surviving after the first the five days of birth.

TABLE II
EFFECT OF SERUM SUPPLEMENTATION DURING IN VITRO CULTURE ON RATE OF ABORTION AND THE TYPE OF CALVING

Embryo culture media	Abort	Type of calving	
		Dystocia	Normal
Serum supplemented media	30.43%, 7/23[a]	52.17%, 12/23[a]	17.39%, 4/23[a]
Chemically defined media	24.24%, 8/33[a]	51.51%, 17/33[a]	24.24%, 8/33[a]

Values with different letters within columns differ, P < 0.05.

vival (rate of calves surviving after the first five days of birth). Gestation length and birthweight of calves means were analyzed with General Lineal Model procedure and abortions, dystocia, normal calving and perinatal survival rates were analyzed with Chi-square procedure of SAS [32].

Logistic regression procedure of SAS [32] was used to calculate the odds ratio of dystocia event, and the model included the effects of culture media, number of embryo transferred and the sex of the calves, while the model to determinate the odds ratio of death of calves, included the effect of culture media, number of embryo transferred, sex of the calves and type of calving.

RESULTS

Gestation length

The gestation length was not affected (P > 0.05) by serum supplementation during *in vitro* culture, (286.98 ± 2.54 days for heifers receiving embryos cultured in serum supplemented media and 289.00 ± 1.96 days for heifers receiving embryos cultured in chemically defined media). The sex of the calf did not affect (P > 0.05) the gestation length (288.27 ± 2.24 days for heifers calving male calves and 288.63 ± 2.41 days for heifers calving female calves.

Calving

Approximately 27% (15/56) of pregnant heifers aborted, 51.79% (29/56) had dystocia and only 21.43% (12/56) had a normal calving process. Serum supplementation did no affect (P > 0.05) the type of calving. Heifers receiving embryos derived from serum supplemented media had 30.43% of abortions, 52.17% of dystocia and 17.39% of normal calving, while heifers receiving embryos cultured in chemically defined media had 24.24%, 51.51% and 24.24% of abortions, dystocia and normal calving, respectively P > 0.05 (TABLE II).

TABLE III
EFFECT OF SEX OF THE CALF ON RATE OF DYSTOCIA

Sex	Type of calving	
	Dystocia	Normal
Male	83.33%, 20/24[a]	16.67%, 4/24[a]
Female	50%, 8/16[b]	50%, 8/16[b]

Values with different letters within columns differ, P < 0.05.

In heifers with gestation to term (dystocia and normal calving) the sex of the calf affected (P < 0.05) the type of calving. The rate of dystocia was highest when the calf was male (83.33% = 20/24) while the rate of dystocia was 50% (8/16), when it was female (TABLE III). These results were confirmed when odds ratio of dystocia were analyzed. There was 4.088 (95% CI: 1.042-22.200, P = 0.0442) greater adds of dystocia when calves were males than females. However, no effect of culture media (serum vs. defined) 0.850 (95% CI: 0.172-4.208, P = 0.8419) or number of embryo transferred (1 vs. 2) 1.217 (95% CI: 0.249-5.935, P = 0.8083) were found on odds of dystocia.

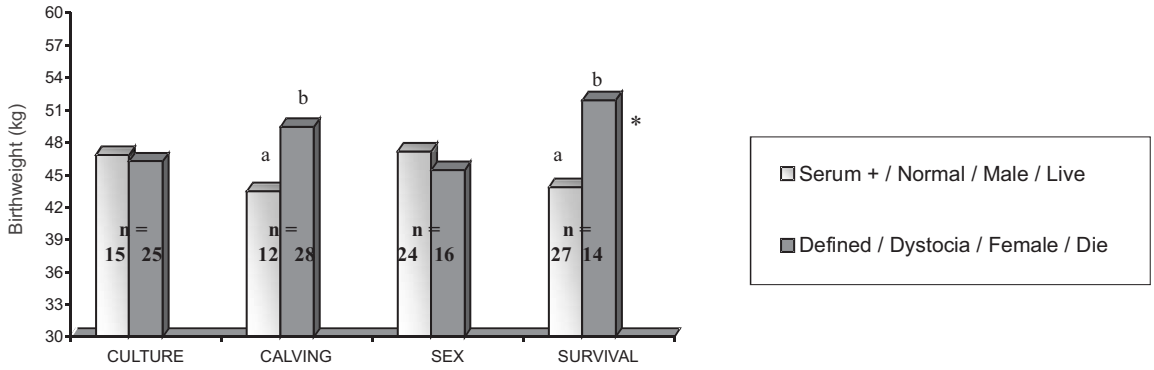

FIGURE 1. EFFECT OF SERUM SUPPLEMENTATION, TYPE OF CALVING, SEX OF THE CALF AND SURVIVAL ON BIRTHWEIGHT. BARS WITH DIFFERENT LETTERS, DIFFER. P < 0.05.

Birthweight

The overall birthweight was 46.1 ± 1.16 kg (n = 40, the weight of one calf was not recorded) and was not affected by serum supplementation during culture. The birthweight was 46.86 ± 2.04 kg (n = 15) and 46.28 ± 1.42 kg (n = 25) for calves derived from embryos cultured in serum supplemented and chemically defined media, respectively, (FIG. 1). The sex of the calf did not cause an effect on the birthweight, 47.20 ± 1.50 kg and 45.45 ± 1.84 for males (n = 24) and females (n = 16), respectively. In addition, the birthweight of calves that survived (43.88 ± 1.22 kg, n = 27) was significantly lower than that of calves born dead or those that died after calving (51.92 ± 1.76 kg, n = 14), (P < 0.05) and the birthweight of calves born by dystocia (n=28, weight of one calf was not recorded) was significantly higher than that of calves born by normal calving (n = 12), 49.45 ± 1.30 kg and 43.51 ± 2.01 kg, respectively, P < 0.05.

Perinatal survival

Serum supplementation during *in vitro* culture did not affect the perinatal survival (68.75% = 11/16 and 64% = 16/25, to calves derived from embryos cultured in serum supplemented and chemically defined media respectively, P > 0.05). No effects of type of calving (TABLE IV) or calf sex (TABLE V) were observed on perinatal survival. In addition, when odds ratio of death were analyzed, no effect of culture media (serum vs. defined) 1.372 (95% CI: 0.295-6.394, P = 0.6868), number of embryo transferred (1 vs. 2) 0.424 (95% CI: 0.084-2.147, P = 0.3001), sex (female vs. male) 0.863 (95% CI: 0.178-4.184, P = 0.8545) or type of calving (dystocia vs. normal) 3.103 (95% CI: 0.510-8.862, P = 0.2189) were observed.

TABLE IV
EFFECT OF SEX OF THE CALF ON PERINATAL SURVIVAL

Sex	Perinatal survival	
	Live	Dead
Male	62.50%, 15/24[a]	37.50%, 9/24[a]
Female	75%, 12/16[a]	25%, 4/16[a]

Values with different letters within columns differ, P < 0.05.

TABLE V
EFFECT OF THE TYPE OF CALVING ON PERINATAL SURVIVAL

Calving	Perinatal survival	
	Live	Dead
Dystocia	58.62%, 17/29[a]	41.38%, 12/29[a]
Normal	83.33%, 10/12[a]	16.67%, 2/12[a]

Values with different letters within columns differ, P < 0.05.

DISCUSSION

Birth of calves derived from *in vitro* produced embryos cultured in a protein-free culture media is a step forward in the development of IVF technology. However, the birth of calves is the final probe to define the embryo quality [21]. This study report the born of calves derived from *in vitro* produced embryos cultured either in serum supplemented media or in a chemically defined media.

In the present study gestation length was not affected neither by the serum supplementation during *in vitro* culture nor by the sex of calves. This is in agreement with Martinez et al. [24], who compared the gestation of Polled Hereford cows pregnant after transfer of *in vitro* produced embryos cultured in a chemically defined media (SOF+PVA) or *in vivo* produced embryos (283.1 ± 1.3 vs. 283.0 ± 1.5 days respectively, P > 0.05). This also agrees with a report by Jacobsen et al. [15], were they compared the gestation length of Holstein pregnant heifers either by artificial insemination (278.6 ± 1.1 days), by transfer of *in vitro* produced embryos cultured in serum supplemented media (280.4 ± 2.6 days), *in vitro* produced embryos cultured in a chemically defined media (282.4 ± 2.6 days) or by transfer of *in vitro* produced embryos cultured in presence of epithelial oviduct cells (277.6 ± 3.5 days). Lazzari et al. [20],

observed no differences in gestation length of heifers and cows receiving of embryos produced *in vitro* in the presence of BSA (281.1 ± 7.1) or serum (280.1 ± 5.9), compared with the recipients of *in vitro* produced embryos cultured *in vivo* (sheep oviduct, 279.2 ± 5.3 days) or product of superovulation (279.4 ± 5.1 days), or with those pregnant by artificial insemination (281.7 ± 4.3). These results and those reported in the present study indicate that culture media (undefined or defined) did not affect gestation length.

Normal calving rates reported in this study were lower than those reported elsewhere with a 53% for recipients of *in vitro* embryos cultured in the presence of serum and 38% for those receiving embryos cultured in a chemically defined media [16]. Fetal loss is the main problem affecting the efficiency of *in vitro* embryo production systems, with only 30% of transferred embryos reaching to term [28]. In the present study, serum supplementation did no affect abortion rates, which were higher than those reported by Hoshi [14], with embryos cultured in presence of BSA whether or not serum is present (14.8% vs. 13.6% respectively). Nonetheless, Lazzari et al. [20], reported 50% of abortion in recipients of *in vitro* produced embryos cultured in the presence of serum and this rate was significantly higher than the rate of abortion for recipients of embryos cultured in the presence of BSA (16.7%), embryos cultured in sheep oviducts (11.7%) and for recipients of embryos produced by superovulation (8.7%).

In the present study no significant effect of serum supplementation was observed on the rate of dystocia. Therefore and under the conditions of the present study, other factors may be affecting the incidence of dystocia. In the present study heifers are used as recipients. Recipients heifers have been showed to have an 18% more probability to present dystocia than adult recipients [16]. Nonetheless, a significant greater percentage of gestations derived from *in vitro* produced embryos resulted in more dystocia than recipients becoming pregnant by transfer of superovulation produced embryos (27.7% vs. 3.8% respectively) [24]. In this study, calves born by dystocia were significantly heavier than calves born by normal parturition (49.45 ± 1.30 vs. 43.51 ± 2.01 kg respectively, $P < 0.05$).

Serum supplementation during *in vitro* culture has been related with the occurrence of LOS [13,35,40]. Nonetheless, in this study the birthweight of calves was no affected by presence of serum. Birthweights reported here were higher than those reported by Aranguren et al. [3], to tropical calves produced by artificial insemination (e.g. 32.37 ± 2.76, 32.21 ± 3.11, 32.06 ± 2.56 and 28.77 ± 3.36 kg to 5/8 Holteins-3/8 Brahman, 1/2 Holteins-1/2 Brahman, Mosaic and Senepol, respectively). Lazzari et al. [20], reported that calves derived from *in vitro* produced embryos cultured in the presence of serum or BSA were significantly heavier than calves derived from either *in vitro* produced embryos cultured in sheep oviduct or produced *in vivo* by artificial insemination or superovulation (52.8 ± 9.4 and 56.7 ± 12.1 vs. 44.1 ± 5.5, 41.1 ± 3.0 and 43.4 ± 4.3 kg, respectively). Jacobsen et al. [15], did not observed significant differences in birthweight between calves derived from *in vitro* produced embryos cultured in the presence of serum (46.9 ± 1.8 kg) and those derived from embryos cultured in a chemically defined media (44.1 ± 1.8 kg) ($P > 0.05$). However, the birthweigh of these groups were not different from the birthweight of calves resulting from artificial insemination. These results and those presented in this study suggest that *in vitro* culture *per se* is able to induce the occurrence of LOS, which has been related with a prolonged *in vitro* culture [19].

The occurrence of LOS is related with alteration of the expression patterns of genes related with development [26]. The gene encoding IGF-II and its receptor has been reported to be associated with the occurrence of LOS [40]. Blondin et al. [5], reported that mRNA levels of IGF-II were significantly higher in fetuses resulting from IVF at day 63 of gestation compared to controls. Metilation of the IGF-II receptor gene was reduce in 30 to 60% in plasma, liver and muscle of ovine giant fetus [41]. The expression pattern of different genes is affected by the presence of serum in the culture media, with a more pronounced alteration when culture media base was TCM-199 [37, 38].

Perinatal survival in this study (65.85%, 27/41) was lower than the 89.5% reported by Agca et al. [2], but similar to the 62% reported by Schmidt et al., [33]. However they observed that the main causes of mortality were enteritis, brain hypoplasia and pneumonia. In the present study, perinatal survival was not affected by serum supplementation but a high birthweight was a factor associated to perinatal mortality. Calves that were born dead or died soon after birth were significantly heavier than calves surviving after birth. In addition, calves born by dystocia were heavier than those born after normal calving. Therefore, in this study, a high birthweight was associated with dystocia and perinatal mortality, and this is in agreement with the reviewed by Holland and Odde [12].

CONCLUSIONS

Serum supplementation of embryo culture media is a common practice in IVF laboratories as a way to increase the efficiency of *in vitro* embryo production but several negative effects have been reported. Nonetheless in this study we conclude that the presence of serum during *in vitro* culture did no affect neither the gestation length nor the rates of abortion, dystocia and normal calving of pregnant heifers after direct transfer of embryos or the birthweight and survival of calves. Probably, *in vitro* culture *per se* is responsible of the epigenetic alteration that results in the LOS. Under the conditions of this study, a high birthweight was the most important factor compromising perinatal survivability. Finally, in this study we have shown that IVF is a tool applicable under tropical conditions and that when embryos are transferred into recipient heifers it can result in live calves.

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