THE EFFECT OF FETAL CALF SERUM CONCENTRATIONS UPON THE *In Vitro* *Bos taurus indicus* X *Bos taurus taurus* CROSSBRED EMBRYO PRODUCTION AND THE CYTOPLASMIC LIPID ACCUMULATION

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ABSTRACT

The aim was to test the supplementation of five different concentrations of fetal calf serum (FCS) upon the *in vitro* production (IVP) and cytoplasmic lipid accumulation in Nelore and Simmental crossbred bovine embryos. The addition of serum started on D3 of embryo culture. Nelore oocytes were submitted to *in vitro* maturation, *in vitro* fertilization with Simmental semen, and *in vitro* cultured. From D1 to D3 it was used the SOFaa media + 0.5% BSA without serum (Base Media). From D4 to D7 the embryos were divided into four experimental groups according to the concentration of serum that was added to the Base media: Group 0-0%: without serum; Group 0 – 2.5%: 2.5% of serum; Group 0-5%: 5% of serum; and Group 0-10%: 10% of serum. As for the Control group it was used Base Media + 2.5% of serum from D1 to D7. Statistical analysis was done with ANOVA followed by the Tukey test ($P<0.05$). Results showed that it is possible the IVP for bovine embryos without the addition of serum in culture media. There was no statistical difference ($P>0.05$) when assessing cleavage (72.74±3.18%, 84.18±0.74%, 68.46±2.35%, 75.72±1.77%, 81.07±3.38%), blastocyst formation (32.62±0.59%, 27.27±0.93%, 27.04±2.25%, 30.31±1.31%, 37.13±1.50%), development stage (5.16±1.12, 5.01±1.10, 5.06±1.02, 4.98±0.99, 5.21±1.04) and embryo quality (2.80±1.39, 2.89±1.36, 2.99±1.33, 2.78±1.42, 2.65±1.39), for the groups Control, 0-0%, 0-2.5%, 0-5% e 0-10%, respectively. However, the increase of serum concentration in the embryo culture media showed an increase of medium and large, lipid droplets. Nevertheless, for the groups with lower concentrations of serum (0-0% e 0-2.5%) the number of small lipid droplets was similar to the ones with higher concentrations (0-5% e 0-10%). We can conclude that it is possible to perform the IVP with no supplementation of serum in culture media, without producing any negative effect upon the production, development, and embryo quality.

Key words: *in vitro* embryo production, fetal calf serum, lipid accumulation, *Bos taurus indicus*, *Bos taurus taurus*.

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EFEITO DA CONCENTRAÇÃO DO SORO FETAL BOVINO SOBRE A PRODUÇÃO *In Vitro* E ACÚMULO LIPÍDICO CITOPLASMÁTICO EM EMBRIÕES CRUZADOS *Bos taurus indicus* X *Bos taurus taurus*

RESUMO

O objetivo deste trabalho foi estudar a suplementação de cinco diferentes concentrações de soro fetal bovino (SFB), a partir do terceiro dia do cultivo embrionário, sobre a produção *in vitro* (PIV) e o acúmulo lipídico citoplasmático de embriões bovinos oriundos do cruzamento da raça Nelore com a raça Simental. Ovários de abatedouro de vacas Nelore foram utilizados para a obtenção dos oócitos que foram maturados *in vitro*. Decorrida a fertilização *in vitro* com o sêmen de um touro Simental, os possíveis zigotos foram desnudos e transferidos para as placas de cultivo. Do D1 ao D3 utilizou-se o meio SOFaa + 5% BSA com 0% de SFB. Do D4 ao D7 os embriões foram distribuídos em quatro grupos experimentais de acordo com a concentração de SFB adicionada ao meio de cultivo: Grupo 0-0%: SOFaa + 5% BSA (meio base), Grupo 0 – 2,5%: Meio base + 2,5% de SFB, Grupo 0-5%: Meio base + 5% de SFB e Grupo 0-10%: Meio Base acrescido de 10% de SFB. Como grupo controle foi utilizado um sistema de cultivo contendo 2,5% de SFB do D1 ao D7. Para análise estatística utilizou-se ANOVA seguido do Teste de Tukey ($P<0,05$). Os resultados mostraram que é possível a PIV de embriões bovinos sem a suplementação do meio de cultivo com SFB. Não foi observada diferenças ($P>0,05$) quanto a clivagem (72,74±3,18%, 84,18±0,74%, 68,46±2,35%, 75,72±1,77%, 81,07±3,38%), a formação de blastocistos (32,62±0,59%, 27,27±0,93%, 27,04±2,25%, 30,31±1,31%, 37,13±1,50%), o estágio de desenvolvimento (5,16±1,12, 5,01±1,10, 5,06±1,02, 4,98±0,99, 5,21±1,04) e grau de qualidade embrionária (2,80±1,39, 2,89±1,36, 2,99±1,33, 2,78±1,42, 2,65±1,39), respectivamente para os grupos controle, 0-0%, 0-2,5%, 0-5% e 0-10%. Apesar disso, com o aumento da concentração do SFB no meio de cultivo embrionário, foi observada uma elevação no acúmulo de gotas lipídicas médias e grandes. No entanto, nos grupos com menores concentrações de soro (0-0% e 0-2.5%) o número de gotas lipídicas pequenas foi similar ao dos grupos com maiores concentrações (0-5% e 0-10%). Conclui-se que é possível a PIV sem a suplementação do meio de cultivo com SFB, não afetando a produção, o desenvolvimento e a qualidade embrionária.

Palavras-Chave: produção *in vitro*, soro fetal bovino, acúmulo lipídico, *Bos taurus indicus*, *Bos taurus taurus*.

EFECTO DE LA CONCENTRACIÓN DEL SUERO FETAL BOVINO EN LA PRODUCCIÓN *In Vitro* Y LA ACUMULACIÓN DE LÍPIDOS CITOPLASMÁTICOS EN LOS EMBRIONES CRUZADOS *Bos taurus indicus X Bos taurus taurus*

RESUMEN

El objetivo fue estudiar la suplementación de cinco diferentes concentraciones de suero fetal bovino (SFB) en la producción in vitro (PIV) y la acumulación de lípidos citoplasmáticos en embriones bovinos obtenidos desde el cruce de Nellore con el Simmental. Ovário de matadero de vacas fueron utilizados para obtener los ovocitos y fueron madurados in vitro. Después de la fecundación in vitro con semen de un toro Simmental, los cigotos posibles se talaron y se transferirán a las placas de cultivo. De D1 a D3 fue utilizado el SOFaa + 5% de BSA con 0% de SFB. De D4 a D7 los embriones fueron divididos en cuatro grupos de acuerdo con la concentración de SFB en el medio de cultura: Grupo 0-0%: SOFaa + 5% de BSA (medio

base); Grupo de 0-2,5%: medio base + 2,5% de SFB, 0-5% Grupo: medio base + 5% de SFB y Grupo 0-10%: medio base + 10% de SFB. Como control se utilizó un sistema de cultivo que contiene 2,5% de suero fetal bovino de D1 a D7. Para análisis estadística fue usado ANOVA seguido del Test de Tukey ($P<0.05$). Los resultados mostraron que es posible que los embriones de la especie bovina PIV sin suplementación del medio de cultivo con suero fetal bovino. No hubo diferencia estadística ($P> 0,05$) y la escisión (72,74 ± 3,18%, 84,18 ± 0,74%, 68,46 ± 2,35%, 75,72 ± 1,77%, 81,07 ± 3,38%), la formación de blastocistos (32,62 ± 0,59%, 27,27 ± 0,93%, 27,04 ± 2,25%, 30,31 ± 1,31%, 37,13 ± 1,50%), la etapa de desarrollo (5,16 ± 1,12, 5,01 ± 1,10, 5,06 ± 1,02, 4,98 ± 0,99, 5,21 ± 1,04) y el grado de calidad de embriones (2,80 ± 1,39, 2,89 ± 1,36, 2,99 ± 1,33, 2,78 ± 1,42, 2,65 ± 1,39), respectivamente para el grupo de control, 0-0%, 0-2.5%, 0-5% y 0-10%. Sin embargo, al aumentar la concentración de SFB en el centro de desarrollo del embrión, se observó un aumento en la acumulación de gotitas de lípidos medianas y grandes. Sin embargo, en aquellos con menores concentraciones de suero (0-0% y 0-2,5%) el número de pequeñas gotitas de lípidos fue similar a los grupos con mayores concentraciones (0-5% y 0-10%). Se concluye que la PIV es posible sin la suplementación del medio de cultivo con suero fetal bovino, sin afectar la producción, el desarrollo y la calidad del embrión.

Palabras-claves: producción in vitro, suero fetal bovino, acumulación de lípidos, *Bos taurus indicus, Bos taurus*.

INTRODUCTION

Throughout the last decade there had been a great advance in the *in vitro* embryo production (IVP) with the improvement of the culture systems, which are more efficient now. But until the present moment there is the challenge of overcoming the use of non-defined or semi-defined media that are supplemented with either fetal calf serum (FCS) or bovine serum albumin (BSA).

It is known that the FCS provides energy substrate, amino acids, vitamins, growing factors, antioxidants and heavy metal chelants, which are important for the embryo development (1).

However, not only do the components' concentration of FCS varies in between samples, but also the use of FCS is associated to the occurrence of large offspring syndrome (2), metabolic changes and excessive lipid accumulation (1, 3, 4). FCS is also associated with organelle abnormalities (3, 5), including the mitochondrial degeneration (5), premature blastocoel formation (6), and the decrease of embryo survival after cryopreservation (1, 3, 7).

The FCS may as well be a source of pathogenic viruses, and international laws for the embryo transportation focus on the ban on animal by-products from embryo culture media (4).

Comparing the embryos that are produced *in vitro* to the ones produced *in vivo*, it can be observed structural differences that can be the result of the selected culture system (8). Some authors suggest that the increase in the lipid content of IVP embryos occurs due to the supplementation of FCS to the culture media (3, 7), or yet, a result of the mitochondrial insufficient metabolism due to the inability of metabolizing lipid complexes via β-oxidation (5).

Barceló-Fimbres & Seidel (4) showed that it is possible the IVP of bovine embryos in the absence of FCS without affecting both the production and quality of blastocysts. Mucci *et al*. (1) observed that bovine embryo cultures in the absence of FCS had better rates of embryonic survival after vitrification.

The use of embryo culture with FCS in the IVP is widespread in such a fashion that affects the embryo market expansion since it may be a pathogenic microorganism source, and thus the embryo might be a possible pathogenic vector. Apart from that, it facilitates the excessive cytoplasmic lipid accumulation in the embryo thus making it more sensitive to the cryopreservation process.

Having said that, the present study aimed at studying the supplementation of five different concentrations of FCS, starting from the third day of the embryo culture so as to assess its influence upon the *in vitro* production and the cytoplasmic lipid accumulation in bovine embryos coming from Nelore (*Bos taurus indicus*) and Simmental (*Bos taurus taurus*) crossbred offspring.

MATERIAL AND METHODS

Experiment Design

A total of 1100 oocytes were collected from ovaries coming from slaughterhouse. These ovaries were from Nelore animals (*Bos taurus indicus*), and 12 repeats were done testing different concentrations of FCS in relation to cytoplasmic lipid accumulation and IVP of embryos according to figure 1.

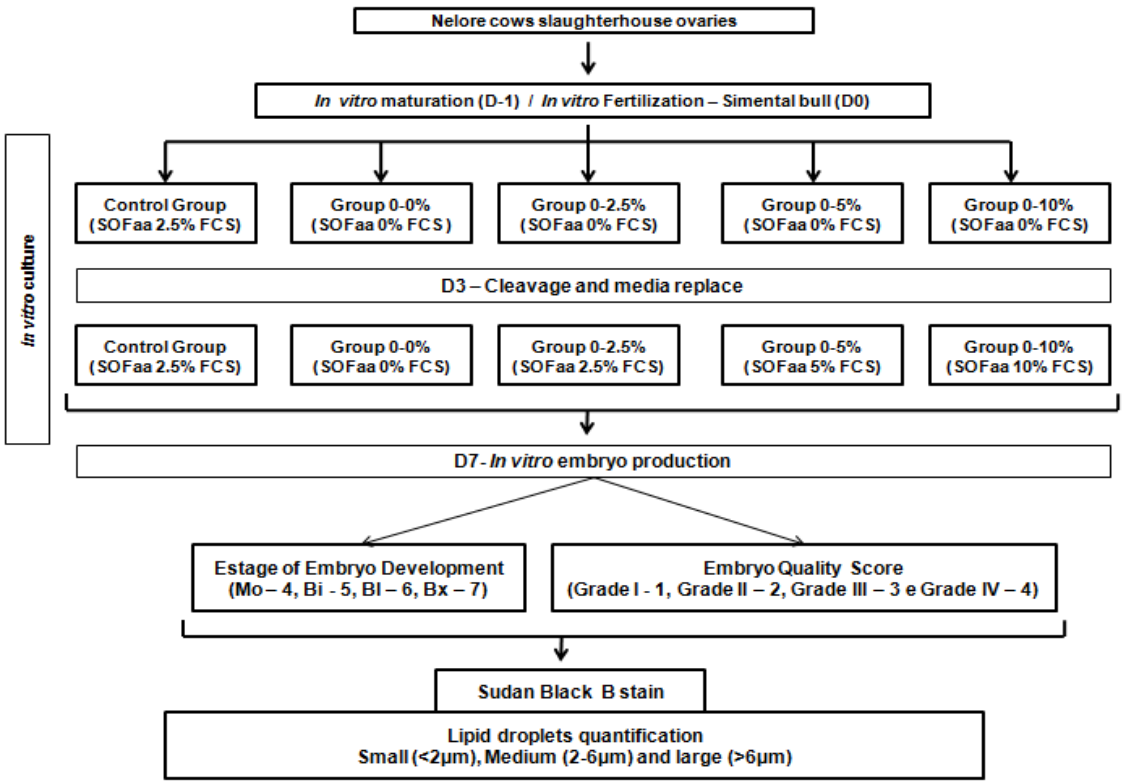

Figure 1. Experiment design.

Reagents

All substances that were used in this experiment were purchased from Sigma (Sigma–Aldrich Corp., St. Louis, MO), except for the ones that are specified in the text.

In vitro maturation (IVM)

Ovaries from slaughterhouse were used in order to obtain oocytes from two to eight mm follicles. Only oocytes with three or more *cumulus* cells layers and with homogeneous color were used.

The selected oocytes were matured *in vitro* (IVM) in a 38.5 ^{o}C incubator, with 5% of CO_2 in air, for 22 - 24 hours. It was used petri dish containing drops covered with mineral oil. For each drop of 90µl of TCM 199 with Earle's salts (Gibco 31.100; Grand Island, NY, USA) supplemented with L-glutamine, 10% of FCS, 2.2 mg/ml sodium pyruvate, 5mg/ml LH (Lutropin-V®, Vetrepharm, Ontario, Canada), 1mg/ml FSH (Pluset®, Calier, Barcelona, Espanha), and 75µg/ml of gentamicin and then it was placed 20-30 oocytes per drop.

In vitro fertilization (IVF)

After the maturation period the groups of 20-30 oocytes were transferred to drops of 90 µl of fertilization media covered with mineral oil. Oocytes were then subjected to IVF with Simmental bull semen (*Bos taurus taurus*) from one single batch. Spermatozoa were selected using the Percoll method and the concentration was adjusted to $1x10^6$ spermatozoa/mL. Fertilization occurred in HTF media (Irvine Scientific, Santa Ana, USA) added by 5mg/mL of BSA, 0.5 mg/mL of caffeine, 3mg/mL of heparin, 2.2mg/mL of pyruvate and 75µg/mL of gentamicin. Oocytes and spermatozoa were incubated at the incubator with the same IVM conditions for approximately 18 hours. The day in which the fertilization took place was considered to be day zero (D0).

In vitro culture

After 18 hours of IVF the possible zygotes were denuded and transferred to 90µl culture media drops in groups of 20 – 30 structures in petri dishes and were covered with mineral oil. The embryo culture was performed in a serial manner. From D1 to D3 of culture embryos were kept in culture media 1 composed by SOFaa supplemented with 0.5% of BSA, 2.2mg/mL of sodium pyruvate and 75µg/ml gentamicin (Base Media) and 0% of FCS. By the end of D3, the cleavage rate was assessed, and the media was changed to culture media 2. Media 2 was divided into four subgroups according to the FCS supplementation: Group 0 - 0%: Base media without the supplementation of FCS, Group 0-2.5%: Base Media with the supplementation of 2.5% of FCS, Group 0-5%: Base media with the supplementation of 5% of FCS and Group 0-10%: Base Media with the supplementation of 10% of FCS. As a Control group it was used a culture system containing Base media with 2.5% of FCS from D1 to D7. Culture media was changed on D5 using the same media and keeping the same concentrations of FCS of D3 for all groups. Embryos were kept in these media until D7 in the incubator with saturated humid atmosphere, at 38.5°C, and with 90% of N_2, 5% of CO_2 and 5% O_2. At that moment the embryo production was assessed and it was attributed scores according to their development stage as follows: Morule - 4, Initial Blastocyst - 5, Blastocyst - 6, Expanded Blastocyst- 7, and embryo quality score: grade I (excellent) -1, grade II (good) - 2, grade III (poor) - 3 and grade IV (degenerated structure) – 4.

Lipid quantification

After the evaluation of the embryo production, a sample of embryos was selected at random throughout the replications of the experiment and was subjected to the Sudan Black B

stain (Sigma S-0395) to quantify the cytoplasmic lipid droplets of the embryos coming from different groups.

The embryos were fixed in 10% formalin solution in PBS with pH 7.4 for 2 hours at room temperature, they were washed with distilled water lately with 0.05% PVA, and after they were transferred to one drop of ethanol at 50% in water. After 2 minutes the embryos were stained with four drops of Sudan Black B at 1% (w/v) in 70% ethanol solution for 1- 2 minutes. Later on, the embryos were washed 3 times (5 minutes each time) in 50% ethanol, and soon after that they were washed in 0.05% PVA in distilled water for 5 minutes. After that, slides were prepared with 10µL drops of glycerol to put embryos and then they were covered with glass cover slide to be then read through the light microscope with a 400x magnification.

It was made in the AutoCAD 2000 software (Autodesk, Inc., California, E.U.A.) a grid with 5 squares measuring 1600 μm^2 each, and they were all subdivided by lines that were at times straight lines, and at times short-dashed (5µm) (figure 2). As for the lipid quantification the cytoplasmic lipid droplets were divided according to their size as follows: small (smaller than 2µm), medium (between 2 and 6µm) and large (bigger than 6µm), adapted from Abe *et al*. (3). The number of lipid droplets was counted in 1000μm^2.

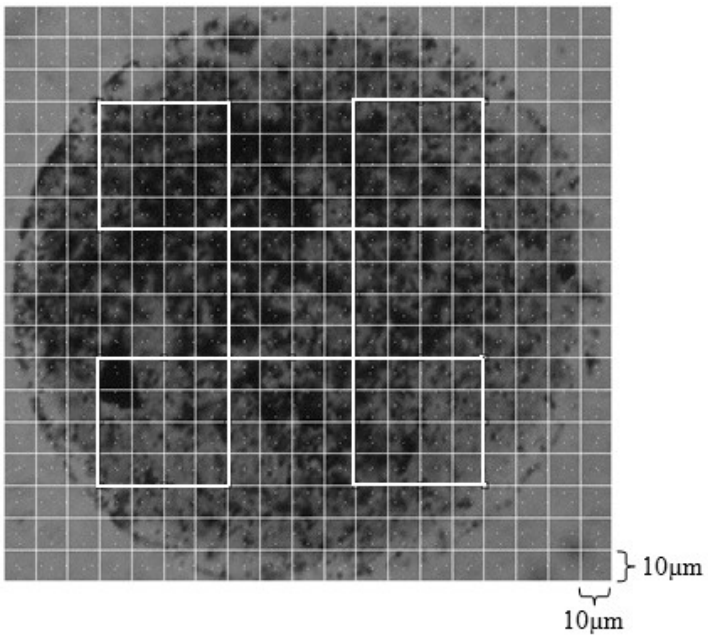

Figure 2. Grid for the lipid quantification of the embryos through Sudan Black B staining.

Statistical analysis

The cleavage rates and the blastocyst production were calculated starting from the total number of oocytes that were put in culture to mature.

In order to perform the statistical analysis the dependent variables that were expressed in percentage (% of cleavage and % of blastocyst production) were transformed using the arcsine ($\sqrt{y}/100$ arcsine transformation) for later analysis using the variance analysis (ANOVA) followed by the Tukey test. The untransformed data is presented on Table 1.

Table 1. Average (sd) of Cleavage %, Blastocyst %, Embryo Development Stage, Embryo Quality and cytoplasmic lipid droplets (Small, Medium, and Large) from Groups Control, 0–0%, 0–2.5%, 0–5%, 0–10%.

Groups/Parameters	Control	0 - 0%	0 – 2.5%	0 – 5%	0 – 10%
% Cleavage	72.74 (3.18)	84.18 (0.74)	68.46 (2.35)	75.72 (1.77)	81.07 (3.38)
% Blastocyst	32.62 (0.59)	27.27 (0.93)	27.04 (2.25)	30.31 (1.31)	37.13 (1.50)
Development stage	5.16 (1.12)	5.01 (1.10)	5.06 (1.02)	4.98 (0.99)	5.21 (1.04)
Embryo quality	2.80 (1.39)	2.89 (1.36)	2.99 (1.33)	2.78 (1.42)	2.65 (1.39)

Data did not differ (P>0.05). Number of repeats = 12. Stage of embryo development-Score: Morule-4; Initial Blastocyst-5; Blastocyst-6; Expanded Blastocyst-7. Embryo Quality Score: Grade I (excellent)-1; Grade II (good)-2; Grade III (poor)-3; Grade IV (degenerated)-4.

For the discrete dependent variables (development stage and embryo quality score), were performed the Kolmogorov and Smirnov adherence test to check if they had normal distribution. Finally, data were analyzed by analysis of variance (ANOVA) followed by the Tukey test. The significance level of 5% (P<0.05) was used. Statistical analysis was performed in the GraphPad InStat 3 software (Graph Pad Software Inc., San Diego, CA).

RESULTS

The supplementation of FCS disregarding its concentration did not show any influence upon the cleavage and blastocyst formation rates. It did not also affect the development stage and embryo quality score (Table 1).

With the increase in the concentration of FCS in the embryo culture media it was observed the increase in the number of medium and large, cytoplasmic lipid droplets (Figures 4 and 5). However, the Groups that contained a lower concentration of FCS showed a similar amount of small cytoplasmic lipid droplets to the ones from the Groups with higher concentrations (Figure 3).

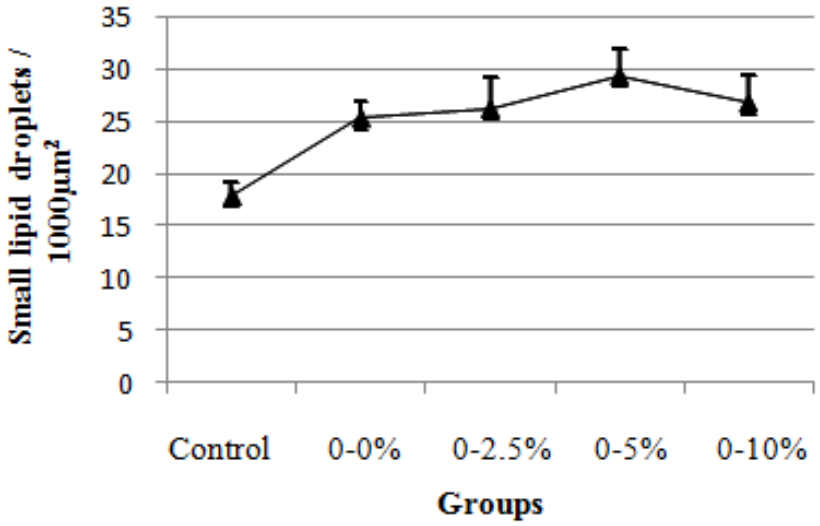

Figure 3. Average (sd) of the amount of small cytoplasmic lipid droplets (<2μm) from all groups. There was a significant increase in the number of small cytoplasmic lipid droplets in the Group 0-5% when compared to the Control Group (P<0.05).

DISCUSSION

The present study assessed for the first time the effect of five different concentrations of FCS in the embryo culture media starting from the third day of culture and their influence upon the *in vitro* production and the cytoplasmic lipid accumulation in Nelore (*Bos taurus indicus*) and Simmental (*Bos taurus taurus*) crossbred bovine embryos.

Results show that the supplementation of FCS regardless of its concentration was not crucial for the *in vitro* embryo development since there had been no difference (P>0.05) concerning the cleavage rates and blastocyst production. On the same way the development stage and the embryo quality score did not differ between groups (Table 1).

Nevertheless, the increase in the concentration of FCS in the embryo culture media led to an increase (P<0.05) in the lipid accumulation, thus appearing a bigger number of medium and large lipid droplets (Figures 4 and 5, respectively) in the produced embryo with concentrations higher than 5%. In spite of that, the groups with lower concentration of FCS showed that the number of small cytoplasmic lipid droplets was similar (P>0.05) to the ones with higher concentration (Figure 3).

According to the literature the presence of FCS may on the one hand inhibit the first cleavage divisions, and on the other hand accelerate the embryo development until the blastocyst stage (7, 9, 10). In the present study the serum biphasic effect was not observed since there was no significant difference between groups. This occurred not only to the cleavage rates but also to the blastocyst production going along with the results found by Barceló-Fimbres & Seidel (4) and Mucci *et al.* (1).

Not like in the literature (7, 9, 11), the present study did not notice that the supplementation of FCS would favor the embryo development since there was no difference in the embryo scores between groups even though the concentrations were increased (5.16±1.12, 5.01±1.10, 5.06±1.02, 4.98±0.99, 5.21±1.04 - Control group, 0-0%, 0-2.5%, 0-5%, 0-10%, respectively - Table 1).

The same lack of FCS influence was observed for the embryo quality score since the scores from different treatments did not differ (P>0.05) (2.80±1.39, 2.89±1.36, 2.99±1.33, 2.78±1.42, 2.65±1.39 - Control Group, 0-0%, 0-2.5%, 0-5%, 0-10%, respectively - Table 1).

Some authors have already reported that the supplementation of FCS increased the number of cells per embryo, hence improving its quality (1, 11, 12). However, other authors observed a negative effect upon the number of total cells and embryo quality (13, 14). Some authors mention no influence of FCS (15), agreeing with the result of the present experiment. This results' variability found in the literature indicates that the effect of FCS upon the embryo quality is too complex, so it is a must to develop further studies with a view to enlarging our knowledge.

Apparently, the major drawback of the FCS supplementation to the embryo culture media is the excessive cytoplasmic lipid accumulation in the *in vitro* embryo production, which according to literature (1, 3, 4, 7), directly affects the embryo cryotolerance.

There was no significant difference in the number of small cytoplasmic lipid droplets between groups with lower (25.25 and 26.18 lipid droplets / 10^3 μm^2, for the groups 0-0% and 0-2.5%, respectively) and higher (29.33 e 26.71 lipid droplets / 10^3 μm^2, for groups 0-5% and 0-10%, respectively) FCS concentrations (Figure 3). Apparently, the supplementation of FCS

to the embryo culture media is not responsible for the small lipid droplets accumulation, since there was no difference between groups with higher concentration of FCS and the groups Control and without supplementation of FCS. The small cytoplasmic lipid droplets may probably come from a different embryo development stage since a great number of lipid droplets are present in bovine oocytes and embryos in the first cleavages after the IVM and IVF (16, 17). It is known that immature oocytes have 59 ng of triglycerides, and this number decreases to 46 ng after their maturation, and later to 34 ng in two-cell embryos (18).

However, with the increase of FCS concentration in the culture media, the number of medium cytoplasmic lipid droplets also increased (Figure 4), being the group 0-10% the one with greater lipid accumulation (17.13 lipid droplets / 10^3 μm^2) when compared to groups Control (9.25 lipid droplets / 10^3 μm^2; $P<0.001$), 0-0% (10.5 lipid droplets / 10^3 μm^2; $P<0.01$), 0-2.5% (11.8 lipid droplets / 10^3 μm^2; $P<0.01$) and 0-5% (10.83 lipid droplets / 10^3 μm^2; $P<0.01$). On the very same way the number of large cytoplasmic lipid droplets, also showed an increase when the concentration of FCS was increased for the group 0-10% (14.71 lipid droplets / 10^3 μm^2) when compared to the groups 0-0% (7.3 lipid droplets / 10^3 μm^2) and 0-5% (7.04 lipid droplets / 10^3 μm^2) ($P<0.05$ - Figure 5).

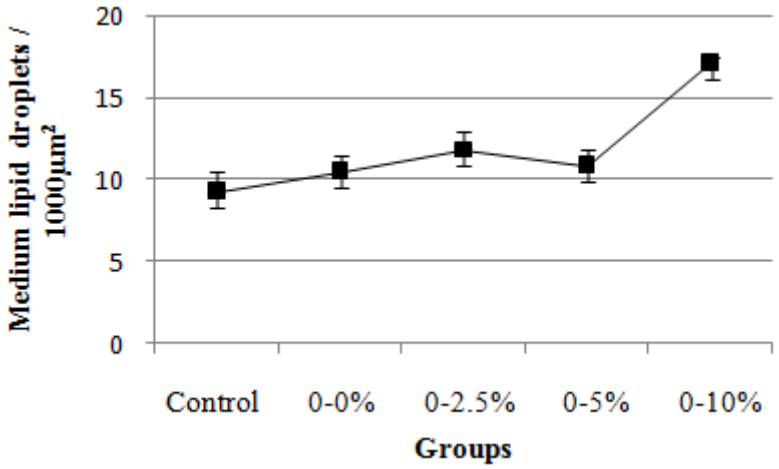

Figure 4. Average (sd) of the amount of medium cytoplasmic lipid droplets (2-6µm) from all groups. There was an increase in the number of medium lipid drops in the Group 0-10% when compared to the Control Group ($P<0.001$), 0-0%, 0-2.5% and 0-5% ($P<0.01$).

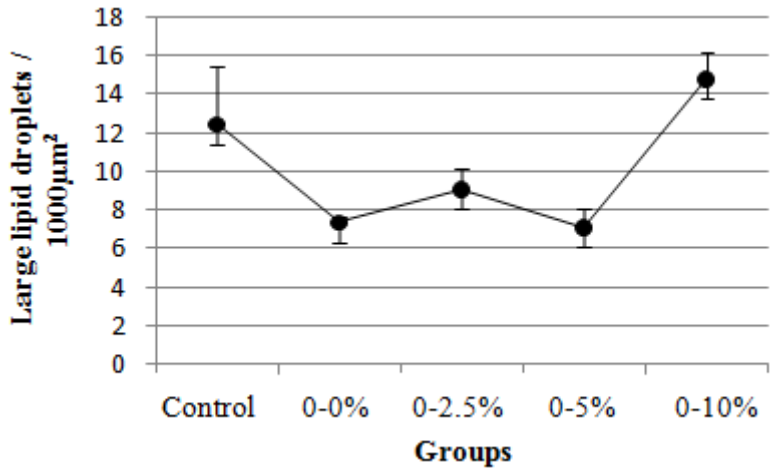

Figure 5. Average (sd) of the amount of large cytoplasmic lipid droplets (>6μm) from all groups. There was a significant increase in the number of large lipid droplets in the Group 0-10% when compared to the Groups 0-0% and 0-5% ($P<0.05$).

These results were in accordance with the literature since the lipid accumulation in *Bos taurus taurus* embryos and in embryos of unknown origin were higher with either the 5% (3, 19) or 10% supplementation of FCS (20), throughout that period of embryo culture when compared to serum-free media.

On the same manner, it is estimated that the bovine blastocyst coming from culture systems containing a supplementation of 10% FCS possess 62 ng triglycerides, whereas the ones coming from systems without FCS possess only 36 ng (18). Sata *et al*. (21) showed that the supplementation of 5% of FCS in the culture media results in the increase in the proportion of palmitic and stearic saturated fatty acids, and the oleic and palmitoleic monounsaturated, in bovine blastocysts.

The mechanism and the source of cytoplasmic lipid accumulation in bovine embryos cultured with FCS is quite complex and it is still unclear. Some mechanisms may explain this event such as a) the lipoproteins present in the serum may be internalized by the embryo cells thus increasing the cytoplasmic lipid content (21, 22), b) the presence of serum would change the β-oxidation in accordance with the mitochondrial dysfunction (3, 5, 23), and c) the embryo would be induced to perform a triglyceride neosynthesis according to the presence of serum (24).

CONCLUSIONS

According to the results showed in the present study it is suggested that the supplementation with FCS in the culture media, starting from D3 is not crucial for the embryo development since it did not affect the *in* vitro production rate (cleavage and blastocyst production), the development stage and the embryo quality score coming from a crossbreeding of Nelore and Simmental.

Apart from that, it was seen that the increase in the concentration of FCS in the culture media 2 has produced a bigger cytoplasmic lipid accumulation in crossbred embryos (Nelore x Simmental), since there was a bigger number of medium and large lipid droplets. But the small cytoplasmic lipid droplet accumulation was not correlated to the serum concentration.

These findings support the idea that the decrease in the concentration of serum or even its removal from the embryo culture would hinder the appearance of morphological and functional changes in the bovine embryo, apart from eliminating risks of transmission of diseases that might be caused by its supplementation.

ACKNOWLEDGEMENTS

This study was funded by the State Support Research Foundation of São Paulo, FAPESP. Process number 07/57766-4.

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Recebido em: 25/02/2010
Aceito em: 11/01/2011