Quantification of cytokines in the amniotic fluid at the moment of delivery of Nelore calves conceived by in-vitro production and artificial insemination

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ABSTRACT

The aim of the present study was to compare the concentrations of cytokines in the conceptus’ amniotic fluid at the moment of delivery of Nelore calves conceived from embryos produced by in-vitro (IVP) or after artificial insemination (AI). Forty cows were divided into 2 groups: 1- 20 cross-bred Nelore cows pregnant with Nelore calves derived from IVP embryos produced from oocitum pick up (OPU) oocytes; 2 - 20 pregnant Nelore cows bearing AI Nelore calves. The animals were fed pasture and had a free access to mineral salt and water. Around labor, cows were transferred to a maternal paddock permitting delivery observation. During the expulsion phase of the delivery, the amnion was punctured by 40x12g needle and a 20 mL syringe and the fluid was collected into a sterile plastic tube and stored in a freezer at -20C. TNF-\(\alpha\) and IFN-\(\gamma\) concentrations were measured by immunoenzymatic assay using a commercial kit according the manufacturer’s recommendations. Mann-Whitney’s U-test was used for statistical analysis with a significance of 5%. The median of TNF-\(\alpha\) level in the amniotic fluid was significantly higher in group 1 (398.7pg/mL) than in group 2 (151.2pg/mL). Furthermore, the median for IFN was not significantly different between the groups. In conclusion, the TNF-\(\alpha\) were detectable in the all samples and same in the human species it accomplices of the normal delivery mechanism.
The technology applied to Brazilian beef cattle in response to globalization and the rising of competitiveness has improved reproductive and productive efficiency by means of biotechnology. Thus, the genetic gain obtained in the last 20 years has been basically due to the higher intensity and efficiency of breed selection. The initial impulse to bovine selection was started by artificial insemination (AI) and progeny-test programs followed by the embryo-transfer technique (ET). After that, another increase in selection pressure was obtained when applying the technique of large-scale in-vitro production (IVP) of bovine embryos in commercial herds (Baud et al., 1999; Moya_Araujo et al., 2009; Prestes, 2005; Suffys et al., 1989).

The amniotic fluid is an important source for fetal evaluation. A variety of biochemical, cytological, biophysical and immunological methods allow for determining pulmonary, renal and epidermal maturation levels in the fetus as well for revealing genetic abnormalities and other pathologies (Wagtendonk-Deleeuw et al., 2000).

Cytokines present in the liquid amnótico are produced by the amnion, the fetal organs such as kidney and lung and leukocytes (Bry et al., 1997; In’t Anker et al., 2003). According to the Kiyokawa and Yoneyama (2006) cytokines have diverse functions in many cells and their proper balance in the maternal-fetal relationship is needed to maintain pregnancy success.

The higher incidence of calf problems at the time of delivery and the lack of studies in this field have led to the design of the present study, which aimed to determine, at the time of delivery, the concentrations of the tumor necrosis factor (TNF) and interferon gamma (IFN-γ) in the amniotic fluid of calves conceived by in-vitro production in comparison to the fluid of calves obtained by artificial insemination.
A 100% of the analyzed TNF-α samples presented concentration above the detection limit of the test (11.7 pg/mL). The statistical analyses showed that the animals in group 2 presented lower dosages than those in group 1 (p<0.05), as illustrated in Figure 1.

As to the IFN-γ assay, 55% (11/20) of the samples in the IVP group and 50% (10/20) of the samples in represented in Figure 2. No statistical difference was observed for IFN-γ concentration between the groups (p>0.05).

![Fig. 1. Dot plot of TNF-α of Nelore calves’ amniotic fluid samples taken at the moment of delivery in Groups 1 (IVP) and 2 (AI).](image)

4. Conclusion

In the present study, TNF-α was detectable in all samples and similarly to human specie, this cytokine plays an important role in the normal parturition mechanism, when it is present in an ideal concentration. TNF-α concentration in the IVP group was higher than that in the AI group. Nevertheless, such significant higher concentration of TNF-α was not related to neonatal sepsis, since none of the newborns followed in the peripartum period showed sepsis symptoms or died during the evaluated period. However, Leibfried-Rutledge (1999) and Kjeldsberg and Knight (1993) reported a relation, in humans, between high levels of this cytokine in the amniotic fluid and a higher incidence of sepsis in newborns when compared to newborns with normal TNF-α levels. This fact could be due to the lack of information on normal TNF-α concentration patterns for the bovine species, despite the fact that the higher concentration found in the amniotic fluid of the IVP group as compared to that in the AI group did not lead to a high incidence of sepsis in newborn calves, thus showing that the levels reported in both groups were in an acceptable range and compatible with a normal gestation in this species.

IFN-γ is a Th1 cytokine which, when associated with TNF-α, leads to inhibition of the embryonic and fetal development. It has also been reported to present a cytotoxic effect on the embryonic cells of rodents (Park et al., 2004). The initial objective of the present study was to compare the concentration of IFN-γ and TNF-α in calves’ amniotic fluid samples taken at the time of delivery in order to evaluate the synergism between the levels of such cytokines as reported in the literature. However, 60% of the IVP samples and 50% of the AI samples had a detectable concentration of IFN-γ, showing no statistical difference between the groups. These results suggest that all animals had a similar IFN-γ pattern, which did not lead to the synergism reported in other studies.
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References


