ESTRADIOL-17β QUANTIFICATION IN BLOOD SERUM USED FOR RUMINANT EMBRYO IN VITRO PRODUCTION BY LC-MS/MS

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Overview

The aim of this work was to develop a robust HPLC-MS method to quantify Estradiol-17β (E2) in different blood serum samples used as media supplement for ruminant embryo *in vitro* production (IVP). E2 has the potential to affect embryo gene expression. Therefore, it's quantification is crucial in order to determine E2 role in gene expression aberrancies found in IVP embryos. Method detection limit (LOD) was 0.08 pg/mL and quantification limit (LOQ) was 0.25 pg/mL, showing that this method is appropriated to further studies of E2 influence on embryo gene expression.

Introduction

Blood serum (BS) is a supplement commonly used for ruminant IVP. Due to sanitary reasons and its undefined composition, BS needs to be replaced by synthetic supplements. BS use has been related to offspring abnormalities such as the large offspring syndrome. E2 is known to affect endometrial gland secretion in the pre-implantation period and to induce and hasten trophoblast differentiation *in vitro*. The aim of this work was to develop a robust HPLC-MS method to quantify E2 in different serum samples used for ruminant embryo IVP production. The precise knowledge of E2 concentration is a crucial parameter for the BS composition.

Methods

For sample extraction, 10µL formic acid was mixed to 400µL of BS, then 4mL of petroleum ether:hexane (80:20) was added. After vortexing (2min) and centrifugation (10min), 2mL from the supernatant was dried by N2 gas flow. The residues were dissolved in 400uL of methanol. An API 5000 triple-quadrupole mass spectrometer with its APPI (photospray ionization) source was used in the MRM negative ion mode to monitor *m/z* transitions 271.2>145.1 and 271.2>182.9. Toluene was used as dopant at flow rate of 0.125 mL/min.An Agilent 1100 series HPLC was used to isocratically elute the analyte with methanol/water (70/30; v/v) at 0.5 mL/min by using a Phenomenex Acqua C18 column (50mm x 2mm – 5µm) in 3.0 min total run time.

Results and Conclusion

Calibration curve was constructed with triplicates of 1.5, 2.5, 5.0 and 10.0 pg/mL E2. Method recuperation efficiency was evaluated with the addition of 2.5 and 25 pg/mL of E2 to blood serum samples containing no E2. Thirteen SB samples were analyzed [bovine fetal calf serum (FCS): 10 batches, sheep BS: 2 batches, and goat BS: 1 batch], and 1 sample of commercial serum replacement. E2 was detected in five bovine FCS batches at concentration of 0.09, 0.09, 0.11, 0.32, and 1.9 pg/mL. The other samples presented no detectable levels of E2. The bovine FCS sample containing the highest E2 concentration (1.9 ng/mL) was a commercial mixture of FCS or other serum enriched with components not specified by the supplier, and intended to be used as a substituent for FBS in many applications. In mouse, E2 plays important roles in blastocyst activation and implantation in the progesterone-primed uterus. Action of estrogens on cells occurs by the classical genomic mechanism, which implies hormone binding to a cytosol or nuclear receptor to induce transcription and translation of special genes to produce functional proteins. The nongenomic and fast action of estrogens is also believed to occur in some kinds of cells. In bovine embryos, the E2 role and concentration effect in the culture medium of developing embryos have not been addressed. In this work, we show that E2 may be present in bovine FCS batches. Sensitive and precise HPLC-MS/MS quantification of E2 using negative ion APPI would certainly benefit studies on the effect of E2 on embryo preimplantation development, leading to a better understanding of the E2 hormone metabolic role and its ideal concentration for ruminant embryo culture, as well as to develop ideal defined serum replacements. In conclusion, E2 quantification of BS used for ruminant IVP by a robust HPLC-MS/MS method may increase knowledge of embryo metabolic needs.

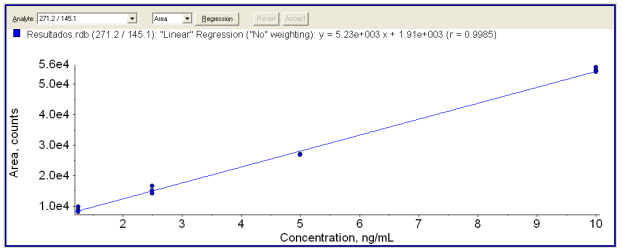

Figure 1. E2 calibration curve constructed with 1.5, 2.5, 5.0 and 10.0 pg/mL

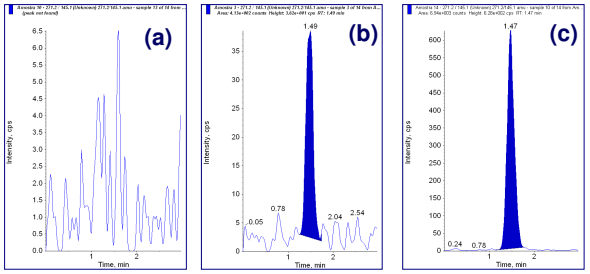

Figure 2. Examples of chromatograms from **(a)** negative sample, **(b)** a sample containing 0.09pg/mL, and **(c)** a sample containing 1.9pg/mL.

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