

# The effect of different zwitterionic buffers and PBS used for out-of-incubator procedures during standard *in vitro* embryo production on development, morphology and gene expression of bovine embryos

A.T. Palasz<sup>\*</sup>, P. Beltrán Breña, J. De la Fuente, A. Gutiérrez-Adán

Ministry of Science and Innovation, Department of Animal Reproduction, INIA, Madrid, Spain

Received 29 November 2007; received in revised form 11 June 2008; accepted 18 June 2008

## Abstract

The effect of the zwitterionic buffers HEPES, TES and MOPS and of PBS used for out-of-incubator procedures during standard *in vitro* embryo production on bovine oocytes and embryo development, morphology and on the expression patterns of eight selected genes: Fgf-4, Lama1, Ube2a, Gsta4, Il6, Sod1, Prss11 and Hspb1, was evaluated. All buffers were prepared at a concentration of 10 mM in TALP medium, with the exception of PBS. The total time of oocyte/embryo exposure to each buffer was ~41 min. The cleavage rates and number of embryos that developed to  $\geq 8$  cells at day 4 were no different among the buffers tested, however, more blastocysts developed at day 7, 8 and 9 in HEPES and MOPS treatments than in PBS and TES ( $P < 0.05$ ). No difference between buffers in total and apoptotic cell number was found. Except for Hspb1 and Ube2a genes, the levels of expression of the six remaining transcripts were higher in *in vivo* than in *in vitro* embryos irrespective of buffer used ( $P < 0.05$ ). In addition, higher expression of Hspb1 and lower expression of Ube2a and Lama1 were observed in PBS and TES than in MOPS and HEPES treatments ( $P < 0.05$ ). Expression of Fgf-4 and Gsta4 in the *in vitro* embryos was lower in PBS than in the remaining three buffers ( $P < 0.05$ ) and the level of expression of the Il6 gene was not affected by any buffer tested but was lower in *in vitro* than in *in vivo* derived embryos. Expression of both Sod1 and Prss11 genes in MOPS were at the level of the *in vivo* embryos. These results showed that the choice of buffer and short exposure time of ~41 min, affects mRNA expression of *in vitro* produced bovine embryos.

© 2008 Elsevier Inc. All rights reserved.

**Keywords:** Buffers; Zwitterionic; Embryo; Culture; Apoptosis; Gene expression

## 1. Introduction

Maintaining a stable pH in culture media in a physiological pH range of ~7.2 ( $pK_a$ ) is a major

prerequisite during the entire *in vitro* embryo production and oocytes/embryo manipulation procedure. Therefore the choice of the proper buffering system may have a significant effect on the final results of embryo culture. It is generally considered that the buffer should be chemically inert, not interact with other components in the media or with the incubated cells, be non-toxic and provide a stable pH in the required range at the required temperatures. There are a few buffers in the physiological pH range between 6.8 and 7.5 ( $pK_a$ ) at

<sup>\*</sup> Corresponding author at: Department of Animal Reproduction, INIA, Ctra de la Coruña Km 5.9, Madrid 28040, Spain.  
Tel.: +34 91 347 40 21; fax: +34 91 347 4014.

E-mail address: [palasz@inia.es](mailto:palasz@inia.es) (A.T. Palasz).

which most embryo manipulations take place. However, to a certain degree all of the buffers exhibit various deviations from the requirements of being called “ideal”. Tris and phosphate buffers have several limitations. Choice of PBS in this study was dictated by the fact that contrary to zwitterionic buffers phosphate is very active chemically in many biochemical processes [1], and even at very low concentrations may alter the physiology and viability of embryos [2]. Tris has a poor buffering capacity below pH 7.5 [3]. Commonly used bicarbonate also has shortcomings related to restricted solubility and spontaneous liberation of carbon dioxide, requiring a constant bicarbonate atmosphere in order to maintain a stable pH [4]. For such reason bicarbonate buffers are generally used with CO<sub>2</sub> gas controlled incubators for oocytes maturation and embryo culture; however, because of the volatility of the CO<sub>2</sub> in an air atmosphere such as is generally used for manipulation of oocytes and embryos out of the incubator and the resulting changes to medium pH, non-bicarbonate buffers must be used for such procedures. Also, the inability of bicarbonate ions (HCO<sub>3</sub><sup>−</sup>) to maintain a physiological pH range (pH 7.3–7.4) in culture medium in room air (0.03% CO<sub>2</sub>) further restricts the use of this buffer. Nevertheless, inclusion of bicarbonate buffer in oocytes/embryo culture media seems to be necessary and to provide more physiological functions than just pH buffering [5]. It was demonstrated that during mouse embryo culture radio-labeled CO<sub>2</sub> was incorporated into RNA [6], but when HEPES was used gas phase was not required in supporting mouse embryo growth in another studies [40].

The introduction of zwitterionic buffers by Good et al. [7] seemed to solve the problems specified earlier and to satisfy all the criteria for a “perfect” buffer. Especially *N*-hydroxyethylpiperazine-*N*-ethanesulfonate (HEPES) buffer, which was considered to stabilize membranes and not to bind metals, gained quick acceptance for oocytes/embryo culture and manipulation procedures [8,9,10,11]. Zwitterionic buffers, by definition (from German “*Zwitter*”, hermaphrodite), contain both positive and negative ionizable groups. Secondary and tertiary amines provide the positive charges, while sulfonic and carboxylic acid groups provide the negative charges. In addition have a high solubility in water and can maintain stable pH in a physiological pH range between 6.5 and 8.0 (pK<sub>a</sub>) at a wide range of temperatures. It is a common perception that contrary to other non-organic buffers zwitterionians are chemically inert. However, according to Good and Izawa [12] no buffer is truly inert and unexpected side reactions not

related to buffering capacity should be taken into account [13]. Zwitterionic buffers containing hydroxymethyl or hydroxyethyl residues may interact with OH<sup>−</sup> group in the media and produce toxic formaldehyde [14]. In addition, the stability of zwitterionic buffers is not very well defined and aggregation of buffer molecules may occur, limiting buffering efficiency [13,15]. Oocytes treated with a HEPES-buffered medium produced a significantly higher rate of triploid and degenerated oocytes after fertilization with ICSI compared with oocytes treated with a medium without HEPES [16]. Most importantly, all three buffers, HEPES, MOPS and TES, may interact with DNA and affect the molecular integrity of cultured cells [17] that was not previously tested with mammalian oocytes and embryos. Despite these shortcomings, HEPES is the most frequently used buffering agent in mammalian oocytes/embryo culture for bench top procedures [18,19] and seldom used for the long-term culture with or without a CO<sub>2</sub> atmosphere [20,21,10,22–24].

We investigated the potential effect of three zwitterionic buffers, HEPES, MOPS and TES on the mRNA levels of genes highly sensitive to environmental conditions: Fgf-4 (fibroblast growth factor 4 precursor), Lama1 (laminin alpha 1), Ube2a (ubiquitin-conjugating enzyme), Gsta4 (glutathione *S*-transferase A4), Il6 (interleukin 6), Sod1 (superoxide dismutase), Prss11 (IGF binding), and Hspb1 (Heat shock protein binding 1) during out-of-incubator manipulation during standard *in vitro* embryo production. Also embryo development and quality were evaluated.

## 2. Materials and methods

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise stated.

### 2.1. Buffer preparation

All four buffers, HEPES (4-2-hydroxyethyl) piperazine-1-ethanesulfonic acid), MOPS (3-morpholinopropanesulfonic acid), TES (*N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid) and PBS (phosphate buffered saline; Na<sub>2</sub>HPO<sub>4</sub>) were prepared fresh every two weeks at a concentration of 10 mM at 25 °C. With the exception of PBS, zwitterionic buffers were prepared in TALP medium [25]. The final pH was adjusted to 7.2. Buffers were stored at 4 °C until use and zwitterionic buffers were kept protected by aluminium foil during storage but not during oocytes/embryo manipulation procedures. The pH of buffers was checked again before final media preparation and after 2 h at 39 °C. All

Download English Version:

<https://daneshyari.com/en/article/2095676>

Download Persian Version:

<https://daneshyari.com/article/2095676>

[Daneshyari.com](https://daneshyari.com)