Cytotechnology (2013) 65:791–793
DOI 10.1007/s10616-013-9633-8

LETTER TO THE EDITOR

A plea to reduce or replace fetal bovine serum in cell culture media

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Received: 31 July 2013 / Accepted: 6 August 2013 / Published online: 22 August 2013

To the Editor,

Fetal bovine serum (FBS) is a universal growth supplement of cell and tissue culture media. FBS is a natural cocktail of most of the factors required for cell attachment, growth, and proliferation, effective for most types of human and animal (including insect) cells. Although in use for more than 50 years, FBS has never been fully characterized. Recent proteomic and metabolomic studies revealed approx. 1,800 proteins (Anderson and Anderson 2002; Anderson et al. 2004) and more than 4,000 metabolites (Psychogios et al. 2011) present in serum. However, the use of serum in cell culture also bears a number of disadvantages. These disadvantages can either be seen from: (a) a scientific, cell biological point of view, since serum in general is an ill-defined mixture of components in culture media, with qualitative and quantitative, geographical and seasonal batch-to-batch variations, (b) from biosafety aspects, since FBS may contain adverse factors, like endotoxin, mycoplasma, viral contaminants or prion proteins, (c) from ethical perspectives in terms of animal protection arguments regarding the harvest and collection of FBS from bovine fetuses, and (d) in terms of recent concerns about the global supply versus demand of FBS. As a consequence, a number of strategies were developed to reduce or replace the requirement for FBS in cell culture media.

FBS is a by-product of the beef packing industry. Thus, the supply is dictated by many factors, including beef consumption, dairy product consumption, feed prices, environmental factors such as drought, cattle import and export, governmental farm policies (Shailer and Corrin 1999), and the outbreak of diseases (foot and mouth disease, BSE) (Asher 1999; Dormont 1999; Even et al. 2006; Wessmann and Levings 1999). The availability of FBS has changed dramatically over the past few years (Fujimoto 2002). Therefore, all efforts and attempts should be undertaken to overcome the expected shortfall in FBS supply. In the case of FBS, supply versus demand models do not follow typical economic principles. Normally, supply can be adjusted to meet the demand. However, in case of FBS, supply and demand operate independently of each other. In addition, there is a severe geographical mismatch between the supply of and the demand for FBS. Demand is highest in US and Europe, while the major sources of FBS are far away—in Brasil, Argentina, South Africa, Australia, New Zealand, and Central America, since in those countries huge

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meat cattle herds—bulls and cows—roam freely together, and as a result, many cows are pregnant at the time of slaughter (Brunner et al. 2010; Jochems et al. 2002). The same holds for the geographical distances between raw serum producers and FBS processors. The latter are also mainly located in the US and in Europe. It is estimated that approximately 500,000 l of FBS are sold per year, which means that more than 1,000,000 bovine fetuses have to be harvested. And the numbers are still increasing.

The FBS market is only loosely regulated (Hodgson 1991, 1993, 1995; Nielsen 1995). That creates the opportunity for abuse (Hodgson 1991, 1993; Bohn 1995). And abuses have occurred and happen until now. In 1994 it was reported (Hodgson 1995), that around 30,000 l of "New Zealand" serum was sold worldwide. However, only 15,000 l of high-quality FBS were annually collected in New Zealand. Thus, we still do not know whether FBS in general might be blended with newborn or adult bovine serum to meet the quality and/or the increasing demands. And in the past, no attempts have been undertaken to trace the origin of the collected sera by approved methods, e.g. analysis of stable isotopes in the sera, which would give the unequivocal evidence about the geographical origin of the raw serum.

Now, a most recent case of fraud with FBS came up. In 2011, GE Healthcare, a unit of General Electric Co., acquired PAA Laboratories, Linz, Austria. Earlier this year, GE Healthcare published an "Urgent Field Safety Notice", stating that *some lots of PAA Laboratories FBS products are subject to label non-conformances from 2003 until 2011. These old and recent products may contain added adult Bovine Serum Albumin (BSA) of United States origin, water, and/or cell growth promoting additives. For FBS product shipped into countries other than the United States, current product labeling states that the origin of the product is either Australia or EU approved serum sources. In addition to, or instead of product of this origin, the product may contain adult BSA of United States origin and/or may contain FBS from sources including United Sates, Canada, Argentina, Brazil, and/or Mexico*.

Some of these FBS lots, produced within the last 5 years, comprising 143 batches with approx. 280,000 l (FDA 2013), may still be sold under other brand names or other labels.

Obviously, in the last 20 years nothing has changed (Hodgson 1991, 1993, 1995). This latest incident of false FBS supply and quality on the world market might be just the tip of the iceberg. Most importantly, the actual case might also have a substantial impact on the results and the scientific outcome of thousands of cell and tissue culture experiments, which can hardly be ignored!

Thus, this recent incident should be taken as an opportunity to question the future use of FBS in cell culture media and/or to increase quality and safety of those sera, which are still in use, e.g. because of directives in the vaccine production. We therefore appeal to cell and tissue culturists to reduce or completely avoid FBS in their cultures, e.g. by alternatives to FBS, like serum-free cell and tissue culture (Brunner et al. 2010, Gstraunthaler 2003; Gstraunthaler and Lindl 2013), or the replacement of FBS by the use of serum substitutes (Rauch et al. 2011). Especially, cultures that will be initiated in the future, like induced pluripotent stem cells (iPS) (www.stembancc.org), should be grown from the very beginning under serum-free culture conditions. In 2003 and 2009, cell culture experts from all over Europe gathered at two Workshops discussing options for, and methodologies of serum-free cell culture. Two comprehensive Workshop Reports (van der Valk et al. 2004, 2010) were published, in which clear recommendations for a replacement of FBS, and the design of serum-free media, respectively, are provided. Following those recommendations will result in scientifically better results, safer products, contribution to ethical research without harming animals, and better availability of cell and tissue culture media and components with transparent and traceable composition.

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