Universiteit Utrecht

Faculty of Veterinary Medicine

3Rs-Centre Utrecht Life Sciences

Fetal Bovine Serum-free Media and Alternatives

Jan van der Valk[1]

[1] 3Rs-Centre Utrecht Life Sciences, Utrecht University, Utrecht, NL

Roman Kolar[2]

[2] Deutscher Tierschutzbund, Neubiberg, DE

Gerhard Gstraunthaler[3]

[3] Innsbruck Medical University, Innsbruck, AT

In vitro methods are widely used tools to study physiological, biological and pharmacological activities at the cell and tissue level. To achieve good experimental reproducibility, the composition of the cell culture medium is essential. To keep cells alive for longer periods of time and to evaluate proliferation, migration and differentiation a basal medium, serum is most commonly used. Fetal bovine serum (FBS) serves most purposes and is the present standard. FBS is a complex mixture of different factors and contains a large number of components, like growth factors, proteins, vitamins, trace elements, hormones, etc., essential for the growth and maintenance of cells.

The use of FBS in cell culture presents significant issues:

1. **the suffering experienced by the calf during blood collection:** *common practice involves hypoxia and heart puncture of the calf; in the EU, this constitutes a procedure under Directive 2010/63 if carried out in the last third of fetal development.*
2. **inappropriate cellular growth profiles and physiological responses of cells:** *In some cases negative effects of FBS are reported as blocking maturation of (stem) cells or leading to delayed cell differentiation.*
3. **FBS contamination with viruses, prions, etc.:** *serious safety concerns in terms of endotoxins, mycoplasma, viral contaminants or prion proteins.*
4. **the large variability of FBS:** *Batch and lot variations can ultimately lead to experimental variability and limit inter-laboratory reproducibility.*
5. **the fraud-problem:** *In spring 2013, a severe case of fraudulent blending of FBS batches came into public.*

These issues have been discussed at three different workshops. The main conclusions are presented below.

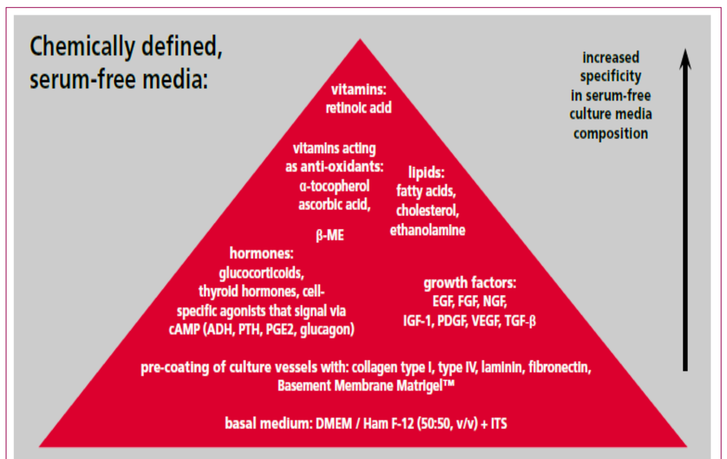
Solutions to replace FBS

- **Serum-free media:** Serum-free media do not require supplementation with serum, but may contain discrete proteins or bulk protein fractions (e.g. animal tissue or plant extracts) and are thus regarded as chemically undefined.
- **Protein-free media:** Protein-free media do not contain high molecular weight proteins or protein fractions, but may contain peptide fractions (protein hydrolysates), and are thus not chemically defined. Protein-free media facilitate the down-stream processing of recombinant proteins and the isolation of cellular products (e.g. monoclonal antibodies), respectively.
- **Animal-derived component free media:** Media containing no components of animal or human origin. These media are not necessarily chemically defined (e.g. when they contain bacterial or yeast hydrolysates, or plant extracts).
- **Chemically defined media:** Chemically defined media do not contain proteins, hydrolysates or any other components of unknown composition. Highly purified hormones or growth factors added can be of either animal or plant origin, or are supplemented as recombinant products.

Platelet lysates

- Recent years showed increased efforts in the establishment of human platelet lysates (hPL) as alternatives to FBS as cell culture supplement.
- Human platelet lysates (hPL) are obtained by freeze-thaw of human platelet concentrates. Platelet releasates are the growth factors released after activating platelets from platelet concentrates.
- With hPL, bulk thrombocytic growth factors are added to basal culture media, providing a human-based, xeno-free culture system.
- The use of hPL as culture media supplement offers a fully humanized, animal-derived component-free (xeno-free) culture system, that openes new avenues in biomedical research.

Develop defined-media



Media Pyramid: a modular approach for the development of serum-free media. **Abbreviations**: ADH, antidiuretic hormone; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF-1, insulin-like growth factor 1; ITS, insulin-transferrin-sodium selenite supplement; ß-ME, ß-mercaptoethanol; NGF, nerve growth factor; PDGF, platelet-derived growth factor; PGE2, prostaglandin E2; PTH, parathyroid hormone; TGF-ß, transforming growth factor-ß; VEGF, vascular endothelial growth factor.

Conclusions and recommendations:

- There is an inconsistency when serum, produced by means that are harmful to animals, is used to establish a method that aims to replace animal experiments.
- The harvesting of FBS from live bovine fetuses in the last third of their development, if it takes place on EU territory, adds a legal issue to the moral one, as it would require regulatory project evaluation including a cost-benefit analysis.
- To be able to work serum-free in daily routine of a cell culture lab, it is desirable to establish (new) cell lines under serum-free conditions.
- Funding should become available for the further development of serum-free media.
- Cell suppliers, such as stem cell banks, should make specific requirements in culture method descriptions, addressing the need to apply FBS-free media for the cell types for which these exist.
- Commercially available serum-free media should be standardized and rigorously validated for various types of cell cultures.
- On-line serum-free databanks, with comprehensive search functions and free access, should be established.

Workshop reports:

- Van der Valk, J. , et al. (2004) *The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture.* Toxicology In Vitro 18, 1-12
- van der Valk, , J. , et al. (2010) *Optimization of chemically defined cell culture media – Replacing fetal bovine serum in mammalian in vitro methods.* Toxicology in Vitro 24, 1053-1063
- Van der Valk, J. et al. (in prep) *Fetal Bovine Serum (FBS): Past – Present – Future*

The 3rd workshop was supported by:

set Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

DEUTSCHER TIERSCHUTZBUND E.V.