

# Foetal Bovine Serum – A pain in the dish?

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The use of animals for research purposes is nowadays not only a dilemma in research but causes also a strong concern, in particular to the general public. In addition, the European Directive on the protection of animals used for scientific purposes (Dir 2010/63/EU) acknowledges that animals have an intrinsic value and their use in research should be kept to an absolute minimum. Furthermore, we currently face a reproducibility crisis in research, in particular in research using animals and animal-derived products. To answer to both issues, the use of animals and animal-derived products should be avoided wherever possible. The application of cell and tissue culture (*in vitro*) methods is generally thought and accepted as one way of addressing the above-mentioned issues. But, do they?

Foetal Calf Serum (FCS), also known as Foetal Bovine Serum (FBS) is a common and an almost universal supplement of cell culture media. However, FCS is harvested, with a reasonable chance of suffering, by a heart puncture from bovine foetuses discovered in pregnant cows during slaughter. Furthermore, since it is a natural product, the composition of FCS is unknown and varies from batch to batch, which may affect the experiments and impedes the reproducibility of results.

Two workshops in the past focussed on foetal pain and distress during blood harvesting for FBS production<sup>1</sup> and on current *in vitro* methods devoid of FBS or other animal components<sup>2</sup>. Recommendations are provided to avoid foetal bovine suffering during collection of blood to prepare serum from. Also strategies are discussed to develop animal-serum free media, and in particular chemically-defined media.

Recent years showed tremendous efforts in the establishment of human platelet lysates as a valuable alternative to FBS as cell culture supplement, although being undefined. This development, together with successful serum-free applications in microphysiological systems and organ-on-chips technologies, led to a 3<sup>rd</sup> workshop on FBS, serum alternatives and serum-free media<sup>3</sup>. It was concluded that:

- 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 foetuses 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) *in vitro* methods under serum-free, preferably chemically defined, conditions.
- On-line serum-free databanks, with comprehensive search functions and free access, should be established (See: <https://fcs-free.org/>).

In contrast to FBS, the preferred chemically-defined media are cell type specific. For several cell types, chemically-defined media have now been developed. To quickly identify whether and/or which medium is available for a specific cell type, the FCS-free database ([fcs-free.org](https://fcs-free.org/)) was established. This database provides an overview of commercially available serum-free media for cell and tissue culture, as well as medium formulations for specific cell types obtained from scientific literature. Furthermore, the website serves as a platform to exchange information on the quality and applicability of each product. Since it is not only a database for, but also by scientist, information on missing media is appreciated. The database is offered by the 3Rs-Centre Utrecht Life Sciences in collaboration with Animal Free Research UK.

It should be realised that not only FBS contributes to possible animal harm and irreproducible results from *in vitro* methods. Several other animal-derived products are used in cell and tissue

culture. Therefore, good *in vitro* methods should address many issues to deliver reproducible and reliable results<sup>4,5</sup>.

**Literature:**

- <sup>1</sup> J. van der Valk, 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. <https://doi.org/10.1016/j.tiv.2003.08.009>
- <sup>2</sup> J. van der Valk, et al. (2010) Optimization of chemically defined cell culture media – Replacing foetal bovine serum in mammalian in vitro methods. Toxicology in vitro 24, 1053-1063. <https://doi.org/10.1016/j.tiv.2010.03.016>
- <sup>3</sup> J. van der Valk, et al. (2018) Fetal Bovine Serum (FBS): Past – Present – Future. Report of the 3rd Workshop on Fetal Bovine Serum, Serum Alternatives and Serum-free Culture Media. ALTEX Vol 35 No 1, 99-118. <https://doi.org/10.14573/altex.1705101>
- <sup>4</sup> OECD (2018) Guidance Document on Good In vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, No. 286, OECD Publishing, Paris. <https://doi.org/10.1787/9789264304796-en>
- <sup>5</sup> T. Hartung et al. (2019) Toward Good In Vitro Reporting Standards. ALTEX Vol 36 No 1, 3-17. <https://doi.org/10.14573/altex.1812191>

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