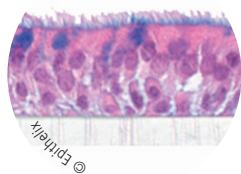


ALTERNATIVES *to the* USE of FOETAL BOVINE SERUM *in* CELL CULTURE APPLICATIONS

Each year, 600,000 litres of foetal bovine serum (FBS) are collected from up to 1.8 million foetal calves worldwide for laboratory use.¹ However, the use of FBS poses significant scientific, legal, and animal-welfare challenges.



Foetal Bovine Serum and Its Uses

FBS is used as a supplement for cell culture media. It provides an undefined mixture of macromolecules that maintain cell viability and facilitate cell metabolism, growth, proliferation, and spreading in culture.^{2,3,4} It is collected when a pregnant cow is slaughtered – a large-gauge needle is used to draw blood from the beating heart of the foetus.

Problems With the Use of FBS

Scientific Concerns

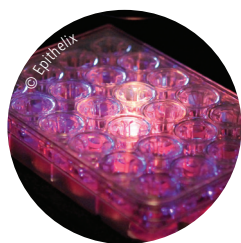
- Batch-to-batch variability: An estimated 1,800 proteins and more than 4,000 metabolites are present,⁵ which can lead to reproducibility issues.⁶
- Contamination, e.g. of biologics with animal proteins, pathogens, and exogenous agents such as endotoxins, mycoplasma, and viruses^{7,8,9}

Animal-Welfare and Legal Concerns

Unborn calves, who may be in the final trimester of development, are not anaesthetised at the time of blood collection and may experience severe pain.^{10,11,12,13} A European Food Safety Authority Scientific Opinion suggests that the collection of foetal blood in this way is not permitted in the EU under Regulation (EC) No 1099/2009 and that authorisation of such a procedure is legally questionable according to Directive 2010/63/EU.^{14,15}

Alternatives to the Use of FBS

Serum-free medium or human platelet lysates can replace FBS in cell culture medium. For optimal definition, the goal should be to use chemically defined serum-free medium and to avoid the use of any animal-derived supplement. Recombinant proteins have the benefit of being analogous to proteins from specific species.¹⁶



Implementing alternatives to FBS

Before implementing alternatives to FBS, several factors must be considered, depending on whether research is conducted for regulatory or non-regulatory purposes.

Regulatory Testing

- The EURL ECVAM Scientific Advisory Committee advocates the use of non-animal supplements for *in vitro* studies.¹⁷
- Serum-free alternatives should be incorporated into new test methods early in the development process. In fact, several OECD test guidelines (TGs) use cells maintained in serum-free media (e.g. TGs 431, 439, 442D, and 492).^{18,19,20,21}
- Where TGs specify cell culture medium components, testing with FBS-free media in parallel will allow assessment of the medium as a replacement for FBS and eventually lead to the revision of the TGs.

Non-Regulatory Testing

- For some cell types, it is possible to adapt the cells directly from growth in serum-containing to growth in serum-free medium while maintaining cellular function, and guidance is available on making this change.^{22,23}
- Optimal concentrations of recombinant proteins have been determined for many cell types.^{24,25,26,27}
- As part of an institutional animal care and use committee or through other review processes, universities should ensure that alternatives for each use of FBS have been considered.

For more information on FBS, please visit [PISCLtd.org.uk/fbs](https://piscltd.org.uk/fbs).

For information on FBS-free media and products, see the Fetal Calf Serum-Free Database at fcs-free.org.

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