uniform gravitational field. Thanks to the large arm separation in the experiments performed by Overstreet *et al.*, the gravitational field of the massive ring at the top of the atomic fountain influenced the upper arm much more than the lower one, thus producing a measurable proper-time difference between the two. In principle, this difference could also be obtained by comparing two clocks following the same trajectories as the interferometer arms, but the difference would be far too small to be resolvable. Notably, interferometry experiments with atoms acting as quantum clocks can be sensitive to the substantially larger time dilation due to Earth's gravitational field by initializing the clock once the arms are spatially separated (*10*).

Measuring the effect of gravitational time dilation on matter-wave interference is a major step in the emerging field of gravitational quantum mechanics. Furthermore, the impressive sensitivity achieved in these experiments could be exploited in future measurements of Newton's gravitational constant (*3*), gravimetry applications (*11*), and tests of the universality of free fall (*12*, *13*). Yet, important challenges remain because gravity gradients also lead to the dependence of the interference signal on the initial position and velocity of the atomic wave packets. This unwanted sensitivity to initial conditions is a major source of systematic uncertainties for precision measurements in nonuniform gravitational fields. Fortunately, a very effective technique to overcome these difficulties has recently been proposed (*14*) and is already playing a key role in high-precision tests of the universality of free fall (*13*). The prospects for improved measurements of Newton's gravitational constant based on atom interferometry are therefore very promising (*15*). ■


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CELL BIOLOGY

# Fetal bovine serum—a cell culture dilemma

## Ethical and possible reproducibility issues arise when using fetal bovine serum in cell culture media

By **Jan van der Valk**

Fetal bovine serum [FBS, also known as fetal calf serum (FCS)] is a popular supplement to the basal medium used in cell and tissue culture. FBS is sourced from unborn calves at the slaughterhouse, raising ethical concerns about animal welfare. Recently, two different laboratories performed in vitro experiments that applied an identical experimental procedure and used cells and FBS from the same suppliers (*1*). The results they obtained were very different. Further analyses revealed that one cause for the difference in cell response was the supplementation of the cell culture medium with FBS, which had originated from different batches. Given the ubiquitous use of cell culture throughout research, it is important to ensure reproducibility as well as ethical sourcing of research products, such as the development of synthetic media.

To maintain and proliferate cells and tissues outside the body, an optimal environment with growth factors and nutrients is required. This is often a liquid medium. Since the first in vitro cell culture experiments that maintained frog nerve fibers in frog lymph fluid (*2*), there has been a search for the optimum medium composition. Over the years, both animal-derived media and artificial media were developed. Examples of artificial media are the Eagles medium and its improvements modified Eagles medium (MEM) and Dulbecco's MEM (DMEM), as well as Ham's F10, F11, and F12. Not all cells flourish in these artificial media. In 1958, it was discovered that cells can be maintained in active growth for longer periods of time in media containing FBS (*3*). Because it was also reported that several different human biopsies and primary cell cultures could successfully be proliferated in such supplemented media, this led to the widespread use of FBS that continues today.

FBS is naturally optimized for prenatal development of unborn calves, and it contains a wide range of nutrients and growth and adhesion factors in excess and has a low antibody content. Combined with its relatively inexpensive availability, FBS is historically the first choice for supplementing almost all eukaryotic cell culture media. Most cell types appear to respond well to FBS with regard to proliferation and viability. Unfortunately, two important observations from the first report of its use in cell culture have been largely overlooked: FBS can contain "toxic factors" that affect the quality of experiments, and the serum obtained in different seasons showed "appreciable variations of performance" [(*3*), p. 946].

That different batches of FBS, which are produced in different regions and during different times of the year, have different effects on cells is not surprising, given that it is a biological product with a largely unknown composition. This variance may become apparent in cell culture through effects on cell morphology, growth rate, and viability, as well as by altering responses in experimental settings (*1*). To avoid intralaboratory disparities in cell performance when a new batch of serum must be obtained, laborious batch-testing to check in-house quality criteria with the available cell lines in the lab are required. Although this solves in-house inconsistency, it does not address interlaboratory consistency, because different laboratories will not have access to the same batch of FBS. Interlaboratory reproducibility becomes crucial when in vitro methods are used in applied research, such as preclinical studies with human cells, or for regulatory safety testing of pharmaceuticals and chemicals. Because of these interlaboratory reproducibility issues, the Organisation for Economic Co-operation and Development (OECD) began to discourage the use of FBS in 2017, especially for human health risk assessments of chemicals (*4*).

Since 1989, the bovine spongiform encephalitis (BSE) crisis fueled efforts to replace bovine-derived material, particularly in clinical or pharmaceutical products. Because of the potential contamination with nonhuman pathogens, the risk of eliciting an unwanted immune response, and issues with product reproducibility, the US Food and Drug Administration (FDA) (*5*) and the European Medicines Agency (EMA) (*6*) discourage the use of FBS in cell and tissue culture for human clinical application.

There is a need to replace FBS supple-


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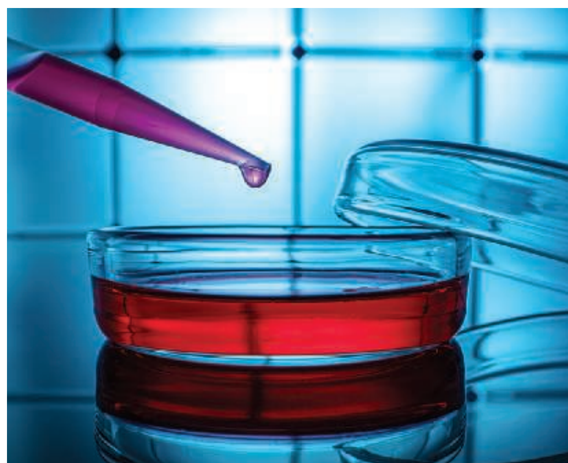
mentation of tissue culture media with media that is more physiologically relevant, is safe to use, and has consistent composition. Different options, such as human or plant extracts or fully chemically defined media, are now available. FBS-free media are preferred for experiments involving human tissues and cells because FBS may contain zoonotic pathogens and xenogeneic proteins and so may be particularly incompatible (*7*). Where in vitro methods are used for clinical applications, xeno-free media, such as human serum or human platelet lysates, have been introduced. However, these are also biological products, and so there are batch-to-batch differences (*8*). In areas where reproducibility and safety are especially important, there is increasing attention on developing and using chemically defined media. Unlike FBS, chemically defined media are generally cell-type specific. Consequently, a different medium is used, or developed when not yet available, for specific cell types.



Cell culture medium is often supplemented with fetal bovine serum (FBS), which may be a source of experimental irreproducibility.

Several existing defined media from commercial sources or described in the scientific literature for a variety of cell types can be identified in the FCS-free database (https://fcs-free.org). For example, for Chinese hamster ovary (CHO) cells, which are often used in the production of recombinant therapeutic products, the development of suitable FBS-free media is extensively described (*9*). At present, 22 different FBS-free media for CHO cells are identified. At least 19 FBS-free media are available for human MRC-5 and primate Vero cells, which are widely used for vaccine production. Mesenchymal cells are isolated from human adipose cells for use in human regenerative therapies, and many FBS-free media have been developed for these purposes.

Synthetic media from commercial sources may have some drawbacks. Primarily, it is often unclear, for commercial and proprietary reasons, what the medium formulation is. It is therefore impossible to identify the relative amounts of specific components and thus their potential effects on cells and the substances to be tested. Furthermore, the composition may be changed, and commercial media are also expensive. In the ideal situation, the composition of the growth medium is published. This might give other researchers the opportunity to adapt media specifically for their cells. Although adaptations of existing FBS-free media for a particular cell type might cause interlaboratory irreproducibility, publication of the full formulations, and comparing these, should allow the cause of any differences to be identified. This is impossible when using FBS and very difficult when using commercial medium. It is hoped that at some point, agreement is reached on the best medium for a particular cell type used for a specific purpose.

FBS-free media are not available for all cell types, so these have to be developed, or, ideally, a universal medium will be developed. The recommended starting point is a rich medium composed of equal volumes of DMEM and Ham's nutrient mixture F12, supplemented with recombinant insulin and transferrin and the mineral selenium (ITS). Other components, such as growth factors, hormones, and proteins, could further improve the medium (*10*). Furthermore, careful adaptation of the cells to the new medium (*11*), and the choice of substrate serving as the extracellular matrix, have been shown to be important to maintain or improve the phenotype of cells in an FBS-free environment (*12*). The cell type–specificity of chemically defined media poses a challenge for multi–organ-on-a-chip researchers when using different cell types within one system. Combining one chemically defined medium with a specific substrate for each cell type allowed a functional human multiorgan in vitro system, which could be maintained for 28 days (*13*).

There is not yet a universal chemically defined cell culture medium. The diversity of existing chemically defined media for some cell types and the work involved in developing those for other cell types are obstacles to replacing routine use of FBS. When new in vitro research models are developed without FBS, and agreements are reached between users about the best chemically-defined medium for a particular cell type and purpose, this will facilitate the acceptance and implementation of FBS-free cell culture.

In what medium cells and tissues express a phenotype that is physiologically relevant and appropriate for a particular study and how new media should be evaluated are questions that remain. Often, results obtained from cells grown in FBS-containing media are used as a reference for comparison. However, these results might not always be relevant and could lead to misinterpretation (*14*, *15*). For human cell and tissue systems, comparison with results from cells cultured in human serum might be the solution, although, as with FBS, possible batch differences should be taken into account.

In vitro research is important to provide animal-free models that are physiologically relevant. Every potential cause for irreproducibility should be identified and a solution found. The use of FBS in cell culture medium is one of those causes, and the development of FBS-free media is crucial to ensure consistent, reproducible, and translatable results and safe products. ■

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# Fetal bovine serum—a cell culture dilemma

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