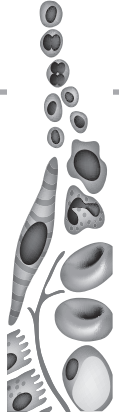

Peak serum: implications of serum supply for cell therapy manufacturing

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KEYWORDS: biomanufacturing ■ bioprocessing ■ cell therapy ■ commercialization ■ fetal bovine serum ■ manufacturing ■ process optimization ■ serum

The cell therapy industry (CTI) is emerging as a distinct and competitive component of global healthcare, creating value for investors and providing life-changing therapies to patients [1,2]. Industry growth has necessitated an increased focus on large-scale manufacturing strategies to meet future demands [3,4]. One major challenge is the limited availability of some crucial raw materials used in cell therapy manufacturing – including bovine serum. Without a sustainable supply or viable alternatives to these components, the commercial-scale production of cell therapies will be impossible, halting the momentum of the industry. We propose that solutions to these challenges are achievable, and can be expedited by industry-wide collaboration.

Bovine serum is currently used in the majority of cell therapy manufacturing processes. Current stocks and production rates of serum suitable for GMP manufacture may only be sufficient to support the production of one blockbuster cell therapy. Limitations in the availability of bovine serum thus act as a major cost driver and significant barrier to the commercial success of the industry as a whole. Thus, without an increase in serum production, or at least a significant increase in the development and implementation of serum-free production strategies, the growth and sustainability of the CTI will be severely constrained.

Uses of serum

Serum has been widely used in cell culture for over a century [5]. Whilst its beneficial impact on cell culturing is universally agreed, its precise mechanisms of action are still not totally known. It serves various functions necessary for effective cell culture, including the provision of important growth and attachment factors, protease inhibitors and protection against shear stress in agitated culture [6]. Without the addition of serum, proteolytic activity within cell culture would ensue, resulting in reduced cell growth and proliferation [7].

There are several potential sources of serum, each with different compositions and characteristics. The most commonly used is fetal bovine serum (FBS) due to its strong growth-promoting capacity and relatively low immunoglobulin levels. However, as multiple calf fetuses are required to make a single liter of FBS, it is also the most expensive type of serum. Cheaper alternatives include new-born, calf, adult, donor calf or donor adult cattle serum [8]. It is also possible to use serum sourced from other animals, including horses and sheep. In general, serum sourced from older animals contains more antibodies and has a greater protein and lipid content.

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The type of serum used can have huge implications in processing and cell characterization. Since 'the product is the process', changes in the type of serum can alter fundamental properties of a cell type, including proliferation kinetics and specific phenotypical characteristics such as cell potency and identity. Furthermore, within the same 'type' of serum there is significant variability from batch to batch, thus in order to ascertain which serum is most suited to a specific cell type it is necessary to test several batches, obtained from either the same or different suppliers. This testing is expensive and time consuming, and often a manufacturer will test up to ten different batches of sera, of which only one or two might successfully meet the

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required specifications. Furthermore, the specific cell characteristics that a serum is selected for (including cell phenotype) can be significantly altered when the chosen batch of serum runs out and a new batch has to be introduced.

> "*The main limitation to serum supply for biotech and the CTI is the huge number of cattle reared for other uses, such as beef and dairy.*"

One fundamental issue with replacing serum is the limited knowledge about what exactly makes it so effective at facilitating cell growth. This lack of understanding, as well as high cost and high associated risk of contamination, is a key driver of research into serum-free alternatives within the commercial cell culture community. These alternatives are mainly based on the addition of a cocktail of specific hormones and growth factors to a base solution in order to create a fully-defined media. A brief comparison of serum-dependent and serum-free media is provided in Table 1. Furthermore, in the case of pluripotent cells, the differentiation factors present in serum have often proved problematic even at the basic research stage, leading to the development of serum alternatives, for example KnockOut™ Serum Replacement (Life Technologies, Carlsbad, CA, USA), and specialized serum-free media such as TeSR™ (Stemcell Technologies, Vancouver, BC, Canada).

Current sources & availability of serum

Approximately 50% of the total global supply of FBS is currently provided by the USA, with a further 20% from Australia and New Zealand [8]. Of particular importance to biotech and the CTI is that these three countries provide more than 90% of all serum used in the commercial manufacture of therapeutics. Australia and New Zealand play a disproportionately large role due to their low reported rates of bovine spongiform encephalopathy (BSE) amongst cattle, therefore, products associated with these countries are perceived to pose lower BSE safety concerns.

In terms of GMP manufacture, there are only a limited number of suppliers that provide serum that has been obtained in suitable, International Standards Organization (ISO)-grade environments. Serum destined for the production of cell therapies has to undergo rigorous testing and be thoroughly documented in terms of place of origin, specific details of the collection facility, government-approved establishment number and information regarding the amount and date of collection.

Serum supply

The limited availability of essential raw materials is a common global problem. The peaking of the supply of important natural resources such as oil and water has been hotly debated over the past decade [9,10] and these concepts provide a framework on how to approach shortages in both renewable and nonrenewable natural resources. The supply of oil, a nonrenewable resource, is stock limited. Thus, peak oil is defined as the point in time when the maximum rate of global petroleum production is reached, after which the rate enters terminal decline. Conversely, water, a renewable natural resource, is flow-rate limited – that is to say it is the amount available per unit time [10]. Peak water is a similar concept describing the growing constraints on the

Table 1. A comparison of serum-dependent and serum-free culture systems.

Serum-dependent	Serum-free
Limited supply	Readily producible at scale using GMP manufacturing
Undefined, high batch-to-batch variability	Defined, reproducible material
Potential for pathogen transmission	Reduced potential for pathogen transmission
Animal welfare considerations	Animal-free
Current material of first choice for basic research due to knowledge base	Transition to serum-free may require resource-intensive optimization
Provides growth factors and hormones, lipids, trace elements and other nutrients	Requires the addition of growth factors, hormones, lipids etc
Provides attachment factors (fibronectin, laminin)	May require the addition of attachment factors for culturing on surfaces
Intrinsically contains differentiation factors	Easier control of differentiation by the addition (or not) of appropriate factors

availability, quality and deployment of freshwater resources. Serum, as a 'renewable commodity', lies somewhere between oil and water.

The main limitation to serum supply for biotech and the CTI is the huge number of cattle reared for other uses, such as beef and dairy. The global supply and cost of serum is, therefore, drastically affected by external events, such as BSE outbreaks or the price of cattle feed (grain). As grain prices increase, the cost of maintaining cattle becomes less financially viable and as a result herds are slaughtered, directly leading to a temporary spike in the availability of serum. Conversely, low grain prices promote the breeding of cattle and maintenance for beef and dairy farming, thus fewer sources available for FBS production results and serum availability decreases.

The recent decline in the vaccine industry has led to reduced global demand for serum, contributing to a decrease in serum production and, therefore, global supply (Figure 1). Aside to this, there has also been significant loss in the infrastructure required to collect and process serum – most notably the specialized, validated abattoirs. Given the decline in both serum processing and its underpinning infrastructure, its role as a key manufacturing component for the future CTI is not tenable. At the very least, there will undoubtedly be a lag between the CTI's serum demand and the rate at which supply can be accelerated. Moreover, the CTI's serum demand is likely to exceed the maximum achievable production rate of serum: 'peak serum' (Table 2). The decreasing availability and thus increasing cost of serum for manufacture will unquestionably result in expensive alternatives, such as serum-free strategies, needing to become economically competitive. This will be through a combination of innovation and increasing economies of scale to meet the rising demand (Figure 2).

> *"Given the decline in both serum processing and its underpinning infrastructure, its role as a key manufacturing component for the future CTI is not tenable."*

Annual serum availability has hovered around 600,000 l per year [8], of which the authors estimate that approximately 200,000 l is produced at a quality suitable for GMP manufacture. However, in recent years, vaccine manufacturers have moved towards methodologies involving

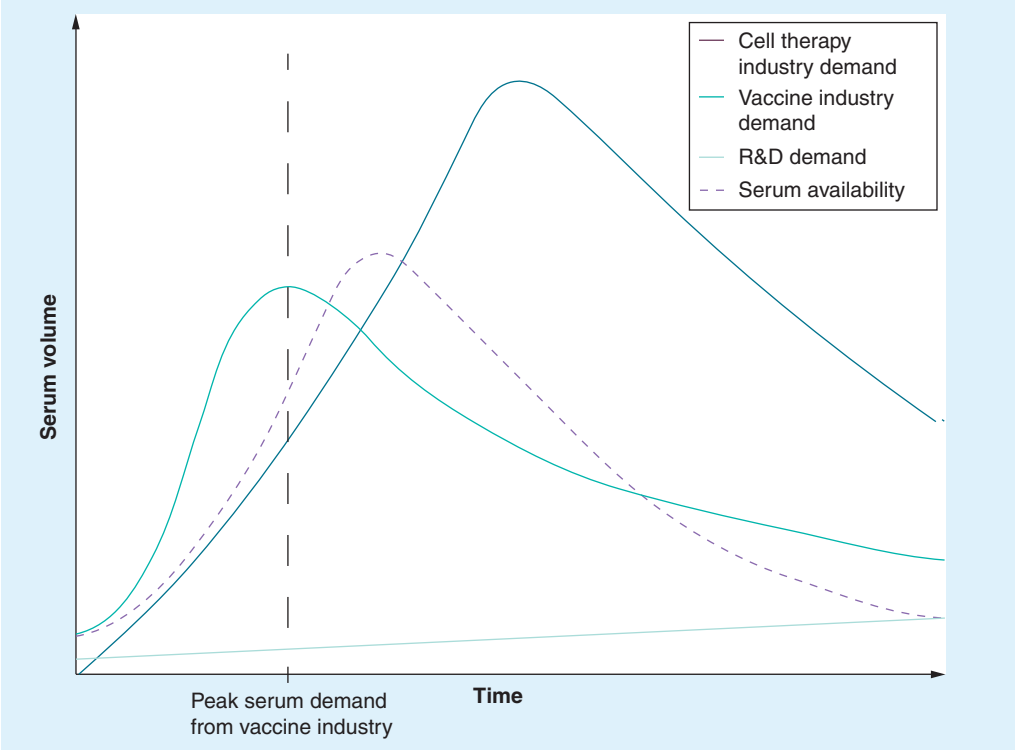

Figure 1. Global serum supply and demand. The peak serum supply driven by demand from the vaccine industry has passed and much of the infrastructure required to sustain this supply has been permanently scaled-back (or lost) to match the reduced demand. Since cell therapy manufacturing intrinsically requires significantly higher volumes of serum, the future cell therapy industry serum demands are likely to significantly exceed peak serum. The lack of essential infrastructure will result in an irreversible mismatch between supply and demand.

Table 2. Predicted cell therapy growth, serum demand and serum availability based on current manufacturing requirements.

Therapeutic doses per year	Serum demand (l)	Serum availability (l)
7000	3500	3500
30,000	15,000	15,000
300,000	150,000	150,000
800,000	400,000	**200,000†**
1,200,000	600,000	**200,000†**
5,000,000	2,500,000	**200,000†**
8,000,000	4,000,000	**200,000†**

†Maximum serum availability at 200,000 l per annum.
Based on the authors' estimates, the availability of serum suitable for cell therapy manufacture peaks at approximately 200,000 l per annum, this will be sufficient to support the production of approximately 400,000 therapeutic doses per year. However, thereafter serum-free manufacturing strategies must be employed in order to sustain industry growth.

the expression of recombinant proteins produced by serum-free microbial or mammalian cell cultures. This decreasing demand from the vaccine sector is one of the key factors resulting in current global serum production and reserves nearing an all-time low [Harshman P, Pers. Comm.].

During the early years of the 21st century, despite decreased demand from the vaccine industry, serum production remained constant; therefore, a global surplus accumulated and was used to produce frozen stockpiles of serum that, because of the CTI's activities, are now being depleted at a higher rate than they can be replenished. This shrinkage in stock-piles has led to a threefold increase in the cost of GMP-grade serum over the last 2–3 years.

> *"At current elevated prices of approximately US$250 per liter ... where 1–3 fetuses are required to produce each liter of serum, it is not cost effective to breed cattle purely for serum production."*

Even if hypothetically the infrastructure were adequate, it is difficult to envisage a sudden and substantial increase in serum availability solely in response to predicted GMP demands, as serum is purely a by-product of abattoirs. At current elevated prices of approximately US$250 per liter [Harshman P, Pers. Comm.], where 1–3 fetuses are required to produce each liter of serum, it is not cost effective to breed cattle purely for serum production. Individual animals are worth thousands of dollars when bred for meat or milk. Furthermore, farmers do not receive any compensation if their slaughtered cattle are harvested for serum production – this margin is retained by the abattoir. Therefore, there is little or no incentive for farmers to assist in increasing worldwide serum production.

For the abattoirs, while serum production and the sale of offal are useful sources of revenue, such byproducts only capture limited value. First, the necessary infrastructure, including GMP processing suites for serum, is expensive to install and maintain. Second, halting the 'production line' to remove carcasses for their serum decreases the throughput of the facility and thus profitability. Subsequently, as smaller local abattoirs have consolidated into larger regional facilities, serum processing facilities have either not been transferred or existing facilities have not been maintained, in order to maximize the throughput of cattle.

A further compounding factor is that not only is the number of batches of serum produced per year falling but also the average batch size is declining. Thus, a greater proportion of each serum lot is used in quality assurance testing, further reducing the availability of GMP serum for manufacturing.

The point of maximum worldwide availability of serum, peak serum, has passed. Indeed, as supply becomes more constrained and the cost increases, the replacement of serum with alternatives in cell therapy biomanufacturing processes may be driven primarily by availability and secondarily by cost. This is contrary to the common thinking that the transition to serum-free manufacturing in the CTI will be primarily driven by regulatory pressures. However, it is our opinion that unless there is a major adverse event associated with serum-based bioprocessing in the next 5–10 years, peak serum will have a hugely significant impact on the commercialization of cell therapy products.

We propose that the global serum supply has reached somewhere near its peak, and this is occurring at the same time as the CTI enters its first sustainable period of growth. There is certainly a pressure across the entire industry to reduce or remove serum from bioprocessing, driven by the perceived US FDA aversion to the presence of animal-derived components in cell culture. However, here we argue that the use of serum in commercial-scale cell therapy manufacturing is less of a regulatory issue and more of a major supply issue.

Predicted demand

There are now a small but growing number of FDA/EMA-approved cell-based products available on the market. Research into the development of serum-free protocols for cell therapy

manufacturing has grown significantly; however, the majority of cell therapy manufacturing processes for products in or nearing the clinic continue to use bovine serum.

For emerging companies, time saved by not switching to serum-free, and the regulatory and technical risk of serum inclusion currently outweighs the short-term economic risk [11]. This conservatism is predicated by the absolute requirement to secure the necessary rounds of funding from angel and venture investors – speed to exit for such investors is critical in their investment making strategies.

Crucially, as we near global peak serum output, reverting from a serum-dependent process to a serum-free equivalent from Phase III clinical trials onwards would represent a 'major process change.' This would, at best, need comparability studies and, at worst, require the repetition of some clinical studies [HURSH D, PERS. COMM.].

Peak serum supply may well have passed, with peak serum demand, driven by CTI growth, still to come. Serum availability is a growing challenge that the CTI cannot overlook and to defend against this becoming a significant supply threat, CTI companies must begin to engineer serum out of their processes at the earliest possible opportunity.

The technical challenge & a potential solution

Today there are several published manuscripts and patents [12,101], as well as commercial media related to serum-free conditions for cell therapy culture. However, establishing completely serum-free processes, from cell isolation through to final product, remains challenging. But how necessary is it to completely eliminate serum?

> "*Peak serum supply may well have passed, with peak serum demand, driven by CTI growth, still to come.*"

We believe that the concern over the total avoidance of serum primarily from a regulatory perspective is potentially questionable. By directing development programs towards processes that are completely serum-free, companies are accepting unnecessarily high technical and comparability risks, for relatively moderate reduction in overall regulatory risk. A more pragmatic approach (in the short-term) would be to adopt a mixture of serum-containing and serum-free steps in the whole bioprocessing of cell therapies. For example, the serum-free expansion of adult cells from cell banks that were isolated deploying serum-containing media has been extensively documented, and is considered to have low technical risks [13,101]. Thus, by moving to serum-free expansion after master cell bank production, the technical risks are significantly reduced. In conventional, allogeneic cell banks that generate 100–300 master cell bank vials, less than 1% of all media (and thus serum) is used to generate the cell bank, compared with the total media required to produce all final products manufactured from that cell bank. Thus, 99% of all serum could be removed from the overall manufacture of a specific cell therapy by initially using serum in the production of master cell banks, but subsequently reverting to serum-free protocols for all post-cell bank expansion and processing. In the

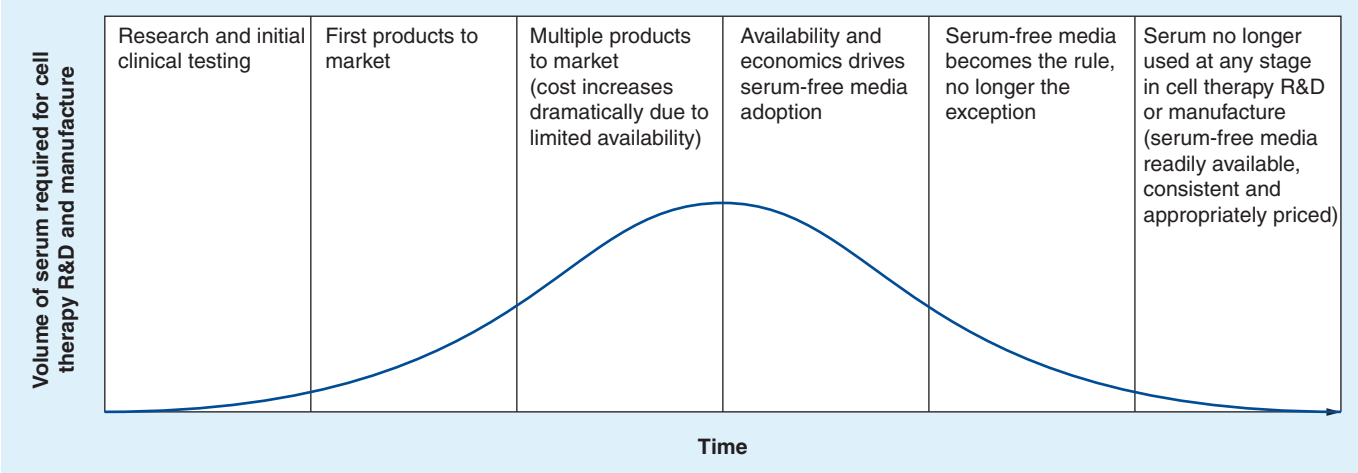

Figure 2. Projected serum life cycle for cell therapy R&D and manufacture. Serum demands exclusively for the cell therapy industry are likely to follow the classical life cycle trend, starting from a basal supply level driven by research applications, peaking as the number of products on the market and thus manufacturing demands increase, and ultimately declining as supply limitations force second-generation products to utilize serum-free alternatives.

short term, this progressive approach would both mitigate the supply risk driven by peak serum, while minimizing the short-term technical and development risks of 100% serum-free processing from cell isolation.

> "*The serum availability bottleneck is not theoretical but already starting to impact the sector, as can be witnessed by the significant rises in the price of GMP-grade serum in recent years.*"

This progressive approach to serum removal in cell therapy manufacturing will help to reduce impact of serum supply and costs on the CTI. Furthermore, by managing these technical and financial risks, the industry can focus more clearly on the other regulatory, safety and ethical issues relating to the use of serum in bioprocessing.

Conclusion

The concepts of peak oil and peak water have spurred innovation, not disaster. We predict the same will be true for peak serum. Whilst existing serum stocks and production rates might be sufficient to support the commercialization of a few first-generation cell therapies, they will grow increasingly inadequate and unable to support future industry demands. The serum availability bottleneck is not theoretical but already starting to impact the sector, as can be witnessed by the significant rises in the price of GMP-grade serum in recent years.

For CTI companies, most of whom are resource constrained, addressing the serum-free challenge individually will be a significant drain on both time and resources. Therefore, working with other members of the CTI and key stakeholders via the main networking organizations such as the International Society for Stem Cell Research (ISSCR), the International Society for Cellular Therapy (ISCT), the BioIndustry Association (BIA) and Alliance for Regenerative Medicine (ARM) will be vital. Research into the production of novel serum substitutes is rapidly growing [14]. One significant step forward would be to educate basic research scientists as to the challenges that industry faces with respect to the use of serum-based processes. A single change at the beginning of the discovery chain might avoid a life-changing therapy being significantly delayed in development, too expensive to manufacture or even lost in translation.

The progressive reduction in serum utilization is highly desirable from both clinical and manufacturing perspectives. The serum-related issues; safety, efficacy, scalability and cost of goods are factors critical to the future success of cell-based therapies. The use of serum and its pragmatic reduction and eventual elimination are essential and achievable steps in the development of a sustainable and competitive CTI.

Acknowledgements

The authors would like to extend their thanks to P Harshman and K Warren (Lonza Walkersville, Inc.), B Reeve (Harvard Stem Cell Institute), W Sahlman (Harvard Business School), G García-Cardeña (Center for Excellence in Vascular Biology, Harvard Medical School), J Ritz (Dana-Faber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School), D Hursh (CBER, US FDA) and J van der Valk (The Netherlands Centre on Alternatives to Animal Use).

Financial & competing interests disclosure

C Mason is a Principal Investigator and DA Brindley, EJ Culme-Seymour and NL Davie are Investigators on the British Regen Industry Tool Set (BRITS) project funded by the Technology Strategy Board under their Regenerative Medicine Program: Value Systems and Business Modeling. JA Rowley and DW Smith are employed by Lonza Walkersville, Inc., a company that performs cell therapy contract manufacturing and also offers serum-free media products. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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