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Science Writing

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FIBRINOGEN IN CLINICAL TRAUMA

7/20

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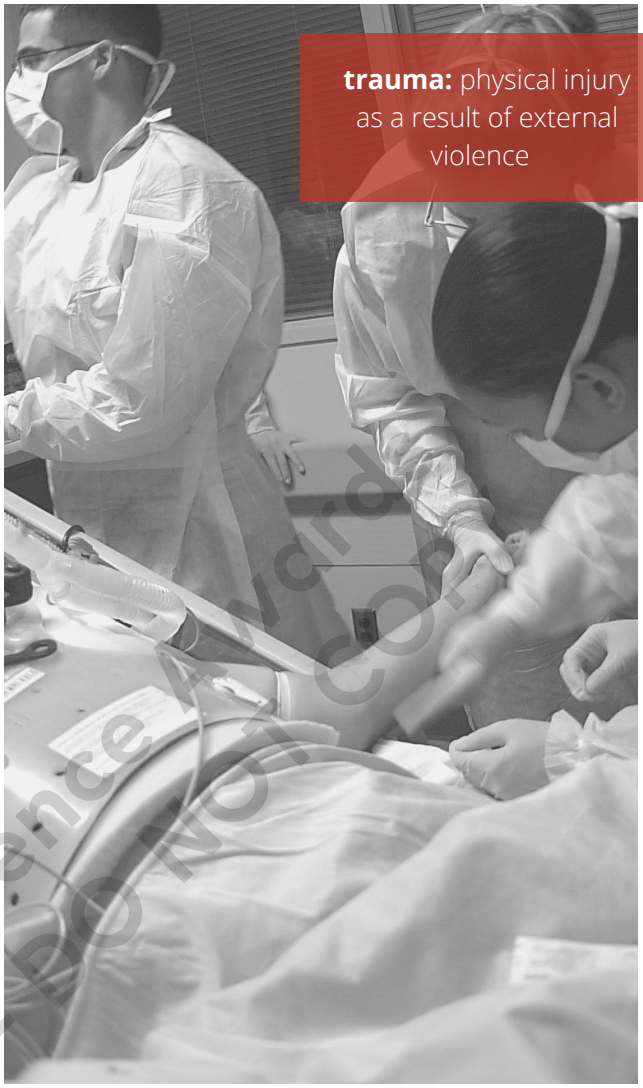
TRAUMA IN MEDICINE



trauma: physical injury as a result of external violence

Medicine is perhaps one of the most dynamic fields of science, which rapidly evolves as new discoveries are continually made. Within this field, traumatic injury is one of the leading causes of death within Australia, with data from the World Health Organisation suggesting that 5.8 million global trauma deaths occur annually. Unlike other symptomatic medical conditions such as cancer and genetically inherited disease, trauma can occur spontaneously to any individual, which makes it unique in its various forms of treatment. Within this article, the *development* of coagulation treatments for trauma patients will be explored. The current transitory progress from cryoprecipitate to fibrinogen concentrate is aided by the development of analytical technologies, which can *influence* a wide population demographic to improve clinical outcomes. Utilising this scientific knowledge, the recent emergence of recombinant fibrinogen products may be *applied* as a form of treatment to reduce ethical and social ramifications - however, this poses many *limitations*, which ultimately, provide leeway for further innovation.

*"**Injuries** affect all age groups but have a particular impact on **young people**. For people between the ages of 5 and 44 years, injuries are one of the **top three causes of death**."*

- WHO, 2004

	5-14	15-29	30-44
1	Lower respiratory infections 224 308	Road traffic injuries 335 805	HIV/AIDS 958 851
2	Road traffic injuries 109 905	HIV/AIDS 333 953	Tuberculosis 367 837
3	Malaria 103 738	Tuberculosis 249 023	Road traffic injuries 329 142

Figure 1 - Top 3 causes of death for individuals ages 5 to 44 years (WHO, 2004)

FIBRINOGEN - A JOURNEY

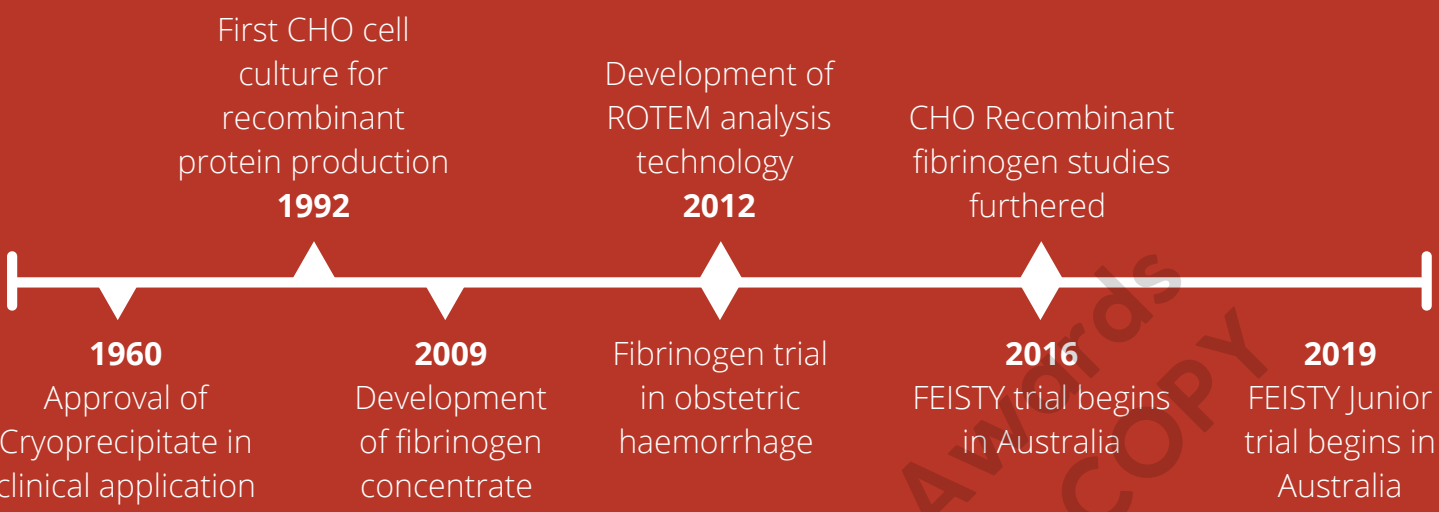


Figure 2 - A timeline of fibrinogen development from the 1960s to present day

BIOLO-GRAPHY

Cryoprecipitate and **Fibrinogen Concentrate** are powerful blood clotting factors, which are both concentrated plasma derivatives that aid in the formation of blood clots (Flores, 2010). Within these derivatives, the glycoprotein fibrinogen is the most fundamental product in promoting blood coagulation, due to its unique hemostatic properties.

So, how does fibrinogen work?

Plasma fibrinogen is primarily synthesised in hepatocyte liver cells, where three specific genes (A alpha, B Beta and gamma, respectively) produce individual protein chains. The interaction and assembly of these protein chains occur in the cell's endoplasmic reticulum, which produces fibrinogen's final six-chain complex (Redman, 2001)

In conjunction with initial platelet aggregation (primary hemostasis), fibrinogen is secreted into the bloodstream towards the site of injury upon appropriate chemical signals. This secondary clotting process is initiated by an enzyme called thrombin, which catalyses the breakage of peptide bonds within the protein structure of fibrinogen, forming two monomers (fibrinopeptides). In optimal pH conditions, these monomers are synthesised into insoluble fibrin. The accumulation of fibrin forms intermolecular cross-links around the platelet plug, which creates a fibrous, mesh sealant. (Haurowitz, 2020). In the event of trauma, fibrinogen is the first blood factor to reach critically low levels, which is why it must be administered in high concentrations to restore haemostasis (Shuttleworth, 2017).

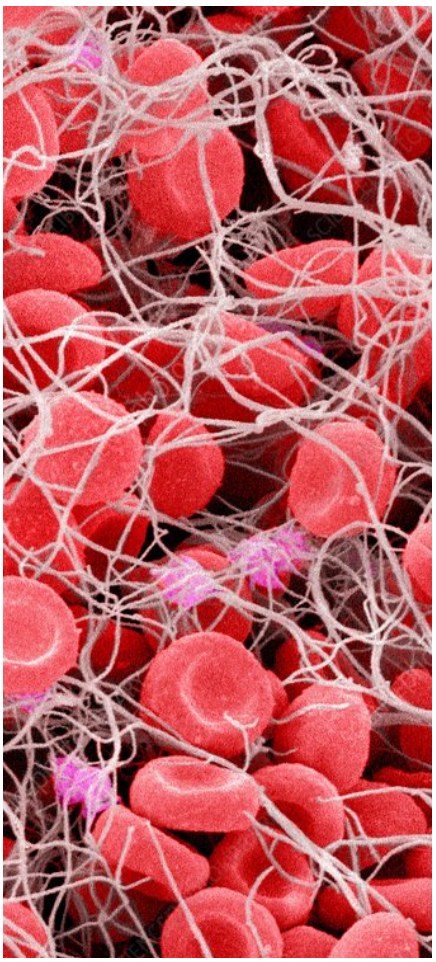


Figure 3 - A fibrous mesh sealant, forming a 'plug' around red blood cells

FIBRINOGEN THROUGH THE AGES

Cryoprecipitate has been accepted as the standard fibrinogen supplementation since the late 1960s (Nascimento, 2014). In order to prepare a solution for administration, fresh frozen plasma from a compatible blood donor must be fully thawed before patient administration. However, such methods are operationally complex and time consuming, which can pose severe risks for haemorrhaging patients in critical need of fibrinogen supplementation (Sachais, 2015). The knowledge of this flawed methodology led to the **development** and use of human fibrinogen concentrate in 2009, which is equally as potent as cryoprecipitate in restoring haemostasis (Schmidt, 2017).

Fibrinogen concentrate is derived from pool plasma and lyophilized into a powdered substance, which unlike aqueous cryoprecipitate, does not require restrictive storage conditions (Silvergleid, 2019). This **development** originated from a shift in plasma product production during 1985, which originally used pasteurisation as a virus inactivation step. However, the resulting product (fibrinogen concentrate) was found to be safe and well tolerated in early clinical studies, which was eventually marketed with the modern brand name RiaSTAP in 2010 (Costa-Filho, 2016). The most significant advancement of fibrinogen concentrate is the improved efficacy of treatment in a clinical environment. In its powdered form, fibrinogen concentrate is simply combined with sterile water and administered as an intravenous fluid (Ryan, 2017). Such theoretical advantages remain in the process of justification, given the **development** of such a complex scientific theory would require a range of comprehensive, clinical evidence before accurate claims can be made. However, this evidence is in the process of collection, with the most prominent and recent investigation being the **Fibrinogen Early In Severe Trauma studY (FEISTY).**

Figure 4 - Unthawed Cryoprecipitate pouches (left) in comparison to prepared fibrinogen concentrate solution (RiaSTAP) (right)

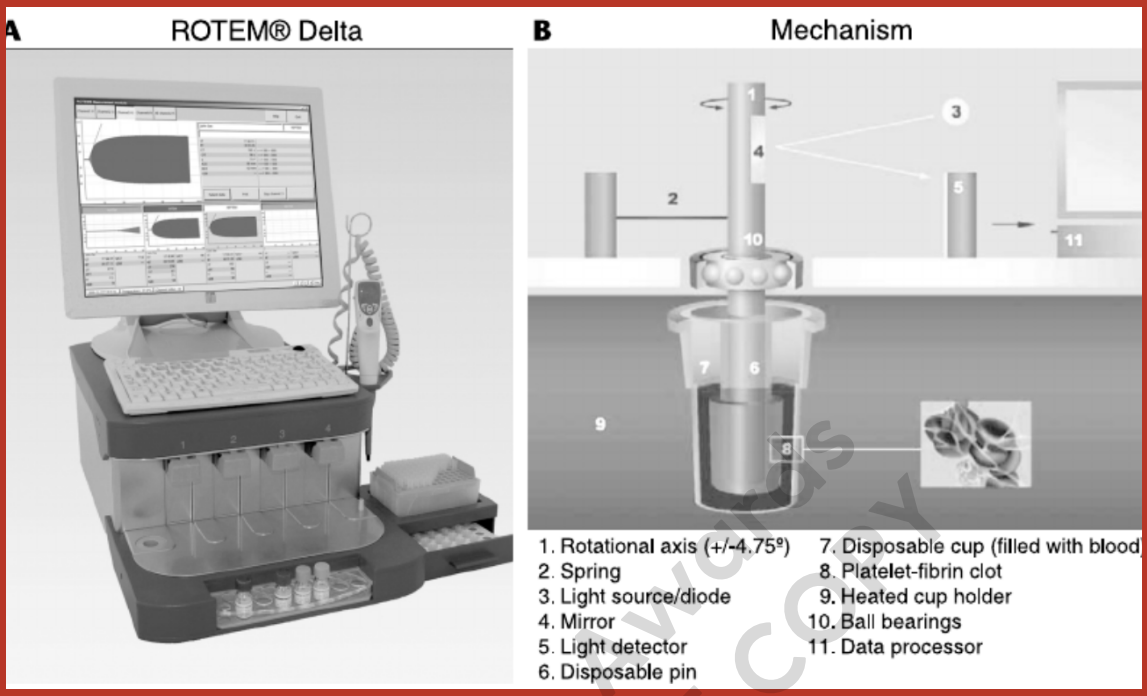


Figure 5 - A Food and Drug Administration approved ROTEM analysis device, measuring the torsion strength of fibrin strands which is indicative of the blood sample's fibrinogen levels (Tanaka, 2012)

TRAUMA GETS FEISTY

The FEISTY study (2016) is a randomised controlled trial in Australia, which has the potential to **influence** conventional cryoprecipitate usage for good. The trial compares the efficacy of Fibrinogen concentrate against Cryoprecipitate in restoring haemostasis to severe adult trauma patients, with a current sample size of 100 adult patients across four national major trauma centers (Winearls, 2017). Within the methodology of this protocol, the whole-blood thromboelastometry (ROTEM) is perhaps the most significant technological advancement. Approved in 2012, the ROTEM analysis vastly improves data collection efficiency, using viscoelastic technology to provide a comprehensive, real-time assessment of blood fibrinogen levels within minutes. (Jilma-Stohlawetz, 2017).

"It's a really important trial in the patient bleeding population... It's also really exciting we are collaborating with all four major hospitals in Queensland"

- Dr James Winearls, 2017

The universal acceptance of this technology is **influenced** by the inefficiency of conventional laboratory coagulation tests, with a turnaround time of 1-2 hours to provide an identical analysis. Evidently, this inefficiency was a significant flaw when confronted with critically injured patients, some of whom may die within a mere hour of hospital arrival (Sobrino, 2013).Holistically, the primary outcome of this 'pilot' FEISTY trial is to compare time efficiency between the two treatments, which will benefit the working age population in society. (Wullschleger, 2017). In terms of economic **influence**, the working age population (ages 18-39) is heavily affected by incidents of trauma, being the leading cause of death for this age group worldwide (WHO, 2020). The treatment of these patients - who are the driving force behind economic growth - will not only decrease their mortality rate, but enable them to return to work, boosting future economic outcomes.

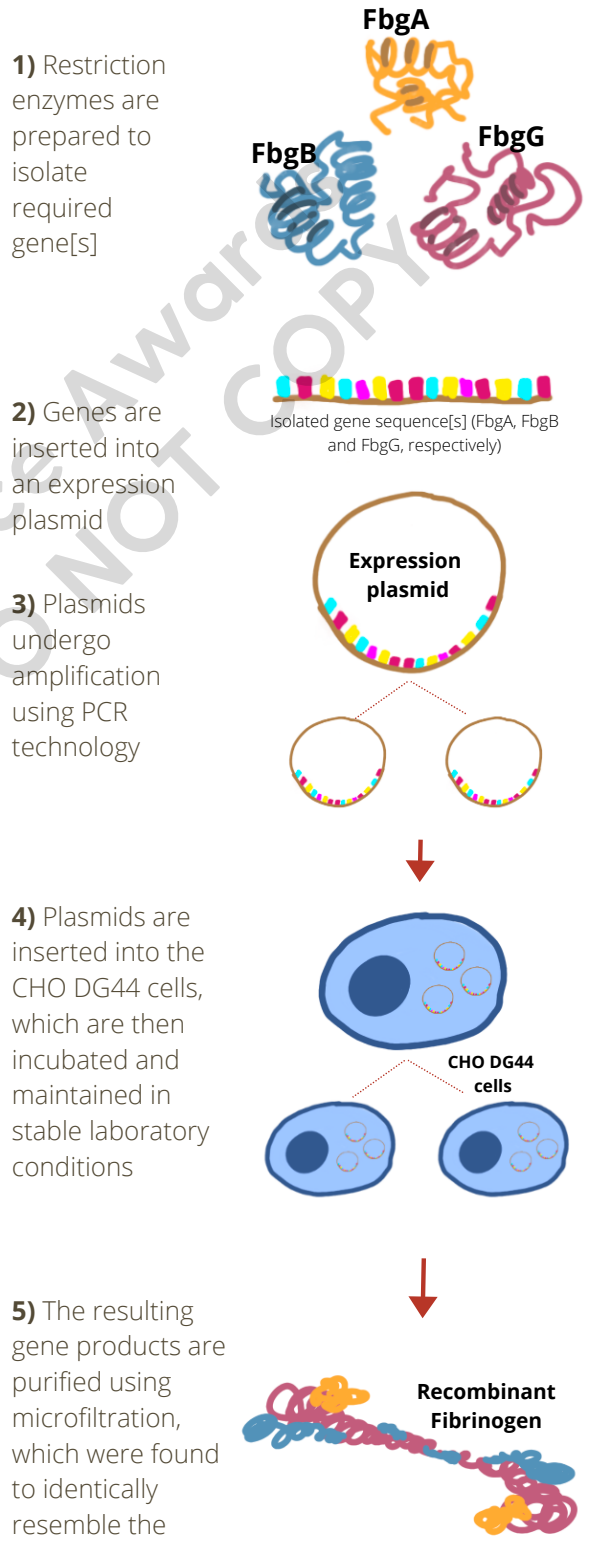
WHO ELSE IS INVOLVED?

Following the randomised trial in adult trauma patients, a smaller, FEISTY Junior trial was initiated in 2019 involving paediatric patients from 3 months to 18 years of age. This investigation is still in progress, involving the same outcome measures as its adult counterpart. Currently, there are yet to be enrolled trauma cases under this trial due to it's recency, in which the data collected will create a holistic evidence base in paediatrics to further **develop** FEISTY's hypothesis. (Wake, 2019). A similar study involving major obstetric haemorrhage was conducted in Ireland, comparing the efficacy of fibrinogen concentrate and cryoprecipitate in treating mothers experiencing uterine atony whilst in labour. Much like the FEISTY study conducted in Australia, the results of this trial contributed to the evidence that fibrinogen concentrate is a valuable alternative to cryo in the treatment of haemorrhaging patients (Ahmed, 2012). Ultimately, this reveals how global, widespread evidence aids in the **development** of complex scientific hypotheses.

RECOMBINANT FIBRINOGEN IN CHINESE HAMSTER OVARY (CHO) CELLS

Human-derived plasma products require blood donors, followed by extensive processing and purification to produce a suitable concentrate. However, the recent approval of a recombinant fibrinogen manufacturing platform may potentially shift the use of plasma products to a scalable method of production (Fibriant, 2019). This scientific model was **applied** through a CHO DG44 cell line, which allows the synthesis of biologically active fibrinogen. However, the application of such complex methodology to achieve large-scale, commercial expression would be inherently challenging - obtaining the highest quality concentrate using limited processes would pose many **logistical challenges** (Imamura, 2016). Additionally, such technology may not foster public trust due to a lack of clinical trialling to validate its effectiveness.

Figure 6 - A Model of the Recombinant Fibrinogen Process



WHAT'S NEXT?

Holistically, the use of both cryoprecipitate and fibrinogen represent positive clinical outcomes. The treatments not only improve the quality of life for trauma patients, but also individuals experiencing brain, gastrointestinal and ulcerated haemorrhaging. Nonetheless, nearly all members of society will be able to receive this treatment, as trauma may occur to any individual regardless of age, gender, race or medical history. Furthermore, Australia's healthcare system allows any trauma patient to receive free treatment, making the treatment inexclusive of social status and eliminating the ethical issue of fair access.However, the applicability of this treatment is not inclusive of all population demographics. Minor religious groups such as Jehovah witnesses decline a majority of blood-derived treatment forms, as ingesting such products contradicts biblical readings.

Such beliefs must be considered in a clinical environment by obtaining informed consent before any form of medical intervention, in order to respect patient autonomy and human rights (Chand, 2014). Despite the administration of human-derived products, some Jehovah witnesses have been recorded to accept fibrinogen concentrate; the product itself contains only a specific clotting factor, as opposed to a combination of blood products in a typical transfusion (Subramanya, 2014). However, this dilemma may be resolved entirely if recombinant fibrinogen were to become commercialised and applied in clinical environments - unlike human-derived concentrate, the product itself is derived from non-animal organisms, which would enable these patients to accept haemostatic treatment. Moving forward, this commercialisation will be considered for future application to mitigate the pre-existing problems with human derived blood products. Such methods of cell culture expression systems pose many theoretical benefits, such as quality control, reduced toxicity risk, and reduced blood donor sample size.

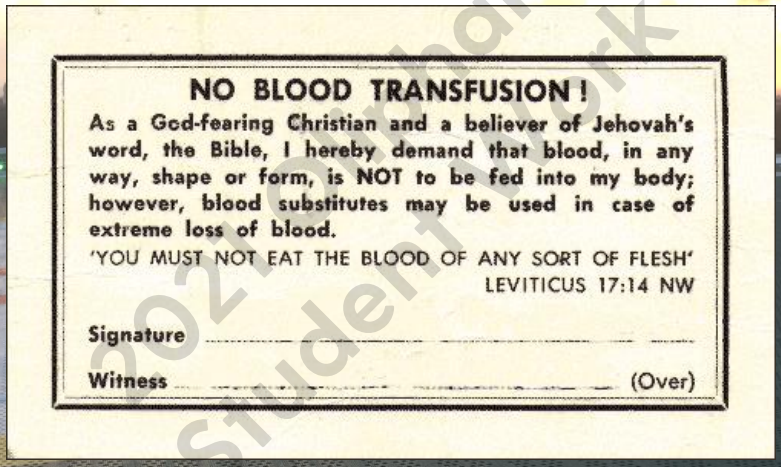


Figure 7 - A Jehovah's witness blood card. These cards are generally carried with personal belongings, which is a common practice for adult witnesses (Migden, 1999)

Evidently, the development of coagulation treatments for trauma patients has made a profound impact on a broad population. Analytical technologies, genetic engineering and clinical trialling all work in conjunction to accelerate scientific progress, ensuring future trauma patients experience improved clinical outcomes. Besides trauma's inherent impact, it exists as a mere speck in the dynamic tapestry of medicine. Thus, all members of science regardless of field are challenged by the same, underlying question - **what may come next?**

REFERENCE LIST

1. Ahmed, S. et al. (2012) 'The efficacy of fibrinogen concentrate compared with cryoprecipitate in major obstetric haemorrhage - an observational study', Transfusion Medicine, 22(5), pp. 344–349. doi: 10.1111/j.1365-3148.2012.01178.x. - Comparison of FC and CRYO
2. Chand, N Kiran et al. "Management of patients who refuse blood transfusion." Indian journal of anaesthesia vol. 58,5 (2014): 658-64. doi:10.4103/0019-5049.144680
3. Feisty. (n.d.). FEISTY Study - Clinical Trial - Fibrinogen Early In Severe Trauma Study. [online] Available at: http://www.feisty.org.au/ [Accessed 18 Jul. 2020].
4. Fibriant (12AD) 'Fibriant Expands License Adding Recombinant Human (pro)thrombin to Existing Recombinant Fibrinogen Capability', Business Wire (English), 2019 May. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=bwh&AN=bizwire.bw43551314&site=ehost-live (Accessed: 21 June 2020).
5. Flores, C. (2010). What is cryoprecipitate? How is cryoprecipitate made? [online] Available at: https://mytransfusion.com.au/sites/default/files/I_need_to_know_about_cryoprecipitate_0.pdf [Accessed 18 Jul. 2020].
6. Haurowitz (2019). Protein - The muscle proteins. In: Encyclopædia Britannica. [online] Available at: https://www.britannica.com/science/protein/The-muscle-proteins#ref593812.
7. Jilma-Stohlawetz, P. et al. (2017) 'Evaluation of between-, within- and day-to-day variation of coagulation measured by rotational thrombelastometry (ROTEM)', Scandinavian Journal of Clinical & Laboratory Investigation, 77(8), pp. 651–657. doi: 10.1080/00365513.2017.1394487.
8. Masaki Hirashima, Takayuki Imamura, Kentaro Yano, Ryoichi Kawamura, Akihiro Meta, Yoshiyuki Tokieda, Toshihiro Nakashima, High-level expression and preparation of recombinant human fibrinogen as biopharmaceuticals, The Journal of Biochemistry, Volume 159, Issue 2, February 2016, Pages 261–270, https://doi.org/10.1093/jb/mvv099
9. Migden DR, Braen GR. The Jehovah's Witness blood refusal card: ethical and medicolegal considerations for emergency physicians. Acad Emerg Med. 1998;5(8):815-824. doi:10.1111/j.1553-2712.1998.tb02510.x
10. Nascimento, B., Goodnough, L. T., & Levy, J. H. (2014). Cryoprecipitate therapy. British journal of anaesthesia, 113(6), 922–934. https://doi.org/10.1093/bja/aeu158
11. Redman CM, Xia H. Fibrinogen biosynthesis. Assembly, intracellular degradation, and association with lipid synthesis and secretion. Ann N Y Acad Sci. 2001;936:480-495.
12. Sachais, B.S. and Shaz, B.H. (2015). Early cryoprecipitate for trauma patients is feasible, but will it improve outcome? British Journal of Anaesthesia, 115(1), pp.3–5.
13. Schmidt, D. E. et al. (2017) 'Relative effects of plasma, fibrinogen concentrate, and factor XIII on ROTEM coagulation profiles in an in vitro model of massive transfusion in trauma', Scandinavian Journal of Clinical & Laboratory Investigation, 77(6), pp. 397–405. doi: 10.1080/00365513.2017.1334128.
14. Schöchl, H., Cotton, B., Inaba, K. et al. FIBTEM provides early prediction of massive transfusion in trauma. Crit Care 15, R265 (2011). https://doi.org/10.1186/cc10539
15. Silverglied, A. (2020). Plasma derivatives and recombinant DNA-produced coagulation factors. [online] UpToDate. Available at: https://somepomed.org/articulos/contents/mobipreview.htm?31/53/32607.
16. Sobrino, J., & Shafi, S. (2013). Timing and causes of death after injuries. Proceedings (Baylor University. Medical Center), 26(2), 120–123. https://doi.org/10.1080/08998280.2013.11928934
17. Tanaka, Kenichi. (2012). A Primer for Clinical Use of Rotational Thromboelastometry. Point of Care The Journal of Near-Patient Testing & Technology. 11. 77-84.
18. Winearls, J., Wullschleger, M., Wake, E. et al. Fibrinogen Early In Severe Trauma studY (FEISTY): study protocol for a randomised controlled trial. Trials 18, 241 (2017). https://doi.org/10.1186/s13063-017-1980-
19. Wong, H. and Curry, N. (2017). Do we need cryoprecipitate in the era of fibrinogen concentrate and other specific factor replacement options? ISBT Science Series, 13(1), pp.23–28.