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TISSUEPAPER

Histotechnology Group of Queensland

President's Report- Mark Bromley

Spring has finally sprung, and as the days slowly start to get longer and warmer, I welcome you to the third 2021 edition of TissuePaper.

Since the last edition, COVID has once again stymied our attempts at a normal programme of scientific meetings. We did manage one face to face, albeit later than originally hoped, at QML. Unfortunately, numbers were limited, but those who were able to attend enjoyed a great tour of the lab and a great presentation by Dr Ben van Haeringen on granulosa cell tumours. However, by the time the joint AIMS/HGQ Scientific Meeting came around in August, we were once again forced to abandon the traditional Pineapple Hotel venue and switch instead to an online forum, which saw some great presentations around the Heart. Another COVID victim was the Trivia night, postponed from 16th of July until the 10th of September. If you haven't already got your tables booked in the form is on the HGQ website, download it, fill it in and whizz it off to Emma. The half day workshop on the 9th of October at QUT is still on schedule and looks to be a great event. Bling it on!

From our nice warm, sunny, and comparatively free seats up here in Queensland, I encourage you all to spare a thought for our histological sisters and brothers in other less fortunate states, particularly NSW and Victoria. I'm sure you will join me in sending your thoughts and hopes for a speedy exit from the ongoing situation they are enduring. Quite how we have managed to avoid the same fate up here is beyond me, but I'm not complaining! Jump on their social media sites and wish them well and stay safe and COVID free yourselves!

In This Top Issue :

Committee Members

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Histotechnology at TAFE Queensland

TAFE Queensland histology teacher Courtney Colless Q and A with 'Promising Histotechnician Award' winner Stephen Humphrys

Do you remember when you started to learn about the fabulous world that is histology?

Histology, rather MSL973020 Perform histological procedures has been offered at TAFE Queensland, Southbank campus since 2018 as a unit of competency in MSL40118 Certificate IV in Laboratory Techniques. Originally starting with a cohort of nine students, it has grown to accommodate over 30 students in a few short years.

On campus the laboratory is set up to be as realistic as possible for a teaching laboratory, with a focus not just on embedding, microtomy, staining, cover slipping, labelling and slide sorting, but also incorporating the importance of laboratory documentation and communication using shift hand over log, equipment temperature logs and maintenance logs. In accordance with www.training.gov.au to be competent in this unit, students must embed, microtome, stain and cover slip a minimum of three different types of tissue in accordance with a set of criteria (for example, no wrinkles or folds on sections).

The Histotechnology Group of Queensland and its members have been extremely supportive of TAFE Queensland and the histology unit, particularly QUT and the gift of three microtomes as well as ongoing assistance from Chris Cazier whom donates tissues no longer required for QUT students.

Then in 2020 HGQ extended their support even further by providing a financial gift for a newly created **'Promising Histotechnician Award'**. This award is given to the student demonstrating the following to the highest standard: a positive attitude during laboratory sessions, produces histological stains of a high standard, and must be a member of HGQ and attend at least one event hosted by the group.

The first recipient of the award graduated last year, when unfortunately, graduation was online due to COVID-19. As RONA continues to wreak havoc on the world and we have been unable to formally introduce the first recipient of the 'Promising Histotechican Award', thus I would like to do that now, for the winner was: **Stephen Humphrys.** Stephen was also the winner of the Graduate of the Year in the Applied Science Facility when graduating with his MSL50118 Diploma of Laboratory Technology.

When did you start at Tafe Queensland?

I started TAFE in 2016, completing the Certificate IV in Laboratory Techniques and Diploma of Laboratory Technology.

Why did you enrol in the Tafe Queensland Course?

While working as a laboratory assistant in the Histology Department at Sullivan Nicolaides Pathology, I decided I wanted to explore further opportunities in the field of histology but was unsure where to start.

Many of my coworkers recommended the Diploma of Laboratory Technology as they had either already graduated themselves or were current students.

Your favourite unit?

I had two favourite units in Histology and Microbiology. In Histology I was able to develop a more in depth understanding of the field and workflow while also gaining confidence in embedding, microtomy and H&E staining across a wide range of tissue. Microbiology demonstrated to me how interesting the diagnostic process and theory was.

When did you start working at the Mater and North West and what do your roles entail?

I'm currently working as a MOHS Technician at North West Private Hospital and as a Scientific Assistant at Mater Pathology.

I started working at the Mater in February 2020 and as a Scientific Assistant I assist the scientific team in roles such as dishing, embedding and data entry while also performing daily tasks such as cleaning stations and equipment, laboratory opening & closing duties, tissue processor maintenance, reagent preparation and xylene recycling.

I began working as a MOHS Technician in July 2021 and work directly with the surgical team to provide a quick and incredibly effective treatment for the patient/s. As a MOHS Technician I'm primarily responsible for producing slides for the dermatologist using frozen section techniques, more specifically using OCT and liquid nitrogen for embedding and microtomy in a cryostat before rapid H&E staining. Throughout the day I'm expected to work independently and therefore I'm responsible for all the daily requirements to running the laboratory by myself such as workstation cleaning, preparation, and quality controls.

Your view on how your practical sessions at Tafe Queensland prepared you for the workplace

My TAFE experience gave me a great start to my practical histology skills and the confidence to take my developed skills to the laboratory, especially microtomy. The free-flowing structure of the histology practicals also helped emphasize just how essential good time management is to a laboratory technician.

Any plans or future study you wish to share

Currently I'm exploring options to obtaining my Bachelor of Medical Laboratory Science so that I can explore more advanced areas of histology as an AIMS accredited laboratory scientist. I hope to one day be able to put all my histology skills together and become a valued member of a great scientific team!

There is More to Me than Histology

Dr **Rachel Maywald – QML Pathology**

1. What did you want to be when you grew up?

A neurosurgeon or an engineer

2. What is the best piece of advice someone has given you?

Everyone needs help / has no idea what is going on some of the time. There is no shame in not knowing. The hardest is not knowing what you don't know.

3. What is your favourite stain and why?

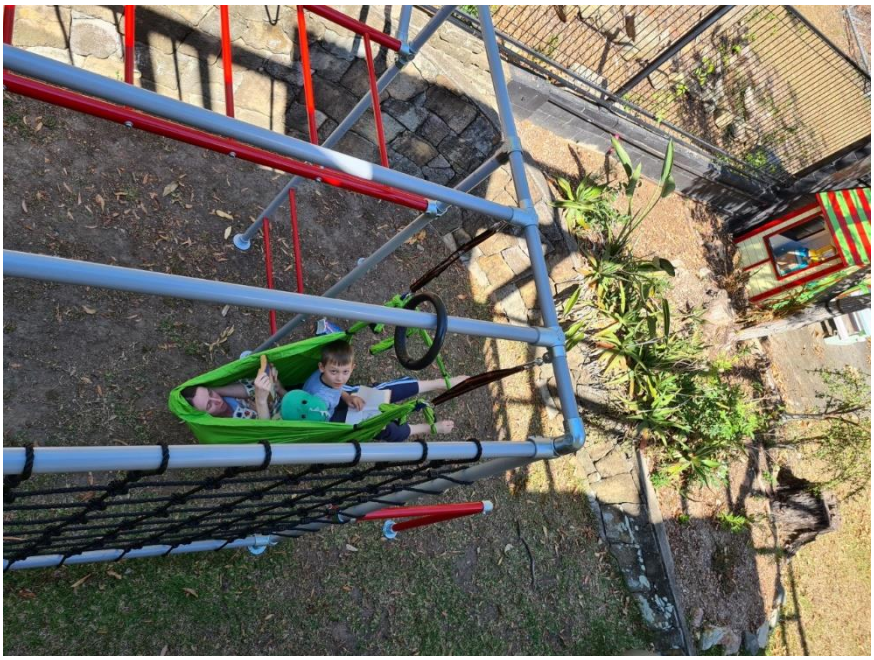
SOX10. Saving sleep every "funny spindle cell" lesion.

4. Do you have any hobbies / What do you do in your spare time?

I enjoy reading (mostly fantasy novels) and if I have a bit more time, building lego sets (but not even vaguely close to Lego masters levels)

5. Do you have a hidden / special skill?

My 8-year-old says it is making people happy.



6. Favourite food?

Hot buttery popcorn (the dentist is VERY disapproving)

7. Dream holiday destination?

Tasmania, with my husband. And a creche/kids club :D

8. We heard you like shoes?

I love shoes!

Save the Date

Date: 9th Oct
Silver Stains Workshop
Venue: QUT Gardens Point

Date: 21st Oct
Scientific Meeting & AGM 4th
Venue: TBA

Scientific Meeting Update

Sharee Durdin

On the 22nd July, Faye Kapoulitsas the manager of the histology department of QML successfully hosted a face-to-face scientific meeting with Dr Ben van Haeringen and Metagene as the nights sponsor.

The night began with some merchandise sharing from Metagene and a round of drinks at BrewDog which was then followed up with some pizza and a walk through of the QML Laboratory.

The walk through was thoroughly enjoyed and it was a great insight into a laboratory practice for those students from Tafe who attended.

After the walk around the laboratory it was then time for Dr Ben van Haeringen to take control of the stage and present his informative speech on Granulosa Cell Tumours of the Ovary.

It was a wonderful night to see everyone, and the committee is looking forward to a night of Trivia and workshops coming up. We hope to see you all there and again a big thank you to Metagene for sponsoring the scientific meeting and to Dr Ben van Haeringen for presenting a very thorough and interesting speech.

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Severe Cytomegalovirus Effect on Placenta Investigation

Krishlin Jaya Pal

Introduction

Cytomegalovirus is a Beta-Herpesviridae virus that affects both adults and babies (congenital CMV); it can cross the placenta and infect the foetus in pregnancies. [1,2] CMV is responsible for a range of poor neurological development, developmental and motor delay, vision and hearing loss, seizures, microcephaly, and death in babies. Symptoms of congenital CMV include low birth weight, jaundice, hepatosplenomegaly, rash, retinitis and/or seizures at birth. Despite low birth rates of congenital CMV foetuses (approximately 6 in every 1,000 live births), a small number of these babies develop any degree of permanent disabilities. [1,2] CMV is transmitted via direct contact with saliva, semen and urine and shed throughout all bodily fluids. CMV has a slow replication rate, therefore, takes longer to detect through viral and microbial testings. We investigated a case of a 25-year-old female with no familial or clinical history of CMV infection who terminated her pregnancy at 19 weeks. The case highlights the importance of decision-making in CMV affected pregnancies and the extent of CMV effect on the placenta visualised through immunohistochemistry (IHC).

Case History

A regional 25-year-old female presented to her doctor at 7 weeks pregnant. The patient underwent numerous general pregnancy screening tests including blood and urine tests. Microbial serology tests identified positive CMV IgM in her urine. Re-sampling of her urine 2 weeks later confirmed the presence of CMV. The patient had not provided any family history regarding CMV. Non-Invasive Prenatal (NIP) Test was requested by the patient and at 11-weeks gestational age, Prenatal Aneuploidy test results showed no chromosomal defects or genetic abnormalities of Trisomy 13, 18 or 21. The foetus was revealed to have two X chromosomes (female). The patient decided to induce delivery of the foetus at 19 weeks and the placenta was removed. Microbiology DNA nucleic acid amplification (NAA) test detected CMV DNA on the placenta. CMV DNA PCR detected the viral load. Cytogenetic microarray analysis identified the foetal karyotype of arr(1 – 22, X) x2; a female with no copy number variation of clinical significance. The placenta was taken to be histologically investigated for macroscopic, microscopic and IHC diagnosis of the extent of CMV effect.

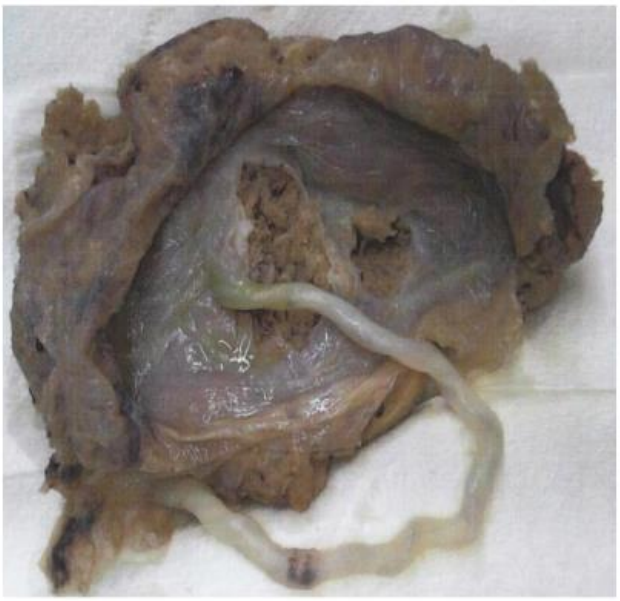

Figure 1a: *Placenta foetal view - consists of foetal surface, umbilical hyper-coiled and point of attachment.*

Results

Upon initial presentation of the CMV-positive placenta in histology, macroscopic descriptions were noted, and the placenta was cut in key locations and blocked as following: the cord, membrane, cord insertion site, central (foetal-side) placenta and peripheral (maternal-side) placenta. Figure 1a shows the placenta foetal side view and 1b shows the disrupted maternal view before cutting.

The placenta measured 141 x 86 x 34mm and weighed 221g. Maternal Cotyledons were visibly ragged in an area compromising approximately 40% of the total maternal surface. The umbilical cord measured 208 x 77mm with 4 coils per 10cm, consistent with hyper-coiling. 3 vessels were present. The amniotic membrane was semitranslucent, disrupted and incomplete. The foetal surface was grey and yellow although no visible infection was noticeable.

Blocked tissue samples were sectioned and stained with Haematoxylin & Eosin (H&E) to demonstrate cellular morphological features for microscopic diagnosis. It revealed the umbilical cord consisted of 2 patent arteries and a patent vein. There were no sightings of intraluminal thrombus, haemorrhage or funisitis which are classic features of chorioamnionitis. The placental surface and membranes showed limited

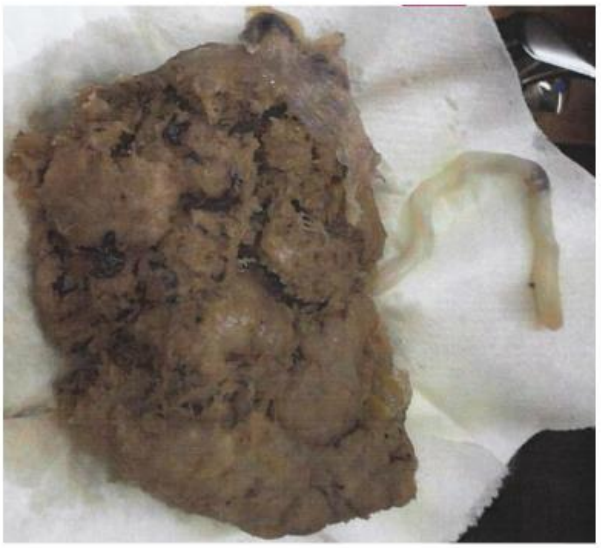

Figure 1b: *Placenta maternal view - consists of disrupted placenta surface.*

evidence of acute chorioamnionitis. Plasma cell villitis with intranuclear CMV inclusions were sighted and identified in Figure 1c. All features aligned with the clinical impression of congenital CMV infection.

Confirmation of CMV infected cells required indirect IHC. Figure 1d demonstrates positive CMV infected cells on the placenta membrane with cytoplasmic and membranous staining pattern. The presence of CMV cells confirmed the diagnosis of Congenital CMV Infection.

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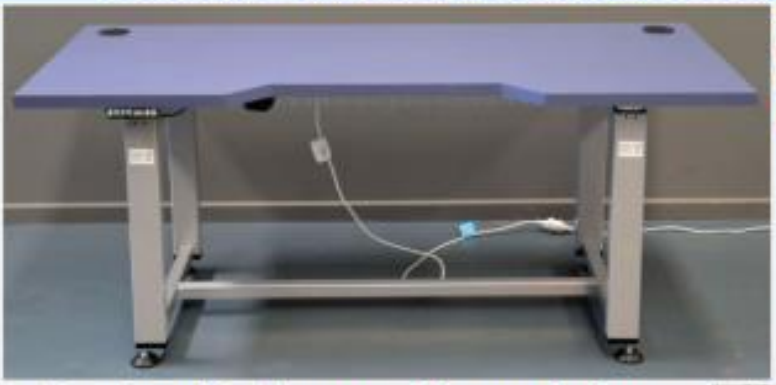

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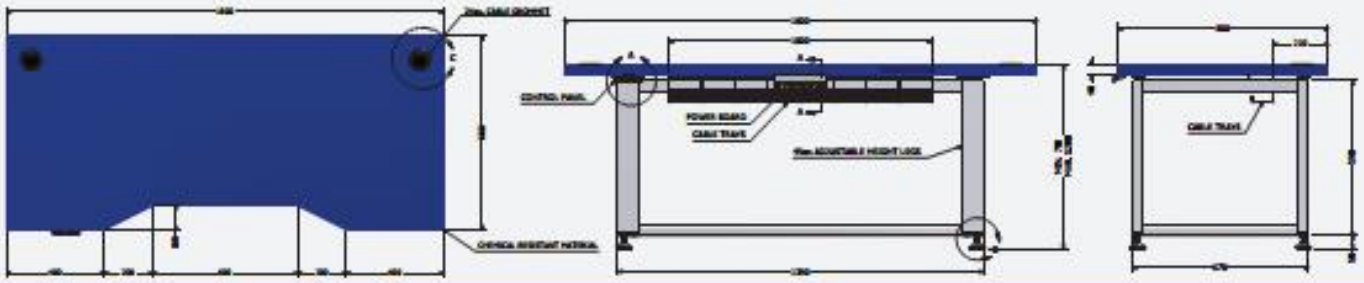

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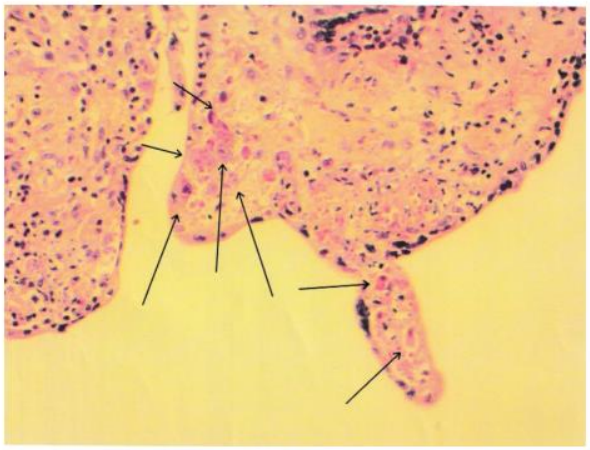

Figure 1c: *H&E stain of Placenta Membrane. Arrows indicating to Plasma cell villitis – classic 'owl eyed' cells with halo clearing.*

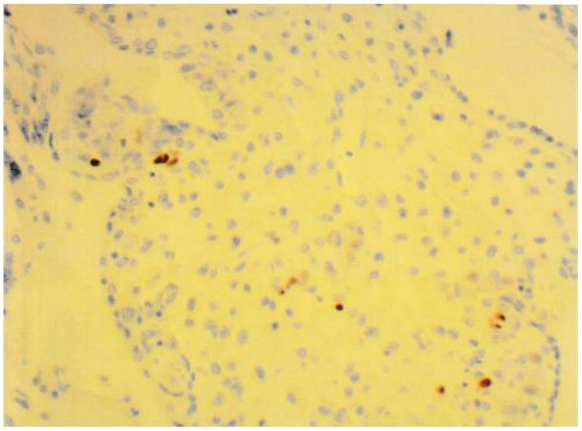

Figure 1d: *IHC of CMV infected cells on placenta membrane. Brown-Red cells are indicative of CMV infection. Muted blue cell stained to visualise-placenta-architecture.*

Discussion

Congenital CMV infection can cause severe permanent disabilities; it can affect neurological and developmental abilities, hearing and vision loss, seizure and even cause death. [1,2] Due to advancing diagnostic testings, the maternal patient can decide how to engage with delivering the baby. Previous tests showed that there were no chromosomal defects or genetic abnormalities of Trisomy 13, 18 or 21 through the NIP test results. This assures that the foetus was genetically normal and not affected by the CMV infection before birth. Upon delivering the baby early, Cytogenetic microarray analysis identified foetal karyotype of arr(1 – 22, X) x2 Female foetus with no copy number variation of clinical significance. Multiple microbial PCR and DNA NAA testings confirmed CMV presence in the patient and on the placenta.

Despite the patient deciding to terminate the pregnancy late at 19 weeks, the extent that CMV has affected the patient has been investigated through histological findings. Several similar histopathological case studies have been published where the extent of CMV effect on the placenta was measured. In previous case studies, the CMV inclusions were graded by counting the number of affected chorionic villi. [3,4]

Macroscopic-findings-included-hyper-coiling of the umbilical cord (Figure 1a). This suggested adverse perinatal conditions or caesarean delivery. In this case, termination of late pregnancies involved caesarean delivery which explained the ragged maternal surface from the difficult placenta removal (Figure 1b). [3,4] Despite the yellow and grey foetal-surface colour, there was no-obvious chorioamnionitis (bacterial infection of the chorion, amnion and amniotic-fluid from delivery).

Microscopic diagnosis included the presence of plasma cell villitis with intranuclear CMV inclusions (Figure 1c). Plasma cell villitis is the result of the inflammatory response caused by the infiltration of lymphocytes, macrophages and plasma cells into the villous tree. [4] Microscopically these are large irregular oval cells with enlarged cytoplasmic components and a prominent eccentrically placed nucleus ('Owl Eye' Inclusion). Supporting indirect

IHC demonstrates CMV infected cell bindings shown visibly brown under the microscope (Figure 1d). [3,4]

These macroscopic, microscopic and IHC features are presented in previous aborted pregnancy findings. The severity of the CMV effect is graded based upon the number of CMV chorionic villi found. [3,4] No proposed differential diagnosis was proposed as results between microbial, cytogenetic, and histological findings confirmed congenital CMV.

This case study shows the extent of changes a severe case of CMV infection on the placenta can develop with a genetically unaffected foetus. The decision-making of the patient to terminate the foetus is based upon information the patient has received through doctor counselling and unrestricted research. Non-existent preventative measures against CMV influences decision-making regarding delivery of the foetus. [1,2] There are currently no approved vaccines available for CMV. [1,3]

Anti-viral CMV suppression medication Valganciclovir is available for confirmed CMV organ-transplant recipients and patients with AIDS/HIV. Valganciclovir is unavailable for most pregnancies due to associated foetal development risks and limited information on the safety and effectiveness of valganciclovir in babies and children. [1,3,5] More research is required to implement a CMV prevention scheme.

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