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ANATOMICAL / MOLECULAR PATHOLOGY

DNA extraction from placental, fetal and neonatal tissue at autopsy: what organ to sample for DNA in the genomic era?

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Summary

Incorporation of genome and exome sequencing into fetal and neonatal autopsy investigations has been shown to improve diagnostic yield. This requires deoxyribonucleic acid (DNA) to be extracted from either the placenta or autopsy tissue for molecular testing. However, the sources and quality of DNA obtained are highly variable and there are no adequate published data on what tissue is most ideal to sample for DNA extraction in this setting. Here we compare the quality of DNA extracted from sampling the placenta and various solid organs at fetal and neonatal autopsy, thereby determining the optimal tissue from which to source DNA for ancillary testing as part of the modern perinatal autopsy.

A total of 898 tissue samples were obtained at autopsy from 176 fetuses (gestational ages 17–40 weeks) and 44 neonates (age range 0–28 days) at our tertiary institution. Fetal tissue was processed using the QIAsymphony DSP DNA Mini kit and placental tissue was extracted using the New iGENatal Kit. DNA concentration was quantified using the Qubit dsDNA BR Assay Kit. DNA integrity, as stratified by gel electrophoresis was classified as high (≥5 kb) or low quality (<5 kb). Genome sequencing was performed on the extracted DNA, together with respective parental DNA from blood samples, and confirmed absence of maternal contamination in all cases. Analyses used logistic mixed models to test for associations between tissue types, intrauterine retention times, delivery to autopsy and death to autopsy intervals with DNA quality.

In the fetal cohort, the placenta had the highest proportion of high quality DNA samples (93.1%), and liver had the lowest proportion (35.3%). Among the neonates, all tissue samples with the exception of liver had over 88% high DNA quality with the placenta also yielding the highest quality (100%). There was statistically significant deterioration in DNA quality with prolonged time interval between demise and autopsy (≥5 days). In the 726 fetal samples, the odds of obtaining higher quality DNA from the placenta, thymus, and spleen were 70.4 [95% confidence interval (CI) 29.2–169.6], 3.6 (95% CI 2.0–6.6) and 3.3 (95% CI 1.8–6.1) times, respectively, more likely than samples from the liver (*p* values <0.001). DNA yield from other fetal solid organs investigated was not significantly superior to that from the liver.

This study shows that, when available, refrigerated unfixed placenta is the most suitable source of high quality DNA during perinatal investigations. Of the solid fetal organs sampled at autopsy, lymphocyte-rich, lytic enzymes-poor organs such as thymus and spleen were significantly more likely to yield good quality DNA than the liver.

Key words: DNA; fetus; neonate; autopsy; perinatal; neonatal; liver; spleen; thymus; placenta; genomic; genome sequencing; pathology.

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INTRODUCTION

The perinatal autopsy remains the gold standard procedure for investigating causes of fetal and neonatal deaths.[1,2] Recent advances in genome and exome sequencing have made it practical to simultaneously interrogate multiple possible genetic aberrations that may cause early death as part of the autopsy procedure. Studies show that incorporation of genomic testing into routine fetal and neonatal autopsy investigations improves diagnostic yield by as much as 25–50%.[3–5] The yield is predicted to increase with time as knowledge of the clinical significance of genomic variants improves. In the setting of perinatal death investigation, molecular testing is performed on DNA extracted from either the placenta or fetal organs, such as the liver, at autopsy. The quality of DNA yielded can be highly variable depending on the organ sampled, duration of intrauterine retention after fetal *in utero* demise and methods of extraction utilised.

Gross and microscopic examination shows that fetal tissues autolyse at different rates after death, a fact that has long been employed by perinatal pathologists to estimate duration of retention *in utero* after demise.[6–8] Therefore, we hypothesised that DNA from various fetal and neonatal organs would not be of uniform quality, with enzyme-rich organs such as the pancreas and liver expected to degenerate earlier, yielding lower amounts of high quality DNA. Surprisingly, the question of what fetal organ is most suitable to sample for molecular testing and cytogenetics during a perinatal or

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neonatal autopsy is not well addressed in current literature. The most recent editions of perinatal autopsy textbooks for practising pathologists offer variable suggestions that include placenta,[9] fascia, lung, Achilles tendon and cartilage,[10] pericardium and gonads.[11] To the best of our knowledge, no studies comparing DNA quality from these organs have been published to back up any of the recommendations.

This study compares DNA quality from various fetal organs and the placenta to determine the best source of DNA material for molecular testing in the perinatal autopsy setting of fetuses and neonates.

MATERIALS AND METHODS

This is a component of a prospective study on whole genome sequencing in perinatal pathology, approved by the Human Research Ethics Committee of Mater Health, Brisbane, Queensland, Australia (HREC/14/MHS/74). Appropriate parental consent for DNA extraction, storage and whole genome sequencing was obtained in all cases.

A total of 898 post-mortem tissue samples were prospectively extracted from 176 fetuses and 44 neonates between 2009 and 2018 as part of a previously published study on the utility of genome sequencing in perinatal death investigation.[3] Gestational ages of the fetuses at birth ranged from 17 to 40 weeks and those of the neonates from 22 to 41 weeks. Of the fetuses, 21 (12%) were under 20 weeks gestation and 155 (88%) were ≥20 weeks. The ages of the neonates at death ranged from 1 minute to 28 days. Of the 44 neonatal deaths, 18 (41%) died on day 1 of life (perinatal deaths).

Before fixation, placentas were refrigerated at 4°C from the time of delivery and were sampled for DNA soon after arrival to the laboratory. Fetal tissue and chorionic villous samples of at least 25 mm^3 in volume were collected and fresh frozen at −80°C before being processed for DNA extraction. Chorionic villi were obtained by sampling under the chorionic plate in the central area of the placental disc. Proteinase K and ATL tissue lysis buffer (Qiagen, Germany) were used at 56°C, to homogenise the tissue in the pre-treatment step. Fetal tissue was extracted using the QIAsymphony DSP DNA Mini Kit (Qiagen) and placental tissue was extracted using the New iGENatal Kit (iGEN Biotech, Spain) as per the manufacturer's instructions.

The DNA concentration was quantified using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, USA). DNA aliquots were electrophoresed at 100 volts on 0.8% agarose gels stained with GelRed Nucleic Acid Gel Stain, 10,000X in water (Biotium, USA) and images captured using the Bio-Rad Gel Doc XR System (Bio-Rad Laboratories, USA).

High molecular weight DNA with samples having a single band at or above the 5 kb band of the GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher Scientific) were classified as being high quality DNA. Degraded DNA with no high molecular weight DNA band or where the majority of the DNA was below the 5 kb band of the ladder was classified as low quality DNA.

Tissue sample types that had low frequencies were combined into one group called 'other'. These tissue types included adrenal gland (n=3), blood (n=1), brain (n=1), cultured fibroblasts (n=1), heart (n=1), lung (n=8), skin (n=9) and umbilical cord (n=1). Sampling from the umbilical cord, skin, adrenal, blood and cultured fibroblasts was discontinued early in the study due to consistently low DNA yield or technical difficulties in sampling and processing. For the most part, these 'other' tissues were sampled in addition to the more common tissues for each case.

Previously published methods[6–8] have estimated the *in utero* demise to delivery interval by examination of placental histopathological changes, extent of fetal skin desquamation, and differential loss of nuclear basophilia in fetal tissue histology. These methods were used to categorise the intrauterine fetal deaths (IUFDs) into the following six retention interval groups: (<6 hours; 6–23 hours; 24–47 hours; 48 hours–6 days; 1–2 weeks; and ≥2 weeks). Information on clinical history, including the duration of absence of fetal movements and in cases of medical terminations and neonatal deaths, the exact time of death were incorporated to assess time of demise. The retention period for IUFDs was re-categorised into <48 hours and ≥48 hours retention, from the six interval groups.

The post mortem interval [PMI, defined for the neonates as the time from neonatal death (NND) to autopsy, or for IUFDs as the time from delivery to autopsy] was categorised into two groups: <5 days or ≥5 days. For IUFD samples, the retention and PMI were combined into a four-category variable:

- retention <48 hours and PMI <5 days
- retention <48 hours and PMI ≥5 days
- retention ≥48 hours and PMI <5 days
- retention ≥48 hours and PMI ≥5 days

Frequency (%) was reported for categorical variables of interest and median [interquartile Range (IQR)] for continuous variables. Chi-square tests were used to assess the association between categorical variables when performed separately by tissue type. For low cell frequencies, Fisher's exact test was used.

Logistic mixed effect models were used to test for associations between key variables with the primary outcome of DNA quality at the individual IUFD/NND level, rather than at the tissue sample level. As individual IUFDs/NNDs had samples collected from multiple tissue types (hierarchical structure), the logistic mixed model takes into account the correlation between samples from the same individual by including a random intercept term for each IUFD or NND. Due to small numbers in the 'other' tissue type category, samples in that category were excluded in all analyses. The effect sizes were presented as odds ratios (OR) with 95% confidence intervals (CI) of high DNA quality with reference to low quality/failed extraction. Statistical analyses were performed separately for IUFDs and NNDs. Significance was defined at the 5% level. All analyses were performed in SPSS version 22 (IBM Corp, USA).

RESULTS

The frequency of the tissue types with low and high DNA quality for IUFDs and NNDs are presented in Table 1. Due to the small percentage of extraction failures (~0.3%), DNA quality was collapsed into two groups: failure/low quality, or high quality. Placental tissue had the highest proportion of high quality DNA samples for IUFDs (93.1%), and liver had the lowest proportion (35.3%). For the NNDs, all tissue

Table 1 Frequency of tissue type and DNA quality separately for IUFD and NNDs

Tissue type	IUFD (n_s=741 samples)		NND (n_s=157 samples)	
	DNA quality		DNA quality	
	Low/Failure	High	Low/Failure	High
Liver	99 (64.7%)	54 (35.3%)	11 (30.6%)	25 (69.4%)
Kidney	95 (62.5%)	57 (37.5%)	2 (6.3%)	30 (93.8%)
Spleen	65 (46.1%)	76 (53.9%)	4 (11.4%)	31 (88.6%)
Thymus	65 (43.6%)	84 (56.4%)	3 (10%)	27 (90%)
Placental tissue	9 (6.9%)	122 (93.1%)	0 (0%)	14 (100%)
Other[a]	6 (40.0%)	9 (60.0%)	1 (10%)	9 (90%)

IUFD, intrauterine fetal deaths; NND, neonatal deaths; n_s, number of tissue samples.
[a] The 'other' category is the combination of adrenal gland, blood, brain, cultured fibroblasts, heart, lung, skin and umbilical cord.

samples with the exception of liver had over 88% of high DNA quality. In particular, the 14 placentas from NNDs had 100% high quality DNA. The percentage of samples with high DNA quality was higher in the NND group (86.6%) compared to the IUFD group (54.3%).

DNA quality versus organ/tissue type

Tables 2 and 3 summarise characteristics of the cohort, with information presented at both the individual IUFD/NND level (Table 2) and also at the tissue level (Table 3). The majority of IUFDs had four or more tissue samples collected (81.3%) and the majority of NNDs had at least three samples collected (81.8%) (Table 2).

The results of the logistic mixed model to assess associations between DNA quality and tissue types for the IUFDs are presented in Table 4. Due to the small sample size of the NNDs, logistic mixed models were only performed for the IUFDs. The model used liver as the reference as this was the most commonly used tissue in our practice. The results showed that the odds for placental tissue, thymus and spleen were 70.4 (95% CI 29.2–169.6), 3.6 (95% CI 2.0–6.6) and 3.3 (95% CI 1.8–6.1) times respectively more likely to yield high DNA quality than liver samples from each IUFD. Due to the small percentage of low/failed DNA quality samples from placental tissue in IUFDs (6.9%), the effect size in the IUFD model is large in magnitude with high variability (as indicated by the width of the confidence interval).

Table 2 Descriptive statistics by individual cases

Variable	IUFD N=176	NND N=44	Total N=220
Gestation (weeks)			
Median (IQR)	26.5 (21–34)	32.5 (25–38)	27 (22–35)
No. samples per case			
1	9 (5.1%)	6 (13.6%)	15 (6.8%)
2	12 (6.8%)	2 (4.5%)	14 (6.4%)
3	12 (6.8%)	10 (22.7%)	22 (10%)
4	48 (27.3%)	14 (31.8%)	62 (28.2%)
5	90 (51.1%)	11 (25%)	101 (45.9%)
6	5 (2.8%)	1 (2.3%)	6 (2.7%)
PMI			
<5 days	91 (51.7%)	20 (45.5%)	111 (50.5%)
≥5 days	85 (48.3%)	24 (54.5%)	109 (49.5%)
Retention (for IUFDs)			
<6 hours	28 (15.9%)		
6–23 hours	35 (19.9%)		
24–47 hours	50 (28.4%)		
48 hours–6 days	49 (27.8%)		
1–2 weeks	5 (2.8%)		
≥2 weeks	9 (5.1%)		

IQR, interquartile range; IUFD, intrauterine fetal deaths; NND, neonatal deaths; PMI post-mortem interval (see text for definition).

Table 3 Summary of tissue types and DNA quality for intrauterine deaths (IUFD), neonatal deaths (NND) and the two groups together (total)

	IUFD N=741	NND N=157	Total N=898
Tissue sample type			
Kidney	152 (20.5%)	32 (20.4%)	184 (20.5%)
Liver	153 (20.6%)	36 (22.9%)	189 (21%)
Placental tissue	131 (17.7%)	14 (8.9%)	145 (16.1%)
Spleen	141 (19%)	35 (22.3%)	176 (19.6%)
Thymus	149 (20.1%)	30 (19.1%)	179 (19.9%)
Other[a]	15 (2%)	10 (6.4%)	25 (2.8%)
Adrenal gland	3 (0.4%)	0 (0%)	3 (0.3%)
Blood	0 (0%)	1 (0.6%)	1 (0.1%)
Brain	0 (0%)	1 (0.6%)	1 (0.1%)
Cultured fibroblasts	1 (0.1%)	0 (0%)	1 (0.1%)
Heart	0 (0%)	1 (0.6%)	1 (0.1%)
Lung	5 (0.7%)	3 (1.9%)	8 (0.9%)
Skin	5 (0.7%)	4 (2.5%)	9 (1%)
Umbilical cord	1 (0.1%)	0 (0%)	1 (0.1%)
DNA quality			
Extraction failure	3 (0.4%)	0 (0%)	3 (0.3%)
Low	336 (45.3%)	21 (13.4%)	357 (39.8%)
High	402 (54.3%)	136 (86.6%)	538 (59.9%)

[a] The 'other' category is the combination of adrenal gland, blood, brain, cultured fibroblasts, heart, lung, skin and umbilical cord. These organs are excluded from further the statistical analyses.

Table 4 Logistic mixed effects model of the association of tissue type with DNA quality for IUFD samples

IUFD (n_R=176, n_s=726)	OR (95% CI)	*p* value
Liver	Reference	–
Kidney	1.17 (0.65–2.11)	0.60
Spleen	3.34 (1.83–6.09)	<0.001
Thymus	3.62 (2.00–6.56)	<0.001
Placental tissue	70.41 (29.24–169.59)	<0.001

CI, confidence interval; IUFD, intrauterine fetal deaths; n_R, number of individuals (IUFDs); n_s, number of tissue samples; OR, odds ratio.

DNA quality versus time intervals (IUFDs)

As it is difficult to separate effects of the duration of intrauterine retention interval from PMI on DNA quality, a categorical variable combining retention period and PMI was derived to investigate associations between length of a combination of either time and DNA quality.

Chi-square tests for the association between time period and DNA quality, separately for each tissue type for the IUFDs, are presented in Table 5. Placental tissue had a high proportion of high quality DNA in all groups and no statistically significant association between DNA quality and time period (p=0.50, Table 5). All other organ/sample types had statistically significant associations, with groups that had retention time <48 hours having a higher proportion of high quality DNA than the groups with retention interval ≥48 hours.

The results from the logistic mixed models used to assess the association between time period and DNA quality at the IUFD level are presented in Table 6. Two logistic mixed models were considered, unadjusted (Model A) and adjusted for tissue type (Model B). Model A only included a main effect for retention time, and Model B also included a main effect for tissue type. The results show that even when taking into account differences in tissue sample (Model B), tissue samples from IUFDs that have a retention time of ≥48 hours are more than 10 times more likely (i.e., either 1/0.05 or 1/0.08, Model B) to have a have low DNA quality than when compared to tissue samples that have a time combination of retention <48 hours and PMI of <5 days. These differences are statistically significant (p<0.001).

DNA quality versus time from death to autopsy (NNDs)

The results from the chi-squared test to assess the association between DNA quality and PMI for the NND samples, separately by tissue type, are presented in Table 7. For placental tissue, no statistical test was performed due to all samples being of high quality. The test results show that there is insufficient evidence for any association between PMI and DNA quality in each of the tissue samples from NNDs.

A logistic mixed model was used to assess the association between time from neonatal death to autopsy (PMI) and DNA quality at the tissue sample level (i.e., 157 tissue samples in 44 NNDs). There was no evidence that NNDs with a PMI of ≥5 days (OR=1.4, 95% CI 0.5–4.0, p=0.58) had significantly different DNA quality than those NNDs with PMI less than 5 days. Due to the small number of NNDs, no adjustment for tissue type was considered.

Sampling for DNA in fetuses and neonates with prolonged demise to autopsy interval

To determine suitable organs from which to sample for high quality DNA in severely macerated fetuses, we considered the subset of IUFDs that had a retention time of ≥48 hours and a PMI of ≥5 days (Table 8). This subset had 121 samples across 31 IUFDs after excluding samples from the 'other' type.

As shown in Table 8, placental tissue had much higher DNA quality (90.9%) than any of the fetal organs from IUFDs. Of the fetal organs, thymus and spleen yielded higher

Table 5 Cross tabulation and chi-square tests of association between retention and post-mortem time period and DNA quality, stratified by tissue type

Tissue type N=726	Time period	DNA quality		*p* value
		Low/Failure	High	
Liver	Retention <48 hours, PMI <5 days	24 (45.3%)	29 (54.7%)	<0.001
N=153	Retention <48 hours, PMI ≥5 days	23 (50%)	23 (50%)	
	Retention ≥48 hours, PMI <5 days	28 (100%)	0 (0%)	
	Retention ≥48 hours, PMI ≥5 days	24 (92.3%)	2 (7.7%)	
Kidney	Retention <48 hours, PMI <5 days	22 (43.1%)	29 (56.9%)	<0.001
N=152	Retention <48 hours, PMI ≥5 days	23 (48.9%)	24 (51.1%)	
	Retention ≥48 hours, PMI <5 days	26 (96.3%)	1 (3.7%)	
	Retention ≥48 hours, PMI ≥5 days	24 (88.9%)	3 (11.1%)	
Spleen	Retention <48 hours, PMI <5 days	14 (29.8%)	33 (70.2%)	<0.001
N=141	Retention <48 hours, PMI ≥5 days	11 (25%)	33 (75%)	
	Retention ≥48 hours, PMI <5 days	22 (84.6%)	4 (15.4%)	
	Retention ≥48 hours, PMI ≥5 days	18 (75%)	6 (25%)	
Thymus	Retention <48 hours, PMI <5 days	13 (26%)	37 (74%)	<0.001
N=149	Retention <48 hours, PMI ≥5 days	10 (20.4%)	39 (79.6%)	
	Retention ≥48 hours, PMI <5 days	26 (92.9%)	2 (7.1%)	
	Retention ≥48 hours, PMI ≥5 days	16 (72.7%)	6 (27.3%)	
Placental tissue	Retention <48 hours, PMI <5 days	5 (10.9%)	41 (89.1%)	0.50[a]
N=131	Retention <48 hours, PMI ≥5 days	1 (2.8%)	35 (97.2%)	
	Retention ≥48 hours, PMI <5 days	1 (3.7%)	26 (96.3%)	
	Retention ≥48 hours, PMI ≥5 days	2 (9.1%)	20 (90.9%)	

PMI, post-mortem interval (see text for definition).
[a] Fisher's exact test.

Table 6 Logistic mixed models assessing the relationship between time period and DNA quality in IUFDs, either unadjusted or adjusted for tissue type

IUFD (n_s=726)	Model A: Unadjusted		Model B: Adjusted for tissue type	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Time period				
Retention <48 hours, PMI <5 days	Reference		Reference	–
Retention <48 hours, PMI ≥5 days	1.01 (0.56–1.82)	0.97	1.05 (0.49–2.24)	0.91
Retention ≥48 hours, PMI <5 days	0.13 (0.07–0.26)	<0.001	0.05 (0.02–0.12)	<0.001
Retention ≥48 hours, PMI ≥5 days	0.18 (0.09–0.35)	<0.001	0.08 (0.03–0.22)	<0.001
Tissue type				
Liver			Reference	–
Kidney			1.19 (0.65–2.18)	0.57
Spleen			3.72 (1.99–6.97)	<0.001
Thymus			3.98 (2.14–7.42)	<0.001
Placental tissue			115.08 (44.49–297.64)	<0.001

CI, confidence interval; IUFD, intrauterine fetal deaths; n_s, number of tissue samples; OR, odds ratio; PMI, post-mortem interval (see text for definition).

Table 7 Cross tabulation and chi-square test for association between interval from neonatal death to autopsy and DNA quality, separately by tissue type

Tissue sample (n_s=157)	PMI	DNA quality		*p* values[a]
		Low/Failure	High	
Liver	<5 days PMI	7 (43.8%)	9 (56.3%)	0.16
N=36	≥5 days PMI	4 (20%)	16 (80%)	
Kidney	<5 days PMI	0 (0%)	13 (100%)	0.5
N=32	≥5 days PMI	2 (10.5%)	17 (89.5%)	
Spleen	<5 days PMI	2 (12.5%)	14 (87.5%)	1.0
N=35	≥5 days PMI	2 (10.5%)	17 (89.5%)	
Thymus	<5 days PMI	0 (0%)	10 (100%)	0.53
N=30	≥5 days PMI	3 (15%)	17 (85%)	
Placental tissue	<5 days PMI	(0%)	5 (100%)	–
N=14	≥5 days PMI	(0%)	9 (100%)	

n_s, number of tissue samples; PMI, post-mortem interval.
[a] Fisher's exact test.

Table 8 Frequency table of DNA quality for each tissue type of IUFDs where *in utero* retention is ≥48 hours and post-mortem interval is ≥5 days and NNDs where PMI is ≥5 days

Tissue type	IUFD with PMI ≥5 days (n_s=121, n_R=31)		NND with PMI ≥5 days (n_s=87, n_R=23)	
	DNA quality		DNA quality	
	Low/Failure	High	Low/Failure	High
Liver	24 (92.3%)	2 (7.7%)	4 (20%)	16 (80%)
Kidney	24 (88.9%)	3 (11.1%)	2 (10.5%)	17 (89.5%)
Spleen	18 (75%)	6 (25%)	2 (10.5%)	17 (89.5%)
Thymus	16 (72.7%)	6 (27.3%)	3 (15%)	17 (85%)
Placental tissue	2 (9.1%)	20 (90.9%)	0 (0%)	9 (100%)
Total	84 (69.4%)	37 (30.6%)	11 (12.6%)	76 (87.4%)

'Other' samples are excluded.
IUFD, intrauterine fetal deaths; NND, neonatal deaths; n_R, number of individuals (IUFDs); n_s, number of tissue samples; OR, odds ratio; PMI, post-mortem interval.

quality DNA than liver. Liver tissue had the smallest percentage of high quality DNA (7.7% of all liver samples).

For neonatal deaths, samples that had a PMI of ≥5 days were again considered. With the 'other' samples excluded, there were 87 tissue samples from 23 NNDs (Table 8). The proportion of high quality DNA samples were similar (80.0–100.0% high quality) across tissue types. Similarly, to IUFD samples, placental tissue had the highest percentage of high quality DNA samples (100%), with liver samples again having the lowest (80%). Due to small number of individuals in the prolonged interval groups, logistic mixed models were not performed.

DISCUSSION

With 898 tissue samples from 220 fetuses and neonates, this is the largest study to date comparing DNA quality from solid tissue organs in the setting of modern perinatal autopsy procedure. Addison *et al.* assessed DNA quality from 14 fetuses and concluded that muscle and kidney sampling was

superior to liver tissue at PMI of 2–10 days.[12] Our data show comparable findings, confirming that the liver is the least optimal organ from which to extract DNA. The placenta, thymus and spleen are also examined and show statistically significant higher odds of yielding high quality DNA than the liver. The kidney yielded a larger proportion of low quality DNA compared with the placenta, thymus and spleen, and showed no statistically significant difference when compared with the liver. Other organs that have been suggested in the literature such as gonads, blood and pericardium were considered impractical in our routine practice due to inconsistent accessibility or small amounts of tissue remaining after sampling for histology.

The tissue type with the highest proportion of high DNA quality samples was placental tissue. In comparison to sampling from the liver, placental tissue from an IUFD was 70 times more likely to have high DNA quality. However, the placenta may not always be readily available, such as when it has been prematurely disposed of, fixed in formalin before sampling, or parental preference is to take the intact organ home for cultural, religious or personal reasons. Confined placental mosaicism is a known cause of otherwise unexplained intrauterine fetal growth restriction that occurs in 1–2% of pregnancies[13,14] and in such cases the genetic configuration of the placenta is different from that of the fetus under investigation. Another disadvantage of sampling the placenta is the possibility of contamination by maternal DNA if some of the decidual tissue on the maternal floor is inadvertently incorporated with the chorionic villous sampling. This contamination risk is minimised through microscopic dissection and removal of suspected maternal tissue prior to extraction. In this study, absence of DNA contamination was confirmed by whole genome sequencing subsequently performed on the extracted fetal and neonatal DNA material, together with that from both parents' blood samples, as part of another arm of the study that is published separately.[3]

The logistic mixed effects model for the 176 IUFDs in our study suggested that, of the solid fetal organs studied, the spleen and thymus were more likely to yield high quality DNA than liver samples. For the NNDs, liver samples also had the smallest proportion of high DNA quality (69%) when compared to other tissue types. All samples from placental tissue of NNDs yielded high quality DNA. This may partly reflect handling protocols of placental tissue in our institution, whereby all placentas from the labour ward are refrigerated at 4°C for up to 2 weeks if an immediate decision to forward to the pathology laboratory for testing is not made.

Our study also shows that the PMI did not have a significant effect on DNA quality in NNDs, and that retention interval had a more significant effect on DNA quality for IUFDs than delivery to autopsy interval. These findings have practical implications when triaging perinatal autopsies and surgical workload in anatomical pathology laboratories with labour and time constraints.

We were interested to determine whether the variability of DNA preservation by organ was related to the rapidity of loss of nuclear basophilia identified in that organ on histology. Of the fetal solid organs studied here, the thymus and spleen are unique in being rich in lymphocytes and other haematological cells throughout pregnancy and in postnatal life. On microscopy, lymphocytes in the thymus and spleen appear to be one of the cell types to lose their nuclear basophilia very late in the autolysis process (>2 weeks). Therefore, it is not surprising that with prolonged retention times, these organs would yield better quality DNA. Whilst the fetal liver before 25 weeks is also rich in haemopoietic elements, in contrast to the thymus and spleen, hepatocyte nuclear basophilia starts to disappear as early as 24 hours after *in utero* death.[6] The presence of lytic enzymes in the liver is the likely cause of earlier degeneration seen in this organ.

CONCLUSION

Our data show that the placenta yields the highest quality DNA when compared with selected solid fetal organs sampled at perinatal autopsy. The quality of DNA extracted from solid fetal tissue deteriorates significantly with *in utero* retention time, while delivery to autopsy interval does not. Where fresh, refrigerated placenta is unavailable for sampling or there is suspicion of confined placental mosaicism, preferable alternative sources from which to sample for DNA extraction and subsequent molecular testing may be lymphocyte-rich, enzyme-poor fetal organs such as the thymus or spleen.

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