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Factors influencing RNA yield from placenta tissue

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ABSTRACT

High yield and integrity of placental RNA are crucial for placental transcriptomics studies. We assessed the effects of time to placental collection post-delivery; tissue storage, amount and method used for extraction; mode of delivery; and tissue type on total RNA yield.

The optimal protocol for RNA extraction from placental tissue includes cryofreezing of the sample upon collection and RNA extraction from 50 mg of tissue using *TRI*zol reagent. Decidua yielded highest RNA quantity/mg of tissue, followed by villous tissue and the chorion. Comparisons with murine kidney and HEK293T show lower placental RNA yield, likely due to highly dense and heterogeneous tissue make-up and potential high placental nuclease activity.

1. Introduction

Understanding the placental transcriptome is invaluable for placental and pregnancy health research. However, placental transcriptomics is plagued with issues related to the integrity and quantity of RNA extracted from primary tissue. Access to patient samples is fraught with difficulty, as priority is understandably given to the wellbeing of the mother and baby.

RNA stability is thought to decrease with time outside the body. High levels of RNase activity in the placenta accelerate RNA degradation at temperatures above 4 °C [1]. Furthermore, labour introduces intermittent myometrial hypoxia which causes low placental pH [2], while storage at 4 °C fails to prevent RNA degradation by lysosomal acid RNases [3]. Trauma to placental tissue, from delivery and dissection, reduces RNA yield suggesting lysosomal RNAse activation by tissue disruption [4]. Upon delivery of the placenta, immediate appropriate storage is required to maintain placental RNA integrity but is rarely practical. Importantly, RNA degradation depends more on tissue handling than time delay to storage [5].

Research indicates placental tissue should be stored as soon as possible after collection, either in excess RNA*later* [6,7] or by cryofreezing (>15 min in liquid nitrogen) [8,9]. However, there is disagreement on which of these storage options is best. Here we compared RNA yields from term placental chorionic villous tissue by time post-delivery before dissection, method of tissue preservation and extraction method.

2. Methods

2.1. Placenta tissue samples

Term human placentae were obtained with consent from Lyell McEwin Hospital, South Australia following delivery from uncomplicated pregnancies stratified by unassisted vaginal delivery (UV) or intervention (emergency and prelabour Caesarean sections). Placental biopsies (chorionic villi, chorion, decidual tissue) were washed in phosphate-buffered saline before immersion in *RNAlater* Stabilisation Solution (Thermo Fisher Scientific, Massachusetts, USA) or cryofrozen in liquid nitrogen for 15 min. Samples were then stored at −80 °C. Ethics approvals were obtained from The Queen Elizabeth Hospital Human Research Ethics Committee (TQEH/LMH HREC/1712/5/2008; SCOPE) and Women's and Children's Health Network Human Research Ethics Committee (HREC/14/WCHN/90; STOP).

Notably, the instructions for use of *RNAlater* solution were inconsistent. We followed instructions on page 6 of the protocol [12] and therefore stored our tissue in *RNAlater* solution at −80 °C. However, page 5 of the protocol indicates that *RNAlater* solution should be removed prior to freezing to expedite sample thawing. We therefore

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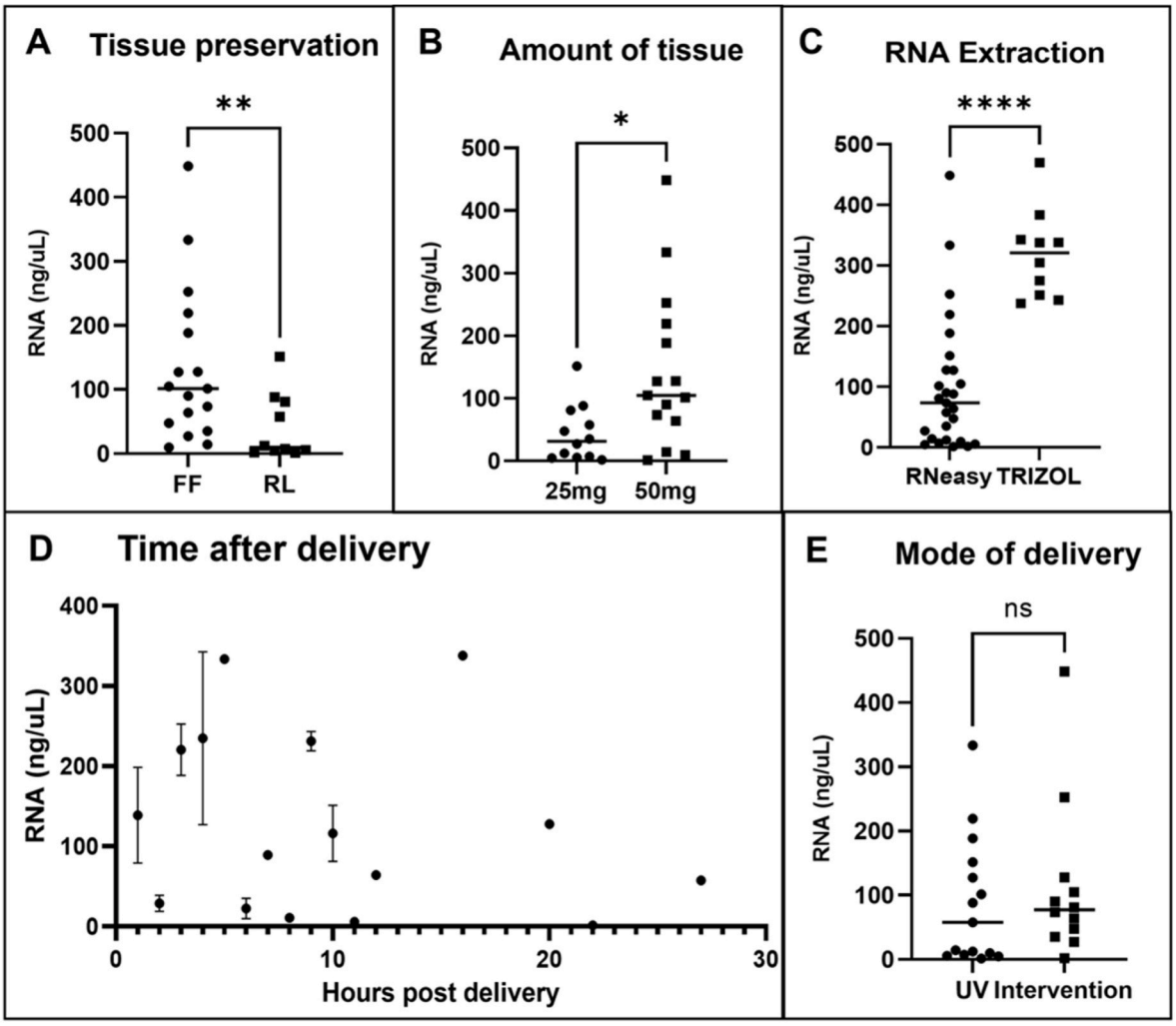

Fig. 1. RNA yields (ng/μL) from placentae stratified by **(A)** tissue preservation method – cryofrozen (FF; n = 17) or *RNAlater* (RL; n = 10) (all extracted using *TRIzol*); **(B)** amount of cryofrozen tissue used for RNA extraction – 25 mg (n = 12) or 50 mg (n = 15) (all extracted using *TRIzol*); **(C)** RNA extraction method for cryofrozen tissue – *RNeasy Plus Mini Kit* (n = 27) or *TRIzol* (n = 10); **(D)** time after delivery (hours) (n = 15; cryofrozen tissue; *TRIzol*); **(E)** mode of delivery – unassisted vaginal (UV; n = 15) or intervention (n = 12; cryofrozen tissue, *TRIzol*). Data are presented as scatter plots with a line representing the median. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

make no inference as to the efficacy of storage at −80 °C with or without excess *RNAlater*.

2.2. Mouse tissue samples

18-week-old C57/Bl/6J female mice (n = 4; Laboratory Animal Services, University of Adelaide) were humanely euthanised according to Wilson, 2017 [10]. The right kidney was cryofrozen in liquid nitrogen for 15 min immediately post-mortem prior to storage at −80 °C. Animal use complied with the Australian Code of Practice for the Care and Use of Animals. Ethics approval was obtained from the University of Adelaide Ethics Committee (M-2014-84).

2.3. HEK293T cells

HEK293T cells (#CRL-3216™) were obtained from ATCC® and cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (37 °C, 5% CO_2). One million cells (n = 4) were harvested at passage 12 and cryofrozen in liquid nitrogen for 15 min prior to storage at −80 °C.

2.4. RNA extraction

When removed from *RNAlater* after −80 °C storage, tissues were thawed on ice and dabbed to remove liquid prior to weighing. When cryofrozen tissues were removed from −80 °C storage, tissues were weighed and placed on ice. 1 mL *TRIzol* was then added prior to thawing.

RNA was extracted from term chorionic villi (25 mg or 50 mg) using either the RNeasy Plus Mini Kit (#74134; QIAGEN) or *TRIzol* Reagent (Invitrogen). The former was used to extract RNA from term chorion and decidua (25 mg). Murine kidney (25 mg) and HEK293T cells (1 million cells) were extracted using the RNeasy Kit.

RNeasy Kit: Tissue was homogenized (3.5 min, 30 Hz (TissueLyser, QIAGEN)) in 600 μL Buffer RLT Plus. Total RNA was extracted from the supernatant according to manufacturer's instructions.

TRIzol: Tissue was homogenized (3.5 min, 30 Hz (TissueLyser)) in 1 mL *TRIzol*. Total RNA was extracted according to Rio, 2010 [11]. DNase I (QIAGEN) treatment was performed on all samples.

All extracted RNA was dissolved in a final volume of 30 μL H_2O. RNA purity and integrity were determined using the Experion™ (BioRad) with RQI >9.

Time after delivery was calculated as time between birth to retrieval of

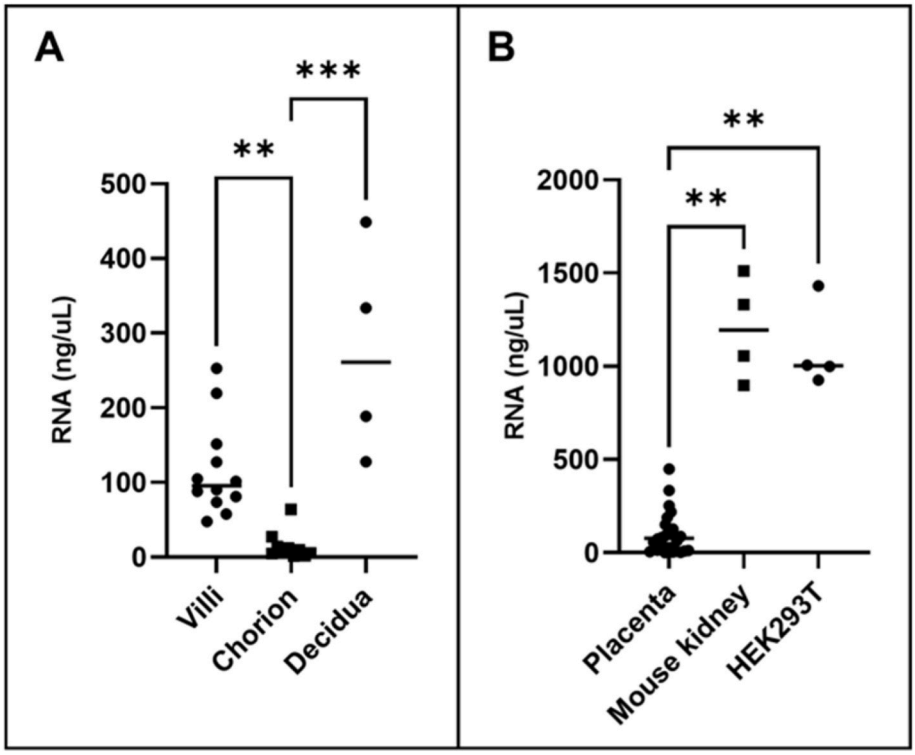

Fig. 2. RNA yields (ng/μL) from **(A)** placental villi (n = 12), chorion (n = 10) or decidua (n = 4), and **(B)** human placental tissue (n = 26), mouse kidney tissue (n = 4) or HEK293T cells (~1 million cells; n = 4). All data are from cryofrozen samples extracted using *TRIzol*. Data are presented as scatter plots with medians. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

the placenta by laboratory staff (range 1–27 h; stored at 4 °C).

2.5. Statistical analysis

Statistical analyses were performed using SPSS Statistics Software (IBM). Data were assessed for normal distribution. Differences between two groups were assessed using Mann-Whitney test and between three groups using Kruskal-Wallis multiple comparisons test. Differences between groups were considered significant at $p \leq 0.05$.

3. Results and discussion

The factors that influenced RNA yield were method of tissue preservation, amount of tissue used for extraction and method of RNA extraction. Cryofreezing tissue in liquid nitrogen for 15 min upon collection, significantly increased RNA yield compared with immersion in *RNAlater* ($p = 0.0067$; Fig. 1A). 50 mg of starting tissue yielded significantly more RNA than 25 mg starting tissue ($p = 0.0102$; Fig. 1B). RNA yield was ~3-fold higher using *TRIzol* reagent than the RNeasy Kit ($p < 0.0001$; Fig. 1C). Time after delivery before tissue collection (Fig. 1D), and mode of delivery (Fig. 1E), did not affect RNA yield.

Types of tissue were also assessed for their intrinsic ease of RNA extraction. RNA yield changed dependent on the type of starting tissue. RNA yields from chorion were significantly lower than those from villous tissue ($p = 0.0022$) and decidua ($p = 0.0003$; Fig. 2A) which likely reflects structure and function.

Furthermore, RNA yield from human placental tissues were significantly lower than those from mouse kidney ($p = 0.0032$) and HEK293T cells ($p = 0.0061$; Fig. 2B) supporting the notion that high endogenous RNase level in placental tissue may impair RNA yield.

It should be noted that whilst changes in the type of tissue, storage and extraction resulted in varied RNA yields, the RNA quality of all samples included in analysis remained high. As determined by the Experion™, all RNA had an RQI>9. This indicates that RNA quality did not influence analysis in this study. RNA quality should be prioritised over RNA yield to ensure precise measurements of placental RNA expression profiles.

In summary, placental RNA quantity is affected by method of tissue preservation, amount of starting tissue and RNA extraction method. We suggest the optimal protocol include cryofreezing tissue in liquid nitrogen for 15 min upon collection and RNA extraction from 50 mg tissue with *TRIzol* reagent. Surprisingly, time to collection after delivery did not affect RNA yield. Yield was highest from decidua tissue, followed by chorionic villi. Finally, it appears likely that the high nuclease content of placenta affects RNA yield, as shown by the comparatively high yields from murine kidney and HEK293T cells.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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