

The factors affecting RNA quality in human placenta tissue samples

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Introduction

Early postnatal adaptation relies on adequate content and functional capacity of respiratory chain complexes, which may play an important role in the morbidity of newborns, especially in very premature neonates. Human placenta represents easily obtainable source of human fetal tissue. The aim of the present study is to determine appropriate options for storage and handling of placenta tissue samples with a view to quantification of mRNA expression by qRT-PCR. The most important requirement for successful qRT-PCR is equal RNA quality in all of samples.

Tab.1 – RNA samples of human term placenta and the RNA sample of rat heart.

blue color marks the normal APGAR samples and yellow color marks the low APGAR samples (3-7 points for the first APGAR interval), the green numbers show different values of rRNA ratio between Experion (E) and Agilent (A). No.10-19 are samples from Caesarean sections and No.10-20 are samples from spontaneous childbearings. RIN – RNA integrity number

Number of sample	RNA quantity (ng/ul)	RNA purity	tissue weight (mg)	RIN	E/A
1	4290	1,62	590	5,3	0,6/0,7
2	4472	1,92	440	4,8	0,6/0,6
3	4411	1,52	830	5,2	0,7/0,7
4	4464	1,98	680	5,4	0,6/0,6
5	655	1,96	360	6,9	1,0/1,0
6	2105	1,98	100	4,1	0,9/0,6
7	4155	1,72	470	4,7	0,8/0,7
8	4286	1,63	550	4,9	0,6/0,8
9	3407	1,92	430	5,7	0,8/0,8
10	4401	1,52	300	N/A	1,0/1,0
11	4223	1,70	270	5,7	0,8/0,8
12	2400	1,97	290	6,3	1,0/1,0
13	453	1,88	360	7,2	1,2/1,1
14	1655	1,97	190	6,3	1,0/0,7
15	775	1,93	470	5,4	1,2/0,9
16	2958	1,95	210	4,9	0,5/0,5
17	1819	1,97	300	5,9	0,8/1,0
18	3518	1,88	470	6,4	0,9/0,9
19	3814	1,83	270	3,0	0,6/0,4
20	2204	1,99	160	3,8	0,5/0,5
rat, heart	2203	2,00	240	9,3	1,2/1,7

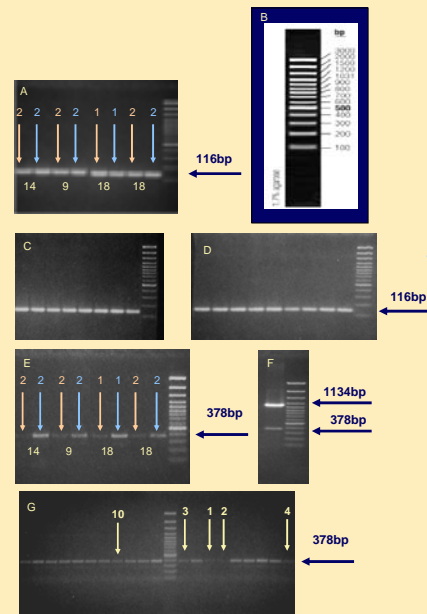


Figure 3 – electrophoresis

A – the samples (No.9,14,18) of the first and the second intervals (blue arrows for 0°C and orange arrows for 24°C, the numbers above arrows mark intervals), amplification of ATP synthase fragment 116bp; B – DNA ladder GeneRuler™ 100bp DNA Ladder Plus (Fermentas); C, D – samples No.1-20, amplification of ATP-synthase fragment; E – the samples (No.9,14,18) of the first and the second intervals (blue arrows for 0°C and orange arrows for 24°C, the numbers above arrows mark intervals), amplification of MECP2 fragments (378bp and 1134bp); F – sample No.14 with added gDNA, amplification of MECP2 fragments; G – the samples No.1-20 (yellow arrows mark the low APGAR samples).

Abbreviations

APGAR – Activity, Pulse, Grimace, Appearance, Respiration. It is score for assessing the health of newborn infants.
COX5a – gene coding subunit Va of cytochrome c oxidase
MECP2 – gene coding methyl CpG binding protein 2
OXPHOS – oxidative phosphorylation
RIN – RNA integrity number

Tab. 2

The first interval (state after 1 hour), blue color marks 0°C and orange color marks 24°C, the red numbers show the low APGAR samples.

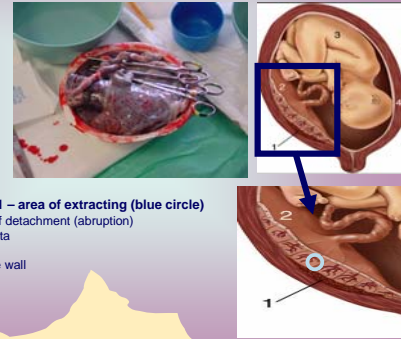
Number of sample	RNA quantity (ng/ul)	RNA purity	tissue weight (mg)	RIN
6	3137	1,96	250	N/A
6	2491	2,00	270	5
12	843	1,92	250	5,3
12	3649	1,89	300	5,8
2	2059	1,96	310	2,7
2	2960	1,92	450	4,9
18	3276	1,91	430	4,9
18	3373	1,89	400	6,7
4	3181	1,94	290	4,5
4	2360	1,98	310	4,4

Tab. 3

The second interval (state after 2 hours), blue color marks 0°C and orange color marks 24°C, the red numbers show the low APGAR samples.

Number of sample	RNA quantity (ng/ul)	RNA purity	tissue weight (mg)	RIN
6	3791	1,86	620	2,3
6	3791	1,87	450	2,9
12	2832	1,98	290	4,8
12	3517	1,92	330	5,8
2	2978	1,91	440	2,9
2	3755	1,84	390	3,3
18	1721	1,97	240	3,5
18	1474	1,97	320	6
9	2743	1,96	360	N/A
9	2532	1,97	280	5,8
4	3817	1,83	380	3,8
4	2619	1,97	250	5,4
14	1020	1,96	100	3,6
14	2518	1,97	230	6,6

Figure 1 – area of extracting (blue circle)



Tab. 4

The third interval (state after 10 hours), blue color marks 0°C and orange color marks 24°C, the red numbers show low APGAR samples.

Number of sample	RNA quantity (ng/ul)	RNA purity	tissue weight (mg)	RIN
6	1750	2,00	550	N/A
6	1228	2,00	360	2,7
12	443	1,93	230	2,3
12	1372	1,96	260	2,5
4	1858	1,82	230	2,5
4	3770	1,84	360	2,6

Methods

The tissue samples (fig.1 and tab.1) were frozen immediately after withdrawal of placenta and washing it by physiological buffer, some samples were frozen according to the time-plan for time-course study (the part of them was kept in 0°C and the other part was kept in 24°C, tab.2,3,4). For RNA isolation was used TRIZOL® Reagent (GIBCO BRL). RNA concentration was measured by instrument NanoDrop ND – 1000 (NanoDrop Technologies) and analysis of RNA quality was checked by two instruments – Experion (Bio-Rad Laboratories) and Agilent 2100 Bioanalyzer (Agilent Technologies) (fig.2). For reverse transcription (SuperScript II, Invitrogen) was used 2000ng of RNA and no-reverse transcription control for all samples. cDNA was analyzed by amplification of two products (fig.3). MECP2 fragment – 378bp which involves the exon-exon boundaries (in case of gDNA contamination there was found the second product – 1134bp, it includes intron, fig.3,F) and ATP-synthase fragment – 116bp with exon-exon boundaries, analysis was provided by electrophoresis. Two products – ATP-synthase fragment – 116bp and COX5a fragment – 116bp were amplified in the runs of pre-analytical quantitative real-time RT-PCR (fig.4). The average C(t)s were determined using DyNAmo™ SYBR® Green qPCR Kit (Finnzymes) on the Opticon 2 System (Bio-Rad Laboratories) (tab.5).

Tab. 5 – Real-time RT-PCR,

average C(t)s of the first interval samples No.6,12,18, the second interval samples No.6,12,18, the low APGAR samples No.1,2,4,10 and the normal APGAR samples No.9,14,17,18; orange color marks 24°C and blue color marks 0°C.

	The first interval	The second interval	Low APGAR samples	Normal APGAR samples
	C(t) avg	C(t) avg	C(t) avg	C(t) avg
COX5a	22,83	23,09	22,33	21,59
ATP-synthase	21,39	21,68	20,80	20,08
	20,23	20,48		

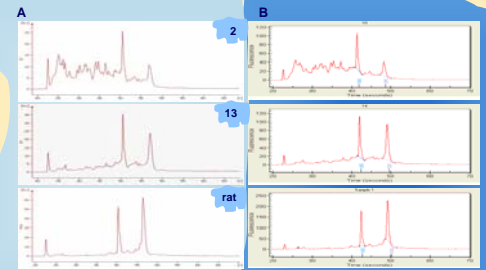


Figure 2 – rRNA peaks

A – graphs of samples No. 2, 13 and rat heart on Agilent instrument; B – graphs of the same samples on Experion instrument.

Results

Nanodrop – RNA purity (protein contamination) and quantity
No correlations were observed in RNA quantity or RNA purity among the placenta tissue samples which were taken after birth of newborns with low Apgar score (group: low APGAR samples), after Caesarean sections (group Caesarean section) and after spontaneous delivery of neonates with normal Apgar score (group: normal APGAR samples). The purity of RNA samples was in range 1,6 – 2, but the purity of the most of samples were around 1,9. In the time course study no connectivity were found between temperature in which the placenta tissue samples were kept and RNA quantity or RNA purity.

Experion and Agilent 2100 Bioanalyzer – RNA quality
The average RIN (RNA integrity number) of 20 samples was 5,4 and RINs of intervals were 5,5/4,4 (first interval 0°C/24°C); 5,0/3,5 (second interval 0°C/24°C); 2,6/2,4 (third interval 0°C/24°C) (tab.1,2,3,4). In rRNA ratio measurements of 10 samples on two various instruments were found differences (tab.1). Despite of fact that the sample of RNA from rat heart was prepared by the same way as the RNA samples from placenta tissue its RIN and rRNA ratio are much better (tab.1 and fig.2).

Electrophoresis – RNA purity (gDNA contamination)
All of samples were found without gDNA contamination, only shorter MECP2 fragments were amplified (fig.3,G). In amplification of MECP2 fragments, significant differences were found between temperatures 0°C and 24°C (fig.3,E). The similar results were observed between the low APGAR samples and the normal APGAR samples (fig.3,G).

In amplification of ATP-synthase, no changes or differences were found in temperatures or low APGAR samples (fig.3,A,C,D).
Real-time PCR – (pre-analytical screening of RNA)
The fragments (ATP-synthase and Cox5a) were amplified in chosen samples and the divergences in C(t)s were found between samples of two different temperatures and similarly between the low APGAR samples and the normal APGAR samples (tab.5).

Conclusion

According to our results, the important factors for storage, handling and acquirement of the best RNA quality of human placenta tissue samples are process of childbirth (Caesarean section or spontaneous delivery, APGAR score (mostly hypoxaemia of newborns with low APGAR score), temperature and time period before freezing of samples.