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Comparative Analysis of RNA Extraction Quality Using TRIzol-Based and Non-TRIzol Methods in Endometriosis Lesion Tissue

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Abstract: Ribonucleic acid (RNA) is a crucial molecule in gene expression analysis, yet its isolation from solid tissues, such as endometriotic lesions, poses significant challenges due to complex tissue structure and high RNase activity. TRIzol is a widely utilized reagent for RNA extraction, known for its efficacy in lysing cells and stabilizing RNA. This study aimed to compare the quality of RNA extracts from endometriotic lesion tissue samples using TRIzol-based and non-TRIzol-based methods. A quasi-experimental design was employed, involving a control group and an experimental group, with non-randomized sample allocation. A total of 28 samples were analyzed in both the control and treatment groups. The results demonstrated that TRIzol treatment significantly increased the median RNA concentration from endometriotic lesion tissues, yielding 288.6 ng/µL compared to 118.2 ng/µL in the non-TRIzol group (p=0.001). Furthermore, there was a significant difference between TRIzol and non-TRIzol groups in RNA purity based on the Å260/280 ratio (median: 2.00; p-value:0.024). However, there wasn't a significant difference in the Å260/230 ratio (median: 2.11; p-value: 0.247). In conclusion, these findings indicate that while TRIzol extraction provides higher RNA yield, the non-TRIzol method ensures slightly superior sample purity, supporting its reliability for downstream transcriptomic analyses.
Keywords: Endometriosis; RNA extraction; RNA quality; tissue homogenization; TRIzol.

INTRODUCTION

Endometriosis is often found in the pelvic peritoneum but can also occur in other sites, such as the ovaries and uterosacral ligaments. The incidence of endometriosis is difficult to quantify due to its often-asymptomatic symptoms and the low sensitivity of diagnostic examinations. Women with endometriosis may present without symptoms, subfertility, or pelvic pain, especially during menstruation (Imanaka et al., 2020; Ocktariyana, 2020; Taylor et al., 2018). Endometriosis affects 10-15% of all women of childbearing age and 70% of women with chronic pelvic pain (Bulun, 2009). Unfortunately, many of these women often experience diagnostic delays for endometriosis, averaging 6.7 years for patients aged 18-45, leading to suffering and reduced quality of life (Nnoaham et al., 2011; Suwartono et al., 2022).

Endometriosis lesions are tissues lining the uterus (glands and endometrial stroma) outside the uterine cavity. Endometriosis lesions form if the endometrium

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attaches to the peritoneal membrane. Endometriosis lesions express integrin and cadherin proteins that may contribute to their development (Laganà et al., 2019). Retrograde menstruation facilitates the extrauterine seeding of endometrial cells onto the peritoneum, initiating endometriosis. Consequently, mitigating estrogen production is a vital therapeutic mandate for halting lesion progression and mitigating patient symptomatology (Becker et al., 2022; Taylor et al., 2017).

Molecular studies of endometriosis rely heavily on the availability of high-quality RNA for downstream applications such as quantitative Polymerase Chain Reaction (qPCR), sequencing, and gene expression analysis. However, RNA is inherently unstable and highly susceptible to degradation by ribonucleases (RNases), which can compromise RNA integrity and affect the accuracy of molecular analyses (Liu et al., 2014; McDowall, 2007). Therefore, effective RNA extraction methods are essential to obtain intact and pure RNA suitable for reliable molecular investigations. In addition to RNase activity, the type and complexity of tissue specimens also influence RNA yield and quality (Pagani et al., 2023). Consequently, optimization of RNA extraction protocols is particularly important for complex pathological tissues such as endometriotic lesions.

In therapeutic contexts, RNase activity poses challenges for RNA-based treatments, such as small interfering RNAs (siRNAs. Strategies to enhance RNA stability, such as chemical modifications or protective complexes (e.g., nanoparticles), are being explored to mitigate RNase-mediated degradation (Haupenthal et al., 2006; Mohanty et al., 2025). Nucleic acid extraction is a critical foundational step in molecular biology, enabling downstream applications such as infectious disease diagnostics, genetic research, and forensic analysis. The process involves isolating DNA or RNA from biological samples and removing impurities such as proteins, salts, and other inhibitors to ensure high-quality nucleic acids for subsequent analyses (Chen et al., 2012; Obino et al., 2021; Widen & Silbert, 2016).

Common extraction methods include cesium chloride (CsCl) density gradient centrifugation, phenol-chloroform extraction, solid-phase extraction, and magnetic bead-based methods. While the CsCl method yields high-purity DNA, it is limited by the need for expensive ultracentrifuges and the use of hazardous chemicals like ethidium bromide. Phenol-chloroform extraction, a phase-separation technique, is relatively simple but involves toxic, corrosive chemicals. Solid-phase extraction, utilizing silica particles or similar matrices, offers advantages in safety, ease of use, and reduced contamination/DNA loss. Magnetic bead-based methods, which bind nucleic acids to magnetic particles, are increasingly favored for their efficiency in eliminating the need for repetitive centrifugation and organic solvents (Hwan Shin, 2018). RNA extraction, a crucial preliminary step in molecular biology, is vital for advanced applications like quantitative PCR (qPCR), sequencing, and gene expression analysis. This process involves isolating RNA from cells or tissues while maintaining its integrity and purity, a critical step given RNA's vulnerability to RNase-mediated degradation. Although RNA-based research drives important biological discoveries and therapeutic advances, the inherent instability of RNA makes high-throughput isolation and purification critical to maintaining its chemical and functional integrity (Martins et al., 2014).

RNA can be extracted from various biological sources, including tissues and biological fluids, including eutopic and ectopic endometrial tissues, which are crucial for gene expression studies in endometriosis (Hongjaisee et al., 2022; Nouvel et al., 123 C.E.; Nur & Yamamoto, 2022; Yin et al., 2022).

RNA extraction from endometriotic lesion tissue remains technically challenging because the tissue contains complex cellular structures and abundant RNase activity that may accelerate RNA degradation. Several nucleic acid extraction methods have been developed, including phenol-chloroform extraction, solid-phase extraction, and magnetic bead-based techniques (Hwan Shin, 2018). Among these methods, TRIzol reagent is widely used for RNA isolation from cells and tissues because it combines phenol and guanidinium isothiocyanate to simultaneously lyse cells, denature proteins, and inhibit RNase activity, thereby preserving RNA integrity during extraction (Lakhotia & Ranganath, 2024; Zhang et al., 2023).

TRIzol Reagent is a ready-to-use reagent for RNA isolation from cells and tissues (Zhang et al., 2023). TRIzol, a monophasic solution of phenol and guanidinium isothiocyanate, effectively isolates RNA from cells and tissues by simultaneously dissolving biological material, denaturing proteins, and inhibiting RNase activity, thereby preserving RNA integrity (Lakhotia & Ranganath, 2024). Although this method enhances molecular research applications by effectively extracting various RNA molecules, common challenges persist, including DNA contamination that compromises RNA purity and integrity and difficulties in dissolving RNA pellets.(Bhat & Hakeem, 2023; Rolfs et al., 2023) Furthermore, TRIzol maintains RNA integrity by robustly inhibiting RNase activity during tissue homogenization while simultaneously lysing cells and disrupting cellular components (Fisher Scientific, 2025; Gautam, 2022). Ensuring high purity and integrity of isolated RNA is critical for accurate quantification and downstream PCR applications, as low quality, contamination (e.g., proteins, genomic DNA), and degradation can lead to variable or false quantification results (Hasegawa et al., 2021).

Although TRIzol is widely used for RNA isolation, evidence regarding its effectiveness in endometriotic lesion tissue remains limited due to the fibrotic and heterogeneous nature of these lesions. Therefore, evaluating RNA yield and purity in this tissue type is essential for molecular studies. Therefore, this study aims to analyze the quality of RNA extracts from endometriosis lesion tissue specimens with and without TRIzol treatment. This research is expected to significantly contribute to optimizing RNA extraction methods for molecular studies of endometriosis.

MATERIALS AND METHODS

This study was an unpaired, quasi-experimental study. Endometriosis lesion tissue was collected from each patient undergoing endometriosis laparoscopy at Fatmawati Hospital and Muhammad Hoesin Hospital. A total of 56 endometriosis lesion tissue samples were included in this study and divided into two sampling groups: those treated with TRIzol and those not treated with TRIzol, with 28 samples in each group. Tissue samples were obtained from women of reproductive age who provided informed consent, had a confirmed diagnosis of endometriosis, and were classified as stage III–IV according to the American Society for Reproductive Medicine classification (ASRM).

Fifty mg of endometriosis lesions obtained at Fatmawati Hospital were taken to the Integrated Laboratory of the Faculty of Medicine, University of Indonesia, and specimens from Muhammad Hoesin Hospital, Palembang, were taken to the Biotek Laboratory for immediate RNA extraction and analysis using Nanodrop. The study protocol was reviewed and approved by the Health Research Ethics Committee of Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia (Ref. No: DP.04.03/D.XVIII.6.11/ETIK/94/2023 and No: DP.04.03/D.XVIII.6.11/ETIK/94/2024).

All procedures were performed in accordance with the ethical standards of the Declaration of Helsinki.

Tools and Material

The commercial RNA extraction kits utilized in this study were the Quick-RNA Miniprep Kit and TRIzol from the Direct-Zol RNA Miniprep Plus Zymo® kit. Key reagents for these procedures included RNA Lysis Buffer, RNA Prep Buffer, RNA Wash Buffer, lyophilized DNase I, DNA Digestion Buffer, DNase/RNase-Free Water, Ethanol, TRI Reagent, DNA/RNA Shield, Proteinase K, and Direct-zol RNA Prewash. The primary instrument for assessing RNA quality was a NanoDrop spectrophotometer, which measured RNA concentration in nanograms per microliter (ng/µL) and purity using the A260/A280 absorbance ratio. Supporting laboratory equipment included microcentrifuge tubes, microliter pipettes equipped with RNase-free tips, a centrifuge, a vortex mixer, and an incubator. All instruments were chosen in accordance with standard laboratory protocols to ensure the accuracy of the research findings.

RNA Extract Procedure

Endometriosis lesion tissue obtained during laparoscopy was immediately stored in 1.5 mL tubes containing 500 mL of DNA/RNA Shield R1100-50 stabilization buffer (ZYMO). DNA/RNA Shield reagent is a DNA and RNA stabilization solution for nucleic acids in any biological sample. Based on the kit protocol, approximately ≤25 mg of high-yield tissue or ≤50 mg of low-yield endometriosis lesion tissue was weighed for the non-TRIzol group, while 10−50 mg was used for the TRIzol group. However, after optimisation in the actual procedure, the homogenized tissue weight was 50 mg in each sample. Tissue homogenization was performed with either direct RNA Lysis or TRI Reagent® groups, using a mortar or a bead beater/homogenizer, respectively. The tissue was homogenized using a digital ULTRA-TURRAX® until it disintegrated in approximately 1–2 minutes. Cell lysis involved mechanical homogenization followed by specific buffer treatments; an enzymatic stage with Proteinase K was included for the TRIzol group, which was incubated at 37∘C for 30 minutes.

RNA extraction was then performed using the Spin Column method following the kit instructions. The TRIzol group used the Direct-zol RNA Miniprep Plus R2071 kit, and the non-TRIzol group used the Quick-RNA Miniprep Plus Kit R1058 (ZYMO). Both kits involved centrifugation stages, with speeds ranging from 10,000−16,000×g for initial separation and column binding and 16,000×g for final elution steps. DNase treatment was incorporated into both protocols to degrade residual genomic DNA. The purification stage followed each kit's specific protocol, involving sequential washes with dedicated buffers.

Immediately after obtaining the RNA extract, RNA concentration was measured using a Nanodrop spectrophotometer and expressed in ng/µL; the A260/A280 absorbance ratio was assessed to determine RNA purity using a Thermo Scientific™ NanoDrop Ultra Microvolume UV-Vis instrument to measure purity and concentration. The RNA was then stored at -80°C for extended periods.

Data Analysis

The Mann-Whitney U test was applied due to the non-normal distribution of RNA concentration, while RNA purity differences were assessed using the Mann-Whitney test. All statistical tests were performed with a 95% confidence level (α=0.05), and conclusions were drawn based on the p-value.

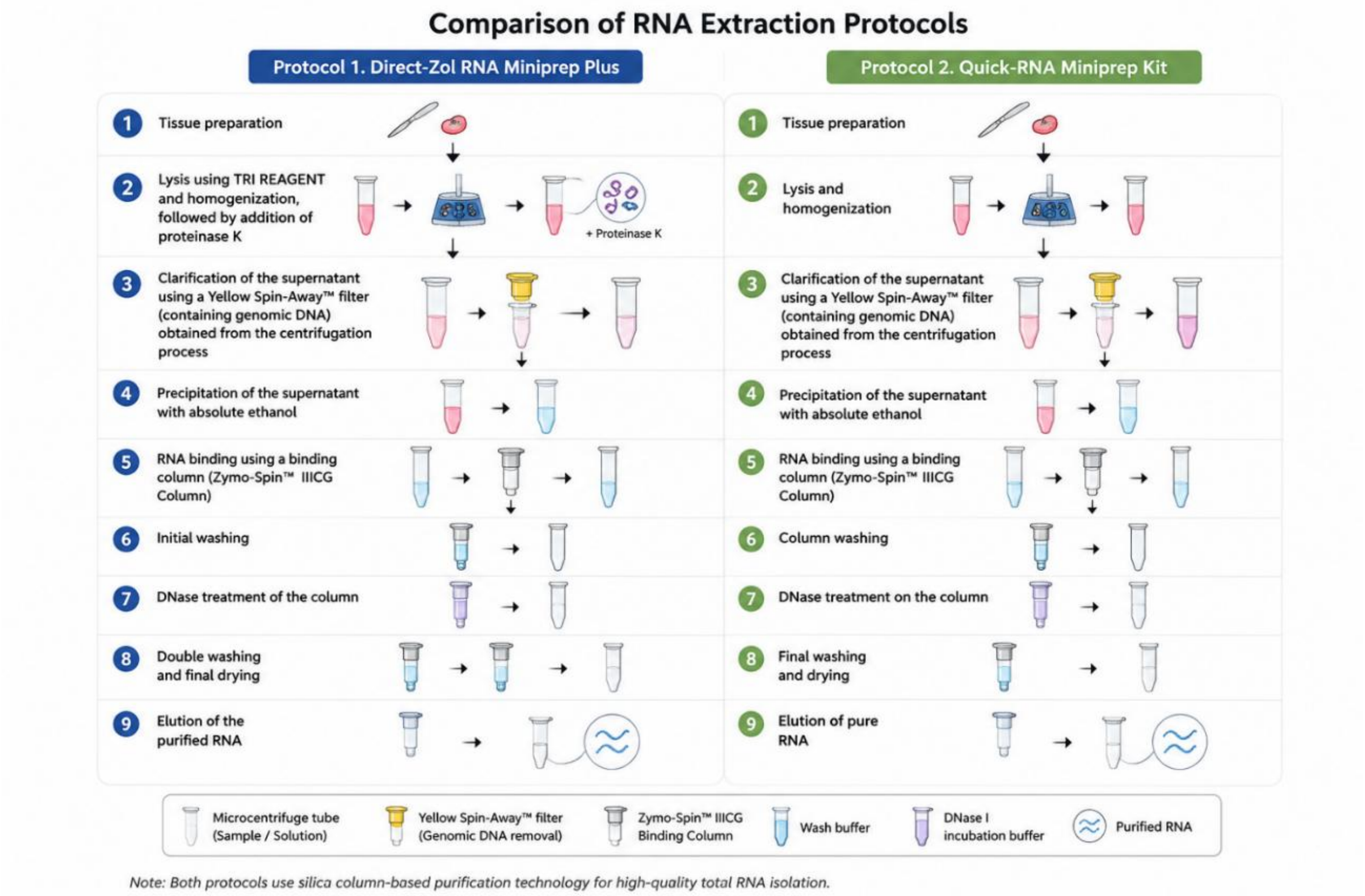

Figure 1. Protocol Flow between TRIZol and Non-TRIZol Groups.

RESULTS AND DISCUSSION

Characteristics of the Sample

Based on the basic characteristics of the patient, including age, body mass index (BMI), number of children (parity), and the level of pain between the two groups, are generally homogeneously distributed (equivalent) (Table 1).

The demographic and clinical characteristics of patients analyzed in this study revealed comparable patterns between the TRIzol and non-TRIzol extraction groups, with minor variations in age, body mass index (BMI), and reproductive history. The mean age of participants in the TRIzol group was slightly younger (33.57 ± 7.20 years) than that in the non-TRIzol group (35.6 ± 4.87 years). BMI distribution showed fewer underweight cases in the TRIzol group (3.6%, n = 1) compared to the non-TRIzol group (10.7%, n = 3), while normal-weight individuals were more prevalent in TRIzol (53.6%, n = 15) than in non-TRIzol (35.7%, n = 10). Overweight and obese categories were relatively similar across both groups, with obesity rates of 28.6% (n = 8) and 31.1% (n = 9) for TRIzol and non-TRIzol, respectively.

From an obstetric perspective, primary infertility was the most frequent condition in both groups—60.7% (n = 17) in TRIzol and 64.3% (n = 18) in non-TRIzol. Unmarried patients were slightly more common in the TRIzol group (10.7%, n = 3) than in non-TRIzol (3.6%, n = 1). Parity analysis indicated that parity 1 occurred in 21.4% (n = 6) of TRIzol cases and 28.6% (n = 8) of non-TRIzol cases, whereas parity > 2 was the least frequent (TRIzol 7.1%, n = 2; non-TRIzol 3.6%, n = 1).

Clinically, pelvic pain and dysmenorrhea were the predominant symptoms, slightly higher in the non-TRIzol group (60.7%) than in TRIzol (53.6%). Dysuria was more frequent in TRIzol (17.9%), while dyspareunia and dyskezea showed identical distributions (17.9% and 10.7%, respectively). Molecular analysis demonstrated that the TRIzol method yielded a significantly higher mean RNA concentration (333.56 ± 288.6 ng/µl) compared to non-TRIzol (150.84 ± 104.5 ng/µl). The mean Å260/280 ratios for TRIzol (1.99 ± 0.07) and non-TRIzol (2.01 ± 0.18) were within the optimal range (~2.0), indicating comparable RNA purity. However, categorical assessment of protein contamination revealed a higher proportion of pure samples in the non-TRIzol group (60.7%) than in TRIzol (50.0%). Organic purity, evaluated through Å260/230 ratios, was optimal in both groups—TRIzol 2.16 ± 0.16 and non-TRIzol 2.09 ± 0.12—with all samples classified as pure.

Table 1. Comparison of Baseline Characteristics and RNA Quality Between TRIzol and Non-TRIzol Groups

Characteristics	TRIzol group (n=28)		Non-TRIzol group (n=28)	
	f	%	f	%
Age (mean±SD)	33,57±7,20		35,6±4,87	
Body mass index				
Underweight	1	3.6	3	10.7
Normoweight	15	53.6	10	35.7
Overweight	4	14.3	6	21.4
Obese	8	28.6	9	31.1
Parity				
Unmarried	3	10.7	1	3.6
Primary infertility	17	60.7	18	64.3
1	6	21.4	8	28.6
≥ 2	2	7.1	1	3.6
Pain level				
Mild- (≤5)	9	32.1	7	25.0
Severe (>6)	19	67.9	21	75.0
Type of pelvic pain				
Dyspareunia	5	17.9	5	17.9
Dysmenorrhea	15	53.6	17	60.7
Dysuria	5	17.9	3	10.7
Dyskezea	3	10.7	3	10.7
Concentration (Mean±SD) ng/µl	333.56		150.84	
RNA Purity rasio Å260/280 (mean±SD)	1.99±0.07		2.01±0.18	
Pure	14	50	17	60.7
Impure	14	50	11	39.3
RNA Purity ratio Å 260/230 (mean±SD)	2.16±0.16		2.09±0.12	
Pure	28	100	28	100
Impure	0	0	0	0

Comparison of RNA Concentration between TRIzol and Non-TRIzol Extraction Methods in Endometriosis Lesion Tissue Samples

These samples were either processed immediately after collection or stored in RNA Shield, thereby minimizing degradation before RNA extraction. The 56 samples were equally divided into two groups: 28 samples constituted the non-TRIzol group, for which RNA extraction was performed using the Quick-RNA Miniprep Kit. The remaining 28 samples comprised the TRIzol-treated group, utilizing the Direct-Zol RNA Miniprep Plus for RNA isolation. Efforts were made to maintain consistency in laboratory protocols, equipment, and researchers involved in the extraction process to minimize potential confounding variables. Normality tests were performed on the RNA concentration data, and both the Kolmogorov-Smirnov test (Sig: 0.0002) and the Shapiro-Wilk test (Sig: 0.000) indicated that the data (n=56) were not normally distributed, necessitating the use of nonparametric tests for intergroup comparisons.

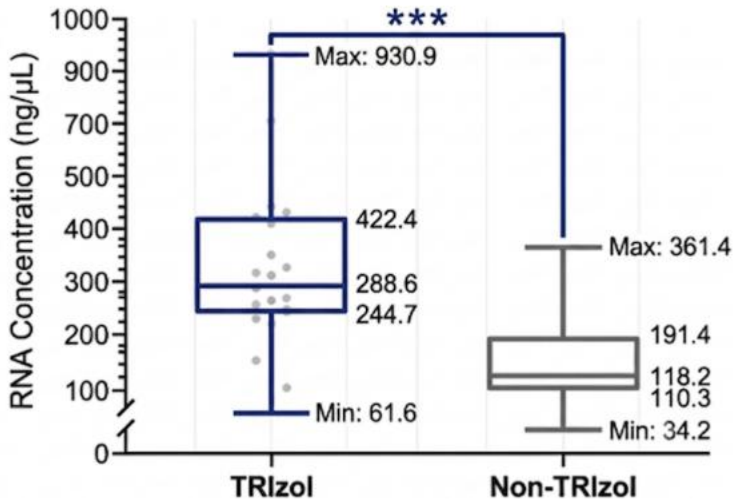

Figure 2. Comparison of RNA concentration between TRIzol and Non-TRIzol extraction methods in endometriosis lesion tissue samples (N = 56). Data are presented as median, 95% confidence interval (CI) with lower–upper range. Max: maximum value; Min: minimum value. ***: p-value = 0.001 (Mann-Whitney test).

The analysis of RNA concentration extracted from endometriosis lesion tissue samples showed a distinct variation between the two methods (Figure 2). The TRIzol extraction method yielded a substantially higher median RNA concentration of 288.6 ng/µL, with a 95% confidence interval (CI) ranging from 244.7 to 422.4 ng/µL. The total RNA yield within this group demonstrated a wide distribution, with a minimum value of 61.6 ng/µL and a maximum peak at 930.9 ng/µL. In contrast, the Non-TRIzol treatment resulted in a lower median RNA concentration of 118.2 ng/µL (95% CI: 110.3 – 191.4 ng/µL). The data distribution for the Non-TRIzol group was narrower, ranging from a minimum of 34.2 ng/µL to a maximum of 361.4 ng/µL (Figure 1). Based on the previous non-parametric comparative analysis (p = 0.001), this difference in concentration between the TRIzol and Non-TRIzol groups is statistically highly significant. These preliminary descriptive findings indicate that TRIzol effectively increases the amount of RNA extracted from endometriosis lesion tissue.

These findings indicate that TRIzol effectively increases RNA yield, producing RNA with sufficient purity and concentration for subsequent applications such as qPCR. TRIzol effectively extracted total RNA, including small RNAs, and yielded stable RNA for gene expression analysis, thereby supporting the concentration differences observed in this study (Masoomi-Aladizgeh et al., 2023; Roy et al., 2020).

Comparison of RNA Purity between TRIzol and Non-TRIzol Extraction Methods in Endometriosis Lesion Tissue Samples

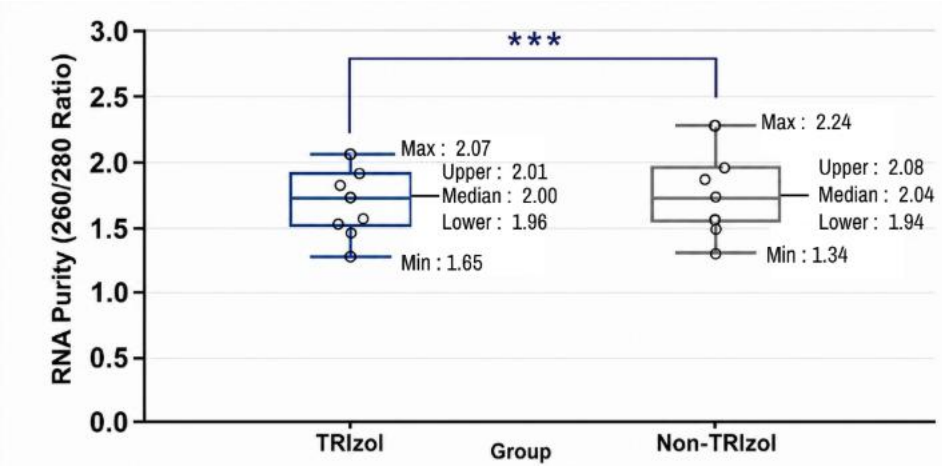

Figure 3. Comparison of RNA purity ratio Å260/280 in the TRIzol and non-TRIzol Treatment in Endometriosis Lesion Tissue Samples (N = 56). Data are presented as median, 95% confidence interval (CI) with lower–upper range. Max: maximum value; Min: minimum value. ***: p-value = 0.024 (Mann-Whitney test).

Analysis of RNA purity in endometriosis lesion tissue showed significant differences between TRIzol and non-TRIzol extraction methods. Based on the results of the Mann-Whitney test, the TRIzol group had a median Å260/280 ratio of 2.00 (range 1.65–2.07; 95% CI = 1.96–2.01), while the NonTRIzol group showed a median of 2.04 (range 1.34–2.24; 95% CI = 1.94–2.08). The value p = 0.024 indicates that the difference in RNA purity between the two methods is statistically significant.

This p-value is significantly smaller than the conventional significance threshold of $\alpha < 0.05$, leading to the rejection of the null hypothesis (H_0). Thus, it can be concluded that there is a significant difference in RNA concentration from endometriosis tissue extracted with TRIzol compared to that extracted without TRIzol. These findings indicate that TRIzol effectively increases RNA yield, producing RNA with sufficient purity and concentration for subsequent applications such as qPCR. TRIzol effectively extracted total RNA, including small RNAs, and yielded stable RNA for gene expression analysis, thereby supporting the concentration differences observed in this study (Hasegawa et al., 2021; Masoomi-Aladizgeh et al., 2023). Biologically, these results indicate that the TRIzol method produces RNA with a more consistent Å260/280 ratio and close to the ideal value of ≈ 2.0, reflecting a high level of purity against protein contamination. In contrast, the wider variation in the NonTRIzol group suggests the possibility of protein or phenol residues affecting RNA quality. These findings reinforce the recommendation for the use of TRIzol as a standard method for RNA isolation in endometriosis tissues, especially for downstream applications such as qPCR and transcriptomic profiling.

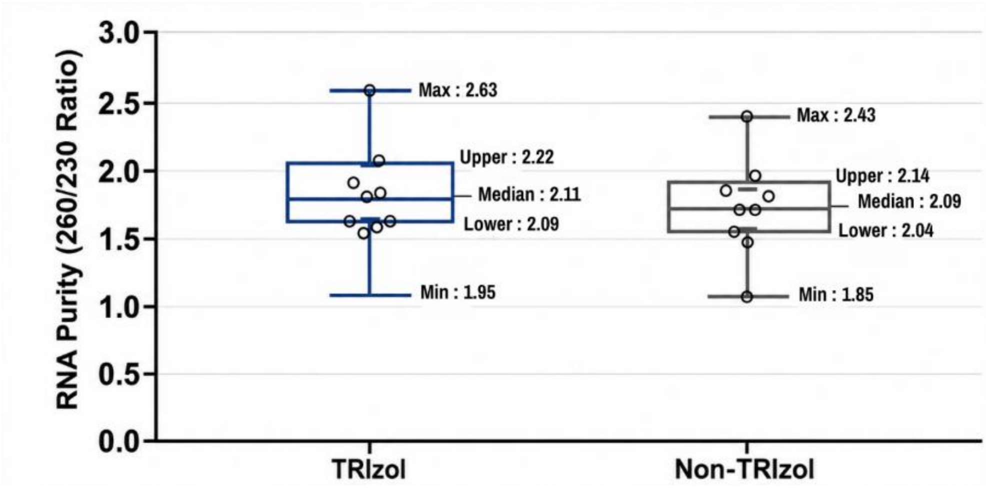

Figure 4. Comparison of RNA purity ratio Å260/230 in the TRIzol and non-TRIzol Treatment in Endometriosis Lesion Tissue Samples (N = 56). Data are presented as median, 95% confidence interval (CI) with lower–upper range. Max: maximum value; Min: minimum value. (Mann-Whitney test). P value > 0.05.

Analysis of RNA purity based on the Å260/230 ratio in endometriosis lesion tissue showed that both extraction methods, TRIzol and NonTRIzole, produced RNA with a high level of purity and were relatively uniform. The TRIzol group had a median of 2.11 (range 1.95–2.63; CI 95% = 2.09–2.22), while the NonTRIzol group showed a median of 2.09 (range 1.85–2.43; 95% CI = 2.04–2.14). The p=0.247 value of the Mann-Whitney test indicates that the difference between the two methods is not statistically significant.

Biologically, the Å260/230 ratio close to 2.0 in both groups indicates that the isolated RNA is free of phenol, carbohydrate, or guanidinium salt contamination that normally affects the purity of the RNA. The narrow range of values in the 95% confidence interval reinforces the consistency of RNA isolation results between samples. Thus, both the TRIzol and NonTRIzol methods can be used effectively for the isolation of RNA from endometriosis tissues, although TRIzol shows a slight advantage in the stability of the purity ratio.

Descriptively, the non-TRIzol group showed higher RNA purity. However, the statistical test for purity, namely the Pearson Chi-Square test, yielded a p-value of 0.093, which exceeds the significance threshold of $\alpha = 0.05$. Consequently, there is insufficient evidence to reject the null hypothesis (H_0), which indicates that there is no statistically significant difference in RNA purity between the two extraction methods. This finding aligns with studies showing that TRIzol extraction yields high-purity RNA, as evidenced by an average A260/A280 ratio of 2.0, indicating optimal purity and minimal contamination (Fisher Scientific, 2025; Gandhi et al., 2020; Hasegawa et al., 2021; Masoomi-Aladizgeh et al., 2023; Roy et al., 2020).

The significantly higher RNA concentration observed in the TRIzol group can be attributed to the mechanistic advantages of phenol-guanidinium isothiocyanate chemistry. This potent chaotropic agent simultaneously disrupts cellular membranes and denatures endogenous proteins, including robust ribonucleases (RNases) that would otherwise degrade RNA during extraction. This highly efficient tissue lysis ensures maximum release of nucleic acids from the cellular matrix, resulting in a superior RNA yield compared to standard detergent-based lysis methods (Non-Experts & 2022, 2022).

Guanidinium isothiocyanate (GuSCN) performs two crucial functions in a single step: lysing cells and viruses and instantly denaturing harmful proteins, including nucleases (DNase and RNase) that can degrade nucleic acids. It also acts as a binding promoter, creating a chemical environment that promotes nucleic acid binding to silica (Boom et al., 1990).

Conversely, while a statistically significant difference was observed in the Å260/280 ratio between groups, no significant difference was identified in the Å260/230 ratio. The relatively comparable RNA purity results may be attributed to the standardization of downstream purification steps, including DNase digestion and silica column-based cleanup systems. The silica membrane selectively binds nucleic acids under high-salt conditions while allowing organic contaminants (such as chaotropic salts or phenol) to be efficiently washed away. When coupled with the enzymatic elimination of residual genomic DNA by DNase, both approaches ultimately yield RNA with comparable purity (Boom et al., 1990).

The *Å260/280* and *Å260/230* ratios are parameters used to evaluate RNA purity and assess contamination in extracted nucleic acid samples(Nouvel et al., 2021). Absorbance at 230 nm indicates organic solvent or TE buffer contamination; absorbance at 280 nm indicates protein contamination; and absorbance at 260 nm indicates nucleic acid contamination (Gallagher, 2011). Although these ratios do not directly measure the potential for enzyme inhibition, they serve as a benchmark for assessing sample suitability for subsequent analysis (Febriyani et al., 2026; Ocktariyana et al., 2022).

This study demonstrated that the use of TRIzol significantly increased the RNA concentration from endometriosis lesion tissue, providing sufficient quantities for molecular analyses such as qPCR. Although there was a descriptive trend toward higher purity in the non-TRIzol group, both methods yielded RNA within an acceptable purity range, and the differences were not statistically significant. Future research should focus on optimizing extraction protocols to further improve RNA yield and purity from challenging tissue types, such as endometriosis lesions, and potentially explore advanced methods that offer greater consistency or reduce cross-contamination.

Furthermore, TRIzol and non-TRIzol group samples were collected from different patients and locations, which is a limitation of this study. We addressed these limitations by tightening the inclusion of participant recruitment and selecting laboratories that have the same type of equipment and standards. Therefore, we suggest that future studies should consider the homogeneity of the treatment groups and tighten the analysis criteria.

CONCLUSION

This study shows that the TRIzol extraction method yields much higher RNA concentrations than the NonTRIzol method (p-value < 0.05) and is consistent with the standard RNA purity ratio (Å260/280) (p-value < 0.05). However, it was not significantly different for RNA Å260/230 (p-value > 0.05). These findings confirm that TRIzol is reliable for obtaining high-quality RNA from endometriosis lesion tissues, making this method recommended as a standard for advanced applications such as qPCR and transcriptome analysis. So, the results of this study make an important contribution to optimizing RNA isolation protocols in biomedical research on endometriosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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