Novel Biome

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Fecal Microbiota Transplantation (FMT) as a Therapeutic Approach for Autism Spectrum Disorder (ASD): A Clinical Evaluation of Safety, Tolerability, and Efficacy in Children

This white paper presents the results of an open-label, observational trial conducted by Novel Biome to assess the safety, tolerability, and effectiveness of fecal microbiota transplantation (FMT) in children with Autism Spectrum Disorder (ASD).

Highlighted Findings:

- **Improvement in ASD Symptoms**: Both subjective and validated assessments (CARS, ATEC, and QoLA) demonstrated significant improvements.
    - Parents reported subjective improvements at all time points (30,60,90,120, and 180 days), with improvements in social interactions, attention, and anxiety reported most consistently.
    - Validated autism assessments demonstrated significant improvements in ASD symptoms following FMT treatment, along with a notable reduction in the family-wide impact of these symptoms, reflecting an overall improvement in quality of life.
- **Safety and Tolerability**: The treatment was well-tolerated with no serious adverse events. Mild, transient symptoms, such as fever and gastrointestinal discomfort, were reported but did not necessitate discontinuation of the treatment.
- **Sustained Benefits**: Improvements in ASD-related symptoms and quality of life were sustained up to 180 days post-treatment

Introduction

The diagnosis and incidence rate of autism spectrum disorder (ASD) continues to rise worldwide [1–7]. As we look to better understand ASD and its core symptom presentation, the connection between the gut microbiome and autism has become a focus. This is partly fueled by increasing evidence indicating that children with neurodevelopmental disorders, including ASD, suffer from increased gastrointestinal problems and dysbiosis of the gut microbiota, which significantly impact quality of life [8–10].

Recently, research has unveiled a potential link between the gut microbiome and autism spectrum disorder (ASD) [8–10]. This emerging evidence suggests that alterations in the gut microbiota composition may play a crucial role in the pathogenesis of ASD [11–19]. Differences in the composition, development and diversity of gut microbiome have been observed in individuals with ASD [5,8,16,19–34]. In addition, there is a higher prevalence of gastrointestinal (GI) issues and an apparent connection between GI issues and ASD-related behaviours reported in children with autism [2,8,25,26,32,35–37]. While the mechanisms are still being elucidated, evidence indicating a role for the gut microbiome in contributing to ASD has led to a growing interest in fecal microbiota transplantation (FMT) as a potential therapeutic approach.

FMT involves the transfer of specially prepared stool material from a healthy donor into the gastrointestinal tract of a recipient [38–41]. Although research on FMT and ASD is in its infancy, and more clinical trials are needed, initial findings are promising. FMT has shown considerable potential in its ability to alleviate GI symptoms [13,42,43], improve gut microbial composition [13,14,42–44], and reduce the severity of certain ASD-related symptoms [13,42,43] and ASD presentation [42]. One longitudinal study reported that GI and ASD-behavioural improvements post-FMT were maintained or further improved 2 years after FMT [42].

An open-label trial was designed by Novel Biome, to investigate the safety, tolerability, and efficacy of FMT for GI and behavioural symptoms in children with ASD. This protocol was based on the groundbreaking work from Arizona State University (ASU) [13]. Long-term FMT treatment was administered to 150 children with ASD. Clinical responses were monitored for 6 months (180 days). Briefly, the FMT protocol involved 2 weeks of oral vancomycin treatment, and often also a 10-day course of Nystatin, followed by 24 h fasting and a bowel cleanse, then 2 days of a high initial dose of FMT either orally (capsules) or rectally (enema) followed by daily, lower maintenance oral doses, either capsules or oral powder for 15 weeks. To evaluate treatment effects, participants were followed for an additional 120 and/or 180 days after treatment ended. This report focuses on the safety and tolerability of FMT and its effects on ASD-related symptoms.

Methods/ Protocol

Overview:
Based on a pivotal study by Dr. James Adams and his team at Arizona State University (ASU), in which they treated study participants with 8 weeks of daily FMT, our study utilized an adjusted protocol. This protocol included individualized pre-FMT treatment plans, an extended 16-week daily FMT treatment involving upfront loading doses followed by maintenance dosing over the treatment period.

Our study design was an open-labelled, observational trial involving 44 patients with ASD (ages 4-19). The treatment protocol included an individualized pre-treatment protocol that included two weeks of antibiotic treatment before FMT followed by 16 weeks of FMT treatment. Three medical consults were also included throughout the treatment period (pre-FMT, midway through FMT treatment, and post-FMT), an in-person MD consult before the first loading dose FMT treatment, and a validated assessment before FMT treatment and at 120 and 180 days post-FMT treatment.

FMT Procedure:
Fecal Microbiota Preparation
Rigorously screened stool donors were sourced for the manufacturing of FMT. The screening involved a review of medical and wellness history, as well as blood and stool screening. Donors underwent rigorous screening that involved regular health questionnaires, a review of medical and wellness history, and physical examinations to rule out infectious disease, metabolic syndrome, gastrointestinal disorders, and neurologic or neurodevelopmental problems, screening for over 34 different diseases. Serologic testing

was performed to rule out infection with HIV-1 and -2; hepatitis A, B, and C; and syphilis, with a total of 54 screening measures in blood. The stool used in preparation was tested for potential bacterial pathogens (i.e. C. difficile toxin B, Campylobacter, Salmonella, toxin-producing Escherichia coli, Vibrio, Yersinia, Multidrug-resistant bacterial), potential parasites (i.e. Giardia, Cryptosporidium, Cyclospora, and Isospora), and potential viral infections (i.e. Rotavirus A, Adenovirus, and Norovirus), with a total of 73 measures in stool. The metabolic health of donor individuals was assessed through physical examinations and serologic testing (i.e., lipid panel, liver function tests, and high sensitivity C-reactive protein). Any single abnormality resulted in the disqualification of the donor and prevented material release. All donors were vaginally born, breastfed, physically active and fit, ate a diverse omnivorous diet and had limited lifetime use of antibiotics.

Our selected donor material was then extensively filtered, standardized, and freeze-dried so that the final product was in powdered form. This powder was then encapsulated using double acid-resistant encapsulation, or to create our oral powder delivery format the powder was placed in single-dose vials.

Patient Pre-treatment (or microbiome prep):

Pre-treatment helps to prepare the gut microbiome by reducing species richness and which microbiota are dominant before FMT to allow for better engraftment of the donor microbiome, which can improve outcomes post-FMT. In our protocols, similar to that of Kang et al. 2017, two weeks before FMT, all children were given Vancomycin, a non-absorbable broad-spectrum antibiotic that stays in the GI tract, a dose of 40 mg/kg per day divided into three doses, which was stopped 48 hours before the start of FMT. A 14-day course of antibiotics was used to help reduce the amount of microbiota in the gut and ensure that pathogenic bacteria were suppressed.

Pretreatment was then individualized, a selection of the below we most commonly applied:

- For children taking the powdered FMT, it was suggested for them to take an acid-lowering medication (i.e. 20 mg Prilosec/day) starting 4 days before FMT to allow more bacteria to survive and colonize the gut
- If the patient presented with a history of elevated yeast (candida) it was recommended to take a 7-10 day course of Nystatin, ending 48 hours before FMT.
- One 1 month before FMT, Biocidin was suggested at 3 times a day with increasing doses with increasing three drops per day every week, stopping 48 hours before FMT

FMT Treatment:

On the day prior to starting FMT, patients performed a bowel cleanse to help clear and remove most remaining gut bacteria and antibiotics before starting FMT. The goal is to create plenty of space and opportunity for the transplanted bacteria to take hold and engraft. Two options were provided for the bowel cleanse: Magnesium (Citrate or Oxide) or MoviPrep. To further enhance FMT effectiveness, a fasting period of 1 day was

implemented, during which participants were only allowed to consume clear liquids or liquid meals

FMT treatment consisted of two options: Oral Capsules or Oral Powder, where patients who could not easily swallow capsules were given the oral powder. Treatment began with a loading dose in person under the direct supervision of the study physician. The loading dose for the capsule group consisted of 35 capsules per day for 2 days, while the oral powder group had a loading dose of 1X60mL enema per day for 2 days. After the loading dose, ongoing treatment was performed at home for 15 weeks. FMT treatment was taken 1.5 hours after and 1.5 hours before (3 hours total) away from food, with FMT treatment consisting of 1 capsule or 1 powder vial per day for 15 weeks. The oral powder was provided as an undiluted powder and mixed before dosing in 5 mL of liquid.

Adverse Events

A mechanism was in place to collect any adverse reactions to FMT, which included virtual physician assistant meetings throughout the treatment period.

Data collection

Patient data was collected between January 2022 through January 2024. Patients were requested to fill out optional surveys throughout the treatment period. The primary endpoint was the CARS assessment. Patients were requested to complete the CARS assessment at intake and 120 days post-FMT treatment with a trained clinical counsellor via online video conference. Secondary endpoints were validated surveys at intake (before treatment) and 30, 60, 90, 120 and 180 days post-FMT treatment. Surveys were collected via an online survey tool, Google Forms, and e-mail reminders were sent to all patients.

Data was summarized with statistical parameters of median, mean, range, standard error of the mean (SEM), and percentage or representation of the total, where appropriate. All comparisons were assessed by paired t-tests, and significance was set at $P < 0.05$, with a trend defined as $P = 0.1 < 0.05$. All statistical analyses and graphs were performed using Prism Version 8.

Validated Assessment Tools

1. Childhood Autism Rating Scale (CARS2)[1,2]

The Childhood Autism Rating Scale **(**CARS2) assessment is widely used and an empirically validated autism assessment. This assessment helps identify where children are on the autism severity scale using quantifiable ratings based on direct observation by a certified professional. The CARS2 includes behavioural observations, interviews of primary caregivers, assessments of intellectual functioning, and a detailed developmental and family history. As part of the treatment protocol, a Childhood Autism Rating Scale (CARS2) assessment is performed pre-and post-FMT.

2. Autism Treatment Evaluation Checklist (ATEC)[5,6]

The Autism Treatment Evaluation Checklist (ATEC) is a caregiver-administered questionnaire designed to measure changes in the severity of ASD symptoms in response to

treatment. The ATEC is an easily accessible method for caregivers to track the changes in ASD symptoms over time. ATEC is comprised of four subscales: (1) Speech/Language/Communication, (2) Sociability, (3) Sensory/Cognitive Awareness, and (4) Health/Physical/Behavior. As part of our treatment protocol, the Autism Treatment Evaluation Checklist (ATEC) is provided pre-and post-FMT.

3. **Quality of Life in Autism Questionnaire (QoLA)**[3,4]

To better understand changes in quality of life (QOL) for both parents and children, we utilize the Quality of Life in Autism Questionnaire (QoLA), giving us a broader understanding of the impacts of FMT treatment. The QOLA is an autism-specific measure of QOL that evaluates all the relevant aspects of living with ASD. It contains two sub-scales 1) QOL sub-scale to measure parents' overall perceptions of their QOL, and 2) Impacts of ASD symptoms sub-scale to assess parents' perception of their child's autism-specific difficulties. The Quality of Life in Autism questionnaire (QOLA) is performed before, during, and after FMT as part of our treatment protocol.

Results

Demographics

Of the 150 patients who completed treatment, 65 patients provided at least intake data (survey or CARS) and follow-up data (30,60,90,120 or 180 days). The average patient age was 9 years, with an age range of 4-22 years, and 74% of the patients were male (17 females and 48 males). Thirty percent of the patients reported their ethnicity to be Northern European. As the route of birth and early diet are factors to consider with ASD, we report that 85% of the patients were breastfed, and 49% were born via c-section.

TABLE 1 | Demographic information.

Age		**Ethnicity**	
4-7	30	Mediterranean	4
8-11	21	South East Asian/Indian	3
12-15	6	East Asian	3
16-19	6	African American	2
20-22	3	East African	5
		West African	3
Biological Sex		Northern European	14
Male	48	Hispanic	1
Female	17	Other	11
Route of Birth		**Breastfed**	
Vaginally	24	Yes	41
C-Section	23	No	7

Reported Improvements

At every point (30, 60, 90, 120, and 180 days), participants reported their subjective measure of improvements with seven choices of improvements. There were 190 responses (63 respondents) over the five-time points, with 171 total improvements reported (271%) and 19 entries listed as no improvements (30%) across all time points, indicating significant reported improvements (t(4)= 2.986 P<0.05 (P= 0.0203)).

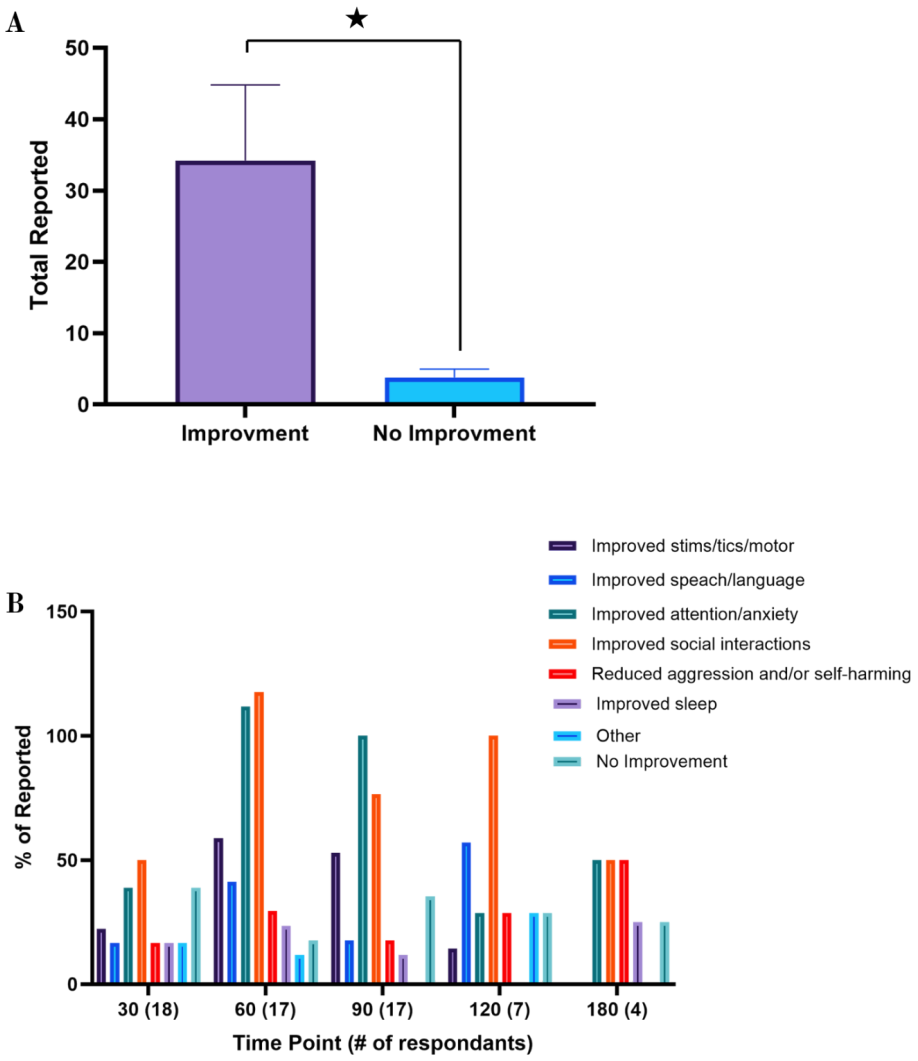

FIGURE 1 | Subjective parent-reported measure of improvement. **(A)** There were significantly more reported improvements than no improvement across reporting periods (t(4)= 2.986 P<0.05 (P= 0.0203)). **(B)** The percentage of subjective parent-reported improvements-by-improvement type grouped by reporting period (where * means significant).

Childhood Autism Rating Scale (CARS2)

A total of 35 patients completed both the intake and follow-up (120 days) CARS assessment. This assessment helps identify where children are on the autism severity scale using

quantifiable ratings based on direct observation by a certified professional. CARS scores (t(34)= 9.72 P<0.05 (P <0.0001)) significantly improved after FMT treatment, showing a 12% improvement. The average pre-FMT (intake) score was 34, which is considered mild to moderate (range 30-36.5), with the post-FMT(120 days) average score of 30, showing the treated population was not considered in the severe category, with only 20% (7 patients) falling within this category.

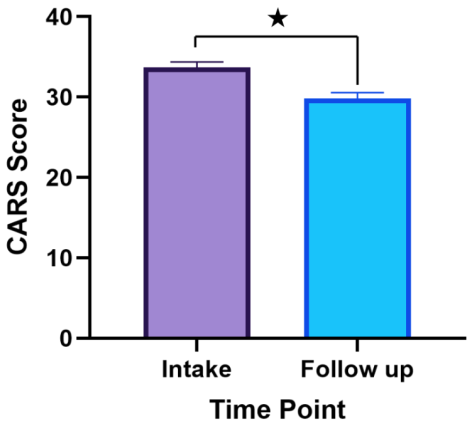

FIGURE 2 | Validated Childhood Autism Rating Scale (CARS2) scores from the intake and follow-up (120 days). CARS2 score significantly decreased after FMT treatment (t(34)= 9.72 P<0.001) (where * means significant).

Autism Treatment Evaluation Checklist (ATEC)

A total of 8 patients completed both the intake and follow-up (120 or 180 days) ATEC assessment. This assessment is a caregiver-administered questionnaire designed to measure changes in the severity of ASD symptoms in response to treatment. To calculate the total ATEC score, the scores from each subscale are combined, which range from 0–179 points, with a lower score indicating a lower severity of ASD symptoms. Total ATEC scores (t(7)= 3.961 P<0.05 (P = 0.0055)) significantly improved after FMT treatment, showing a 23% improvement. The average pre-FMT (intake) score was 68.1, while the post-FMT(120 and 180 days) average score was 61.

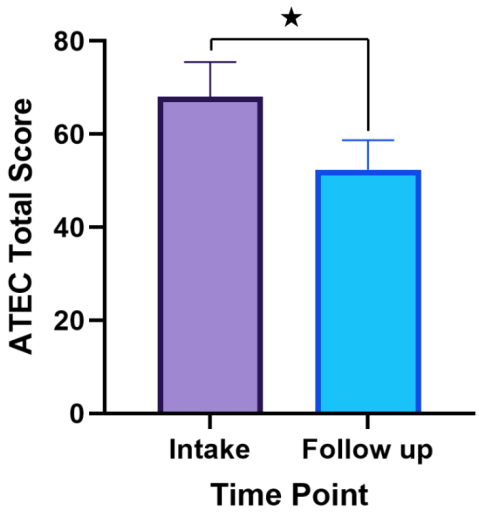

FIGURE 3 | Validated total Autism Treatment Evaluation Checklist (ATEC) scores from the intake and follow-up (120/180 days). Total ATEC score significantly decreased after FMT treatment ($t(7)= 3.961$ $P<0.05$) (where * means significant).

Based on the significant improvements reported by the total ATEC scores, further was completed for the four sub-scales (1. Speech/language and communication, 2. Sociability, 3. Sensory and cognitive awareness, 4. Health/Physical/ Behaviour). Both the sociability ($t(7)= 4.741$ $P<0.05$ ($P = 0.0011$), 39% improvement) and sensory and cognitive awareness ($t(7)= 3.578$ $P<0.05$ ($P = 0.0045$), 27% improvement) scores were significantly improved after FMT treatment. The health/physical/behaviour measure trended towards significance ($t(7)= 1.411$ $P=0.10$), with a 16% improvement), while the speech/language and communication did not show significantly improved scores ($t(7)= 1.040$ $P=0.1664$)

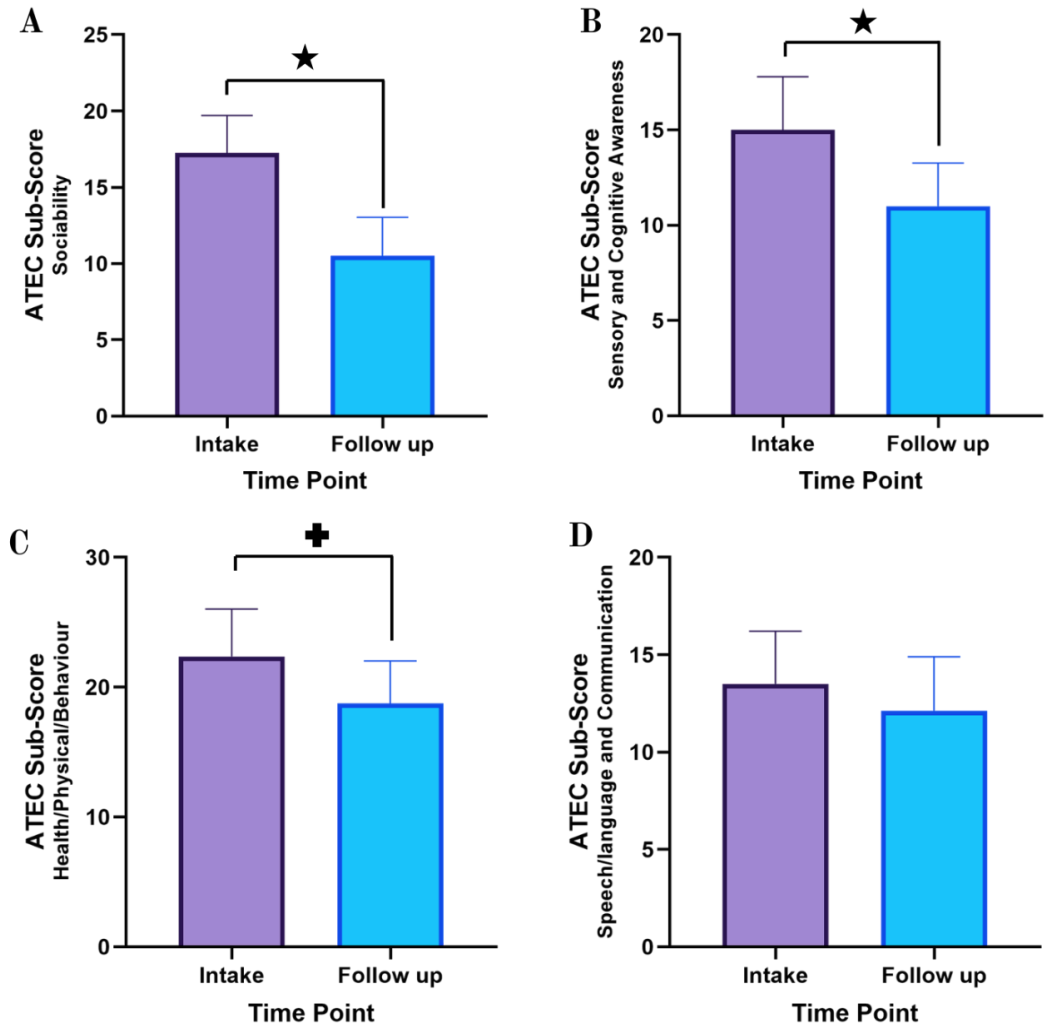

FIGURE 4 | Validated Autism Treatment Evaluation Checklist (ATEC) sub-scale scores from the intake and follow-up (120/180 days). **(A)** The sociability sub-scale score significantly decreased after FMT treatment ($t(7)= 4.741$ $P<0.05$). **(B)** The sensory and cognitive awareness sub-scale score significantly decreased after FMT treatment ($t(7)= 3.578$ $P<0.05$). **(C)** The health/physical/behaviour sub-scale scores tend towards significance after FMT treatment ($t(7)= 1.411$ $P=0.10$). **D)** The speech/language and communication sub-scale scores did not show significant change after FMT treatment ($t(7)= 0.3741$ $P>0.05$) (where * means significant and + means trend).

Quality of Life in Autism Questionnaire (QoLA)

A total of 8 patients completed both the intake and follow-up (120 or 180 days) QoLA assessment. This assessment is a caregiver-administered questionnaire designed to understand changes in quality of life (QOL) for both parents and children. There are two sub-scales of the QoLA, the parents' overall perceptions of their QOL and the impacts of ASD symptoms. The impacts of ASD symptoms scores ($t(7)= 2.97$ $P<0.05$ ($P = 0.0207$))

significantly improved after FMT treatment. The parents' overall perceptions of their QOL scores (t(7)= 0.3741 P>0.05 (P = 0.7194) did not significantly improve after FMT treatment.

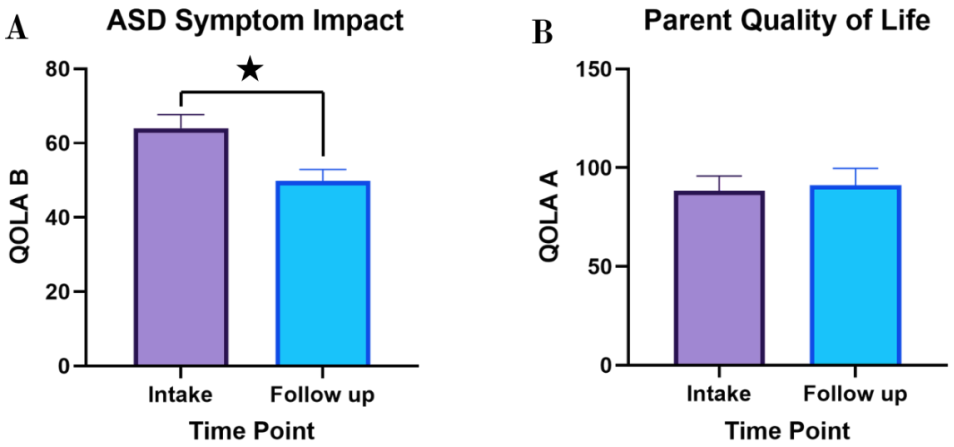

FIGURE 5 | Validated Quality of Life in Autism Questionnaire (QoLA) scores from the intake and follow-up (120/180 days). **(A)** The impact of ASD symptoms sub-scale score significantly decreased after FMT treatment (t(7)= 2.97 P<0.05). **(B)** The parents' overall perceptions of their Quality of Life (QOL) sub-scale scores did not show significant change after FMT treatment (t(7)= 0.3741 P>0.05) (where * means significant).

Adverse Events

No serious adverse events or any nuisance events that led to the discontinuation of treatment were reported. Of the nuisance events were mild transient symptoms, including mild fever, diarrhea, nausea, abdominal discomfort, and bloating, which resolve without intervention, similar to what has been reported in the public domain [41,50,51].

Summary Findings

Aligning with previously published studies [13,43], we report improvements in ASD behaviour and ASD severity after FMT treatment. We report significant improvements in both ASD severity and ASD symptoms, as well as the impacts of ASD-related behaviours on quality of life. Of the 150 patients who underwent treatment, 65 patients went on to complete the intake and follow-up questionnaires and were included in the study. Parents reported significant improvements at all time points, with the most commonly reported improvements being in social interactions (i.e. eye contact, interaction, initiating play) and hyperactivity/anxiety (i.e. calmer, attention span, and anxiety). In terms of validated measures, there were significant improvements in both Childhood Autism Rating Scale (CARS2) scores and Autism Treatment Evaluation Checklist (ATEC), representing improvements in both ASD severity and ASD symptoms, including sociability and sensory and cognitive awareness, respectively. As this study aimed to report not only on FMT

effectiveness but also safety, we report that there were no serious adverse events or any nuisance events that led to the discontinuation of treatment.

Our findings suggest that FMT is safe and well-tolerated in children with ASD ages 9–22 years. FMT led to significant improvements in ASD-related symptoms and severity, and the improvements were sustained at least 17 weeks after treatment. While this study was an open-label observational trial, our results are promising and provide additional understanding of the connection between the gut microbiome and ASD, as well as the role FMT may play as a treatment pathway.

Ready to Explore the Potential of FMT?

Let's discuss how FMT can integrate into your clinical protocols, collaborate on impactful case studies, or support your patients' outcomes. Novel Biome offers high-quality FMT products designed to support clinical and research applications. If you're interested in exploring how our products can enhance current treatments or improve patient outcomes, let's connect!

👉 Schedule a Call Today or email us at support@novelbiome.com to explore research insights and practical applications in your practice.

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