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Faecal Microbiota Transplantation in Anorexia Nervosa: A Systematic Review of Methodologies, Outcomes, and Challenges With Recommendations for Future Studies

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ABSTRACT

Objective: Anorexia nervosa (AN) is a severe psychiatric disorder displaying an altered gut microbiome. Faecal microbiome transplantation (FMT) has emerged as a powerful research tool and potential treatment option in AN due to the microbiome-gut-brain axis. Current studies are limited and reveal variable FMT protocols. This leads to heterogeneous outcomes and complicates drawing definitive conclusions from existing literature. This review aims to compile and assess the different protocols and develop recommendations on ideal donors, handling of faeces, recipients, duration/frequency of FMT, and measuring transfer success for future FMT studies regarding AN.

Methods: We systematically screened three databases (Pubmed, Embase, Web of Science), identifying 13 studies, including two human case reports, one human study protocol, and 10 animal studies.

Results: While all studies demonstrated microbial alterations in the recipients, not all animal studies successfully induced an AN/underweight phenotype, suggesting that precise coordination of study protocol components to allow further refinement is essential.

Conclusion: Researchers should prioritise clear, comprehensive, and transparent documentation to ensure the interpretability and reproducibility of FMT procedures. Detailed reporting will enable more meaningful comparisons across studies, deepen our understanding of the microbiome's role in AN, and help identify methodological factors that influence outcomes. Ultimately, completeness of documentation in FMT studies in AN has substantial potential to support future clinical applications and improve patient care.

1 | Introduction

Anorexia nervosa (AN) is a severe eating disorder that mainly affects adolescent women. The lifetime prevalence is estimated to range from 1% to 4%, though numbers appear to have increased after the COVID-19 pandemic (van Eeden et al. 2021; Devoe et al. 2023). Core symptoms of AN include weight loss due to insufficient energy intake and hyperactivity (Herpertz et al. 2018). These symptoms frequently coincide with body image disturbance, fear of weight gain, and comorbid anxiety and

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Highlights

- Current FMT studies in AN employ heterogeneous protocols, limiting comparability and contributing to inconsistent conclusions.
- Our recommendations for human-to-animal FMT studies emphasise minimising potential confounders, including prior antibiotic, probiotic, or antipsychotic use and gastrointestinal diseases of donors. Age-/sex-matched healthy controls should be included. Starvation of recipients is a crucial aspect of the protocol design. Transfer success can be evaluated using 16S rRNA sequencing. Consistent reporting of phenotypic endpoints such as body weight, food intake, and physical activity enhances cross-study comparisons.

depression (Marucci et al. 2018). Consequently, AN stands as one of the most lethal among psychiatric disorders (GBD 2019 Mental Disorders Collaborators 2022; Keel et al. 2003; Søeby et al. 2024; Neale and Hudson 2020). The current therapeutic approach combines weight recovery, nutritional therapy, and psychotherapy, while pharmacotherapy primarily focuses on treating comorbidities (Herpertz-Dahlmann 2021). Yet, relapse rates reach 9%–52%, especially in the first year after treatment. Only 30%–60% of patients achieve full remission, while 20% exhibit a chronic course (Khalsa et al. 2017; Treasure et al. 2015).

Traditionally, a multimodal interplay of several causes, including the involvement of genetics, epigenetics, social and psychological factors, hormonal, and endocrine pathways, is discussed regarding the pathogenesis of AN (Skowron et al. 2020; Käver et al. 2024; Voelz et al. 2024).

In recent years, research has focused on AN-associated gut microbiome changes. About 100 trillion microorganisms inhabit the human gastrointestinal tract, collectively called 'the gut microbiome' (Valdes et al. 2018). These microorganisms primarily consist of bacteria but also include viruses, fungi, protozoa, and archaea (Barko et al. 2018). Microbial dysbiosis can be caused by genetics, epigenetics, infection, and inflammation, although substantial shifts in gut microbiota may result from changes in macronutrients, as seen in AN (Hrncir 2022). Multiple studies found altered α-diversity (number/relative abundance of species) and β-diversity (difference in species between samples) when comparing patients with AN to healthy controls, though overall results remain heterogeneous (Garcia and Gutierrez 2023; Anton-Păduraru et al. 2023). Specifically, butyrate-producing genera like *Faecalibacterium* and *Roseburia* appear to be decreased in patients with AN, while mucin-degrading species like *Methanobrevibacter smithii* and *Akkermansia muciniphila* seem to be increased (Garcia and Gutierrez 2023; Zhao et al. 2024; Nikolova et al. 2021; Di Lodovico et al. 2021).

Following weight rehabilitation, the microbiome dysbiosis improves, but some alterations remain (Schulz et al. 2021; Andreani et al. 2024). Also, the microbiota composition at admission could potentially serve as a predictive indicator for relapse probability (Andreani et al. 2024). These findings suggest that microbial modifications may substantially influence weight regulation (Wei et al. 2022). Furthermore, the gut microbiome affects the brain via the microbiome-gut-brain axis (Stilling et al. 2014), as various neuropsychiatric and neurological diseases seem to be influenced by this axis (Socała et al. 2021).

Animal studies play a pivotal role in understanding the pathophysiology of AN and developing improved treatment options. The activity-based anorexia (ABA) model mimics AN symptoms seen in patients (e.g., body weight loss, hyperactivity, and amenorrhoea) by combining food restriction with access to a running wheel. This model also induces microbial alterations, apparently with a stronger influence of food reduction than physical activity (Trinh, Kogel, Kneisel, et al. 2023; Breton et al. 2021).

Next to food supplements, faecal microbiota transplantations (FMT) of healthy stool could help to restore the microbiome dysbiosis seen in AN (de Clercq et al. 2019; Prochazkova et al. 2019; Wilson et al. 2023). FMT is a procedure of transferring faecal microbes from donors to recipients to modify the recipient's gut microbiome (Wang et al. 2019). Originally identified to resolve symptoms of *Clostridioides difficile* infections, this approach gained popularity in the past decade of microbiome research and treatment of non-infectious diseases (van Nood et al. 2013; Trinh, Keller, et al. 2023). New findings suggest FMT might also be able to transfer behavioural characteristics of neuropsychiatric and psychiatric disorders to recipient animals, to study underlying pathophysiologies (Kelly et al. 2016). This indicates a causal-effect relationship between gut microbiota and psychiatric symptoms (Borrego-Ruiz and Borrego 2024). In contrast to AN-related diseases like depression and obesity (Kelly et al. 2016; Ridaura et al. 2013), only few studies investigated FMT in AN. Some of these show reduced weight gain in FMT recipient animals (Fan et al. 2023; Hata et al. 2019), while others do not find any phenotypic changes (Glenny et al. 2021; Kooij et al. 2024, 2025). This heterogeneity may have partially occurred due to diverging methodology. While some studies aim to induce an AN phenotype by only transplanting faeces from patients with AN, others subject transplanted animals to food-reduction/starvation. Also, divergent starvation models cause different degrees of starvation. Particularly, fixed starvation models may induce disproportionately severe starvation in adolescents, whose feeding requirements exceed those of younger and older animals. Currently, a standardised protocol to perform FMT in AN does not exist, and the few available studies employed designs tailored to specific research questions. These customisations involve donor and recipient selection (species, sex, age, mothers), animal model (germ-free, antibiotic depletion, laxative depletion), housing, duration, and dose of FMT, as well as sample collection, preparation, storage, and administration. Due to promising results in other metabo-psychological disorders, further investigation of FMT's potential to regulate AN progression is of great scientific interest. Thus, this review aims to evaluate different FMT methodologies of existing publications. Also, recommendations for a more standardised protocol are provided for future FMT studies in AN. These apply only to human-to-animal studies, as evidence-based data for human-to-human transfer is still lacking.

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2 | Methods

This systematic review was designed according to the PRISMA guidelines (Moher et al. 2009). Between 18th June 2024, and 28th November 2025, we systematically screened three different scientific literature databases (PubMed, Web of Science, Embase) with no time limitation. Our search strategy is reported in Supporting Information S1: Table S1. All identified articles were evaluated based on pre-established inclusion and exclusion criteria.

2.1 | Selection Criteria

Studies that met the following selection criteria were included:

- Publication in a peer-reviewed English or German journal.
- Original articles.
- Case reports, cross-sectional, and longitudinal studies.
- Clinical diagnosis of AN according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) version IV or V, OR underweight/malnutrition.
- Investigation of microbial composition.
- Execution of any type of FMT protocol.
- Both animal and human studies were considered.

Figure 1 demonstrates our selection process and search results in a PRISMA flow diagram.

Two authors independently assessed the articles identified by the systematic literature search based on the selection criteria as depicted above. Firstly, non-eligible studies were excluded based on titles, abstracts, and keywords. Afterwards, the full text of the remaining articles was reviewed. In this way, 13 studies were systematically analysed based on defined parameters:

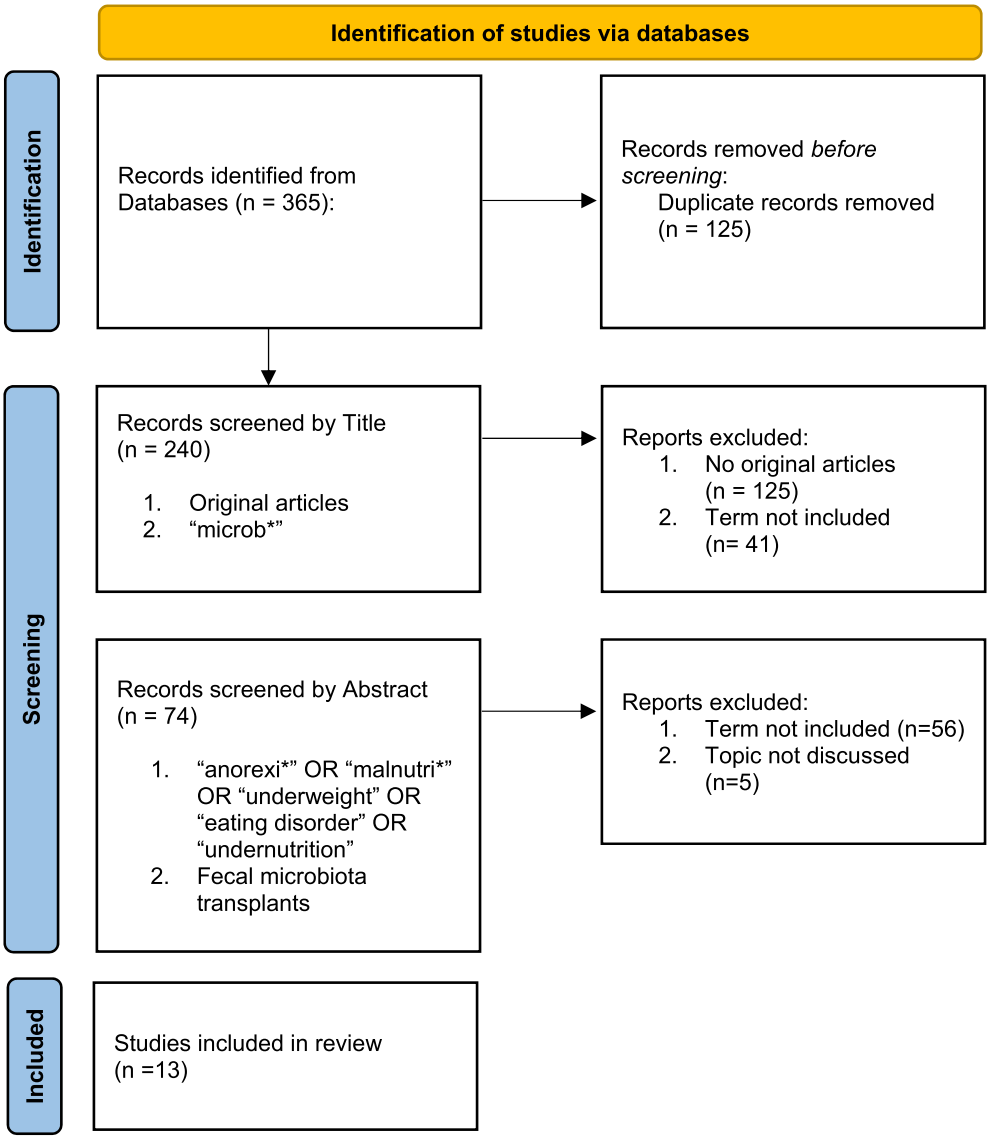

FIGURE 1 | PRISMA flow chart (Page 2021). [Colour figure can be viewed at wileyonlinelibrary.com]

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2.2 | Data Extraction

— Author.

— Publication year.

— Study design.

— Sample (including species; donor/recipient inclusion and exclusion criteria; number of cases and controls; sex; age; mean BMI; diagnosis).

— FMT protocol (including sample collection and processing; application method, dose, and frequency; FMT method; experimental duration).

— Outcome variables (engraftment, body weight, morphological, biological, and behavioural alterations).

Due to the heterogeneity of the methods and results among the identified articles, a quantitative meta-analysis could not be performed. Therefore, a qualitative systematic review was conducted to compare FMT protocols in AN and underweight populations and to assist in designing future FMT trials.

3 | Results

In total, 365 publications were identified in the selected databases. Before systematic screening, 125 duplicates were excluded. After screening for the predetermined selection criteria by title, 166 studies were excluded. Of these, 125 studies were not original articles, and 41 did not address the microbiome. After screening by abstract, 56 studies unrelated to AN or underweight, and 5 studies without an FMT protocol were excluded. As a result, 13 full-text articles and case reports were evaluated for eligibility. Ultimately, these 13 publications with 99 donors (56 patients/43 healthy controls) and more than 540 recipients (exact number can not be reported due to lacking information) were included in this review. Two case reports described FMT in humans with AN, one study protocol outlined a human FMT trial, seven articles conducted FMT from AN patients into animals, and three studies performed FMT from underweight patients into animals (Table 1). Since this review aims to guide FMT animal studies, the protocols of the included animal studies are analysed in detail, while the analysis of the human studies is descriptive.

3.1 | Human Studies

Only two case reports have been published that describe FMT from a healthy donor to a patient with AN.

In 2019, de Clercq and colleagues (de Clercq et al. 2019) reported the case of a 26-year-old female patient with relapsed AN and recurrent weight loss following their discharge after initial weight gain. The treatment was conducted with one application of 150–250 g of fresh faecal material from a healthy female, administered via a duodenal tube after a fasting period of unknown duration (presumably 12 h overnight). This resulted in an increase in body fat by 55% and body weight of 6.3 kg (13.8%) after 6, 12, and 36 weeks, respectively. Furthermore, an augmented phylogenetic diversity was observed at 6 and 12 weeks. Interestingly, the authors identified a reduced resting metabolism, which might have aided in weight gain.

In the same year, Prochazkova et al. (2019) presented a case of FMT from a healthy 5-degree relative (a 67-year-old female) into a 37-year-old female patient with therapy-refractory AN. After a six-hour-long starvation period, three rectal applications of 250 g of fresh (first application) or frozen (following two applications) faecal material were performed within 2 weeks, which resulted in measurable changes to the bacterial taxa, including an increase in species richness and short-chain fatty acid levels over 12 months. However, the patient showed no clinical improvement, but a progressive deterioration in condition.

Furthermore, Wilson et al. (2023) published a protocol for a pilot study on multi-donor human-to-human faeces transfer. Frozen faecal samples from four healthy donors will be encapsulated for application to the recipients. After eight hours of starvation, all patients will receive a mix of five capsules from each donor (20 in total). Shifts in the microbial composition 3 weeks post-FMT will serve as the primary outcome. Meanwhile, engraftment over 12 weeks, physiologic responses, and alterations of clinical AN symptoms will be analysed as secondary outcome criteria.

3.2 | Animal Studies

Seven studies have been published using animals as FMT recipients in AN, and three in undernutrition, with diverse protocols (Table 2).

Hata et al. (2019) conducted FMT from patients with AN into parental mice and subsequently examined female offspring. Four adolescent female patients diagnosed with a restrictive type of AN and four age- and sex-matched healthy controls were recruited as donors. As recipients, germ-free (GF) female BALB/c mice raised under sterile conditions were orally administered with 0.5 mL of freshly collected faecal matter. As no frequency of FMT is reported, a single shot might be assumed. After a vertical transfer from the parental generation into litters, female offspring were examined between four and 10 weeks of age. In the FMT-treated animals' offspring, a decrease in the Bacteriodetes phylum and an increase in the relative abundance of the Firmicutes, Fusobacteria, and Cyanobacteria phyla, compared to the FMT HC mice's offspring, but no significant differences in α-diversity were observed. The AN-like phenotype was evidenced by a reduction in body weight gain, food intake, and a notable alteration in anxiety-like and compulsive behaviour.

Glenny et al. 2021 recruited donors similarly to the criteria described by Hata et al. (2019). However, the faecal material was directly administered to 8–9-week-old GF C57BL/6 mice of both genders. Samples from patients with AN were taken on admission or discharge. In conjunction with samples from HC, FMT treatment with 10 mL/kg (up to 0.25 mL) of frozen faecal material was conducted by oral gavage in three experimental groups (AN at admission, AN discharge, and HC). Again, the frequency of treatment is not reported. No AN-like phenotype could be identified, and no sex-dependent differences were

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TABLE 1 | Data extraction table.

First author	De Clercq	Prochazkova	Wilson
Publication year	2019	2019	2023
Study design	Case report		One arm open pilot trial protocol
Species		Human to human	
Number of donor and recipients	Donor $n = 1$ Recipient $n = 1$	Donor $n = 1$ Recipient $n = 1$	Donor $n = 4$ Recipient $n = 20$
Sex/age	Donor: Female/– Recipient: Female/26 years	Donor: Female/67 years Recipient: Female/37 years	Donor: Female/adult Recipient: Female/adult
Donor characteristics	BMI 25, unrelated	First degree relative	BMI 18.5–25th p., total body fat < 33%, healthy diet, regular exercise, regular bowel habit
Exclusion criteria of donor characteristics	—	—	Gut/metabolic/psychiatric disease, elevated HbA1c, asthma, autism, malignancy, ABX/probiotic/substance use, pregnancy
Recipient characteristics	DSM-5 typical AN, therapy refractory (cognitive bahavioural therapy, weight restoration, mediaction, force feeding), body weight drop after discharge, no medication (except for autoimmune dermatomyositis), no GI disease alcohol/smoking, no diet influencing microbial changes	DSM-5 AN over 20 years, repeated vomiting, abuse of laxatives, therapy refractory (cognitive therapy, hospitalisation, medication)	DSM-5 AN, BMI 13–19th p., medically stable (exclusion: Allergies, ABX/probiotic/laxative use, steroid treatment, immune deficiency, chronic gut/metabolic disease, suicidal ideation, pregnancy)
FMT processing	150–250 g faeces diluted in saline	250 g faeces diluted with 600 mL 10% glycerol, filtered	Aerobic conditions, faeces diluted in 0.9% saline solution 1:2, resuspended in 15% glycerol/0.9% saline, concentration: 1 g/mL, aliquots capsules
Fresh or frozen	Fresh	First dose fresh, second + third dose frozen −80°C	Frozen −80°C
Application method	Duodenal tube/infusion	Rectal	Capsules (oral)
Dose/frequency	150–250 g/1x (day 1)	250 g/3x (day 1/5/14)	10 g gut microbiota (2.5 g per donor), 20 capsules (5 from 4 donors each)/over 2–4 days
Antibiotics use	—	—	—
Starvation	Unknown duration (likely 12 h overnight)	6 h fasting before application	8 h fasting overnight before capsule intake, no food 1 h post intake
Experimental duration	36 weeks	52 weeks	12 weeks

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TABLE 1 | (Continued)

First author	De Clercq	Prochazkova	Wilson
ARRIVE guidelines	—	—	—
Primary outcome	Changes in metabolic parameters (resting metabolism and body weight)	Recovery/changes in bacterial taxa abundance	Shift in bacterial composition
Secondary outcome	—	Side effects	Phenotype and behaviour
Phenotypic success	Yes	No	—

First author	Hata	Glenny	Fan	Kooij	Kooij	Maschek	Gabriel-Segard
Publication year	2019	2021	2023	2024	2025	2025	2025
Study design	RCT	RCT	RCT	RCT	RCT	RCT	RCT
Species				Human to animal			
Number of donor and recipients	Donor $n = 8$ (4 AN/4 HC)	Donor $n = 8$ (4 AN/4 HC)	Donor $n = 6$ (3 AN/3 HC)	Donor $n = 6$ (3 AN/3 HC)	Donor $n = 10$ (5 AN/5 HC)	Donor $n = 6$ (3 AN/3 HC)	Donor $n = 6$ (3 AN/3 HC)
	Recipient $n = -$	Recipient $n = 153$	Recipient $n = 20$	Recipient $n = 46$	Recipient $n = 72$	Recipient $n = 48$	Recipient $n = 24$
Sex/age	Donor: Female/–	Donor: Female/adult	Donor: Female/adult	Donor: Female/adolescent	Donor: Female/adolescent	Donor: Female/adult	Donor: Female/adult
	Recipient: Male and female/adult; examination of female litters (4–10 weeks)	Recipient: Male and female/adult (8–9 weeks)	Recipient: Female/6 weeks	Recipient: Male and female/adult	Recipient: Female/6–7 weeks	Recipient: Female/3–4 weeks	Recipient: Female/4 weeks
Donor characteristics	AN: DSM-4 typical AN, Japanese	AN: DSM-5 AN, T1 = admission, T2 = discharge	AN: DSM-5 typical/atypical AN, Danish Caucasians, dietary plan	AN: DSM-5 typical AN	AN: DSM-5 typical AN	AN: DSM-5 typical AN	AN: Eating Disorder Diagnosis Survey French-validated version
	HC: Mean BMI 21.6th p.	HC: BMI 18.5–24.9th p., no ED history	HC: BMI 18.5–85th p., no regular medication (except birth control)	HC: BMI 20–68th p.; without current psychiatric diagnosis/no ED diagnosis in history	HC: BMI 20–68th p.; without current psychiatric diagnosis/no ED diagnosis in history	HC: BMI 19–23th p.; without history of ED or gut disease	HC: Over 18 years, BMI between 18.5 and 25 kg/m^2, absence of metabolic abnormalities, and a recent weight change
Exclusion criteria of donor characteristics	Digestive diseases, severe physical diseases, ABX use or regular intake of yogurt/probiotics 12 weeks before	GIT surgery (except chole-cystectomy), chronic/recurring bowel symptoms, ABX/NSAD/steroid/probiotics 8 weeks before	ABX or antifungal medication 12 weeks before, acute/chronic somatic diseases	IQ < 80, pregnancy, ABX use 6 weeks before, DM, GI/organic brain diseases	IQ < 80, pregnancy, ABX use 6 weeks before, DM, GI/organic brain diseases	ABX use 3 months before	ABX use 2 months before, purging behaviour with laxative abuse in the month before, somatic comorbidities (diabetes, irritable

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TABLE 1 | (Continued)

First author	Hata	Glenny	Fan	Kooij	Kooij	Maschek	Gabriel-Segard
							bowel syndrome, or other digestive pathology, metabolic disease), history of overweight or obesity
Recipient characteristics	GF mice (BALB/c); female litters	GF mice (C57BL/6)	GF mice (Swiss Webster)	SPF rats (TH-cre +/− Long Evans)	Wistar rats (Crl: WU)	SPF mice (BALB/cJTac)	GF mice (BALB/c)
FMT processing	Anaerobic conditions, 100 mg faeces diluted in 1 mL PBS	Anaerobic conditions, faeces diluted in PBS, filtered	Anaerobic conditions, 250 mg faeces diluted with 5 mL LYBHI media with 20% glycerol	Anaerobic conditions, 4.5 g faeces diluted in PBS with 20% glycerol/0.1% L-cysteine hydrochloride/0.001% resazurin, concentration: 100 mg/mL	Anaerobic conditions, 4.5 g faeces diluted in PBS with 20% glycerol/0.1% L-cysteine hydrochloride/0.001% resazurin, concentration: 100 mg/mL	Faeces diluted 1:5 in 50% glycerol	Anaerobic conditions, faeces homogenised in cryoprotectant media and diluted in maltodextrin-trehalose mixture per gram of stool, pooling of donor samples
Fresh or frozen	Fresh + frozen −80°C	Frozen −80°C	Frozen −80°C	Frozen −80°C	Frozen −80°C	Frozen −80°C	Frozen −80°C
Application method	Oral gavage	Oral gavage	Oral gavage	Oral gavage	Oral gavage	Voluntary feeding	Oral gavage
Dose/frequency	0.5 mL/–	10 mL/kg (250 μL maximum)/–	0.2 mL/2x (days 1 + 3)	0.3 mL/4x (days 1–3, day 7)	0.3 mL/4x (days 1–3, day 7)	0.5 mL/FMT1: 5x (1x/week); FMT2: 4x (1x/week)	0.2 mL pooled faecal sample/2x (day 0 and 2)
Antibiotics use	—	—	—	Metronidazole/Gentamycin/Vancomycin/Ampicillin via oral gavage 8 mL/kg for 7 days	Metronidazole/Gentamycin/Vancomycin/Ampicillin via oral gavage 8 mL/kg for 7 days	Ampicillin via drinking water 1 g/L (FMT1 2 weeks, FMT2 3 weeks)	—
Starvation	—	—	3 weeks (start: Day 3), 30% food reduction	—	7 days (start: After FMT, 1.5 h food access per day)	—	—
Experimental duration	Observation up to 10 weeks	28 days	22 days	65 days	40 days	14 weeks	8 weeks
ARRIVE guidelines	E10 not met	E10 not met	E10 mostly met	E10 mostly met	Not mentioned, but PREPARE guidelines	Mentioned and E10 met	E10 mostly met

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TABLE 1 | (Continued)

First author	Hata	Glenny	Fan	Kooij	Kooij	Maschek	Gabriel-Segard
Primary outcome	Influence of FMT on weight and behaviour	Influence of FMT on weight	Influence of FMT on AN development	Influence of FMT on weight and behaviour	Influence of FMT on ABA model	Influence of FMT on food intake, weight, and behaviour	Influence of FMT on weight and behaviour
Secondary outcome	Addition experiments: Fresh versus frozen, probiotic supplementation	Correlation of bacterial taxa and phenotypic parameters	—	Influence of ABX on behaviour	—	Cross-over from AN and HC FMT in 2 phases	Influence of FMT on immune system and multi-organ-crosstalk, correlation of bacterial taxa and phenotypic parameters
Phenotypic success	Yes	No	Yes	No	No	No	Yes

First author	Smith	Blanton	Fluitman
Publication year	2013	2016	2022
Study design	RCT	RCT	Randomised cross sectional cohort study
Species		Human to animal	
Number of donor and recipients	Donor $n = 6$ (3 underweight/3 HC) Recipient $n = 30$	Donor $n = 19$ (11 underweight/8 HC) Recipient $n = 95$	Donor $n = 17$ (6 low BMI/5 weight loss/6 HC) Recipient $n = 41$
Sex/age	Donor: Male and female/3 weeks–30 month old infants Recipient: Male/8 weeks old	Donor: Male and female/6–18 month old infants Recipient: Male/5 weeks old	Donor: Male and female/older adults Recipient: Male/10–11 weeks old
Donor characteristics	Co-twin with Kwashiorkor Healthy co-twin	Underweight or healthy, Malawian twin/triplet pairs	Undernutrition (BMI < 20 if < 70 years, BMI < 22 if > 70, or > 5% body weight loss in last 2 years)
Exclusion criteria of donor characteristics	Insufficient amount of faecal samples, death of one co-twin	—	Overnutrition (BMI < 30th p./> 2% body weight gain within 2–4 years), active malignancy, MMSE score < 18, ABX use 3 months before
Recipient characteristics	GF mice (C67BI/6J)	GF mice (C67BI/6J)	GF mice (Swiss Webster)
FMT processing	Anaerobic conditions, faeces diluted in 4 mL PBS + 2 mL DTT	Anaerobic conditions, 1 g diluted in 15 mL GMM with 15% glycerol	Anaerobic conditions, LYHBHI media
Fresh or frozen	Frozen	Frozen −80°C	—
Application method	Oral gavage	Oral gavage	Oral gavage

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TABLE 1 | (Continued)

First author	Smith	Blanton	Fluitman
Dose/frequency	200 μL/1x	200 mL/1x	200 μL/2x (days 1 + 3)
Antibiotics use	—	—	—
Starvation	Malawian diet	3 days before sterile Malawian diet	5 h fasting before
Experimental duration	> 5 weeks	4–5 weeks	3 weeks
ARRIVE guidelines	E10 mostly met	E10 mostly met	E10 mostly met
Primary outcome	Influence of FMT on weight development, microbial shift	Influence of FMT on phenotype	Influence of FMT on body weight development
Secondary outcome	—	—	—
Phenotypic success	Yes	Yes	Yes

Abbreviations: ABX, antibiotics; AN, anorexia nervosa; BMI, body mass index; ED, eating disorder; FMT, faecal microbiota transplants; GF, germ-free; HC, healthy control; RCT, randomised control trial.

observed. However, microbial clusters of mice transferred with FMT from the same experimental groups could be identified when analysing male mice with an overlap of the recipient-donor microbiome of 42%. Furthermore, an association between specific bacteria and caecum weight, as well as alterations in body weight and fat mass, was identified, irrespective of the donor group.

Fan et al. (2023) recruited females with AN as donors for transplantation into 6-week-old female Swiss Webster GF mice. On days 1 and 3, 0.2 mL of frozen faeces was administered to the animals via oral gavage, with one day of chow diet interposed. In contrast to other protocols, the animals were subjected to a 30% calorie restriction for 3 weeks, commencing one day after the second transplant. The FMT treatment successfully induced an AN-like phenotype in starved GF mice compared to controls, with a greater decrease in body weight and slower weight gain, as well as elevated hippocampal gene expression, for example of proteins regulating adipose tissue thermogenesis or affecting appetite. Also, engraftment testing was reported with a 20% overlap of amplicon sequence variants (ASV) between the donor and recipients. Interestingly, these phenotypical changes did not appear when ad-lib feeding was conducted in a separate set of experiments.

In 2024, Kooij et al. (2024) reported faecal transplants into specific pathogen-free (SPF) TH-cre$^{+/-}$ Long Evans rats who had undergone antibiotic treatment prior to FMT. Three female adolescent patients with AN from a human cohort of the MiGBAN study and three age and sex-matched controls were recruited to donate faecal samples (L. Keller et al. 2022). Transplants were performed into male and female adult rats. These recipients had been administered an antibiotic cocktail 1 week before FMT to deplete the endogenous microbiome. Transplants were performed on the first 3 days after antibiotic treatment and day seven with 0.3 mL of processed frozen faecal material via oral gavage. As the primary outcome, FMT did not alter cognitive flexibility, anxiety, or reward signalling. Also, a transfer efficiency of up to 22% of successfully transplanted bacteria was found. As a secondary outcome, antibiotic depletion without FMT decreased task engagement but did not affect cognitive flexibility.

Subsequently, in 2025 (Kooij et al. 2025), the same group published another experiment with the same protocol for recruitment, stool sampling, processing, and administration. However, in this experiment, Wistar rats were subjected to a 7-day food restriction of 1.5 h per day following the four administrations of FMT, to provide more insight into the influence of the ABA model in the context of FMT. Again, a (partially) successful transfer was validated by an overlap of up to 31.5% in the microbial composition of donors and recipients. Nevertheless, no significant differences were identified in the phenotypic development of the animals from divergent donor groups. However, a deterioration in condition, as measured by loss of body weight, body temperature, and overall survival, was observed in the antibiotic control group.

In 2025, Maschek et al. (2025) presented a cross-over design in which 3–4 week old BALBcJTAC mice, pretreated with ampicillin orally for 2 weeks, received 0.5 mL of frozen faecal

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TABLE 2 | Evaluation of parameters divergent within protocols and according to study success rate based on phenotypic disease-specific alterations.

Parameter	Phenotypic alterations (sucess rate)			Not mentioned
Anerobic (9/10) versus non-anerobic (0/10)	6/9			1
Glycerol or other media (7/10) versus PBS only (3/10)	4/7	2/3		—
Fresh (1/10) versus frozen (8/10)	1/1	4/8		1
GF (7/10) versus AB (3/10)	6/7	0/3		—
Both genders (2/10) versus female (5/10) versus male (3/10)	0/2	3/5	3/3	—
Oral gavage (9/10) versus different application method (1/10)	6/9	0/1		—
Single shot (2/10) versus repeated FMT (6/10)	2/2	3/6		2
Starvation (5/10) versus no starvation (5/10)	4/5	2/5		—

Note: All 10 animal studies were included. Parameters that have not been reported are classified as 'not mentioned'. Exemplary, one of 10 studies used fresh faecal material (1/10), while eight others used frozen samples (8/10). The transplant of fresh samples successfully induced an AN phenotype (1/1). Contrastingly, only four out of the eight protocols using frozen material reported phenotypic disease-specific alterations (4/8). One protocol did not mention whether fresh or frozen material was used (1/1).

material orally via voluntary feeding from three AN donors or healthy controls each. After 5 weeks of weekly FMT administration, animals were subjected to another 3-week antibiotic treatment, followed by a second FMT period for 4 weeks. During the second FMT, mice either received faeces from the same donor group as before (AN or HC) or as a cross-over from the divergent donor group. Under ad libitum feeding conditions, alterations in food intake, body weight, and behaviour were examined. While reduced food intake was identified in the AN groups after both FMT phases, there were no differences in body weight between groups. Microbial analyses only showed significant alterations after the first FMT phase. Antibiotic treatment resulted in microbial depletion and similar body weight gain in all experimental groups. After the second FMT period, mice that had only been treated with FMT from patients with AN showed higher levels of appetite-regulating hormones than all other groups (Maschek et al. 2025).

Most recently, Gabriel-Segard et al. (2025) published a study with female GF mice receiving pooled frozen faecal material from patients with AN. In both groups, twelve 4-week-old BALB/c mice were given 0.2 mL pooled FMT of three AN or HC donors, respectively, at two time points (days 0 and 2). The animals were administered to ad libitum food for the whole experimental duration of 8 weeks. Faecal sampling allowed microbial analyses at three timepoints. In addition to body weight and food intake, and physical activity, also behavioural tests were assessed. Furthermore, parameters of immune function (cytokines) and integrity of organs other than the gut were analysed. More than 50 days post FMT, microbial alterations and changes in SCFA levels within the transplant groups could be detected. AN like symptoms such as food restriction, anxiety-like behaviour, physical hyperactivity and inflammatory responses was identified in the AN group. Also, disruption of ovarian follicles as well as liver dysfunction could be exhibited. Nevertheless, no alterations in body weight could be seen between the two groups. Interestingly, the AN transplant group even presented with higher fat mass compared to controls (Gabriel-Segard et al. 2025).

In 2013, Smith et al. (2013) presented a twin study on FMT in the context of undernutrition. Transplants of frozen faecal material from twin pairs, with one healthy child and one co-twin diagnosed with kwashiorkor due to undernutrition, were performed into 8-week-old GF C67BI/6J mice as a single-shot intervention. During interventions, mice were fed a typical Malawian diet (donor-matched). Results showed that mice transplanted with two of three twin pairs experienced significant weight loss 3 weeks post-FMT, and a partial weight recovery upon refeeding. Microbial sequencing identified specific bacterial taxa associated with kwashiorkor, and metabolic profiling revealed diet-dependent differences, with stronger responses in recipients of kwashiorkor microbiota, though no full recovery achieved.

Blanton et al. (2016) performed a study on Malawian multiples (twins and triplets) suffering from severe underweight and undernutrition. Frozen faecal material from matched pairs of underweight or normal-weight infants was given to 5-week-old GF C67BI/6J mice. Before the single-shot transplant, recipients had been fed a sterile Malawian diet for three consecutive days. Mice that received FMT from healthy infants presented with an augmented weight gain compared to recipients of 'underweight' FMT.

In comparison, Fluitman et al. (2022) used donors recruited as part of the longitudinal ageing study Amsterdam and found a defined weight loss in FMT recipient Swiss Webster mice due to undernutrition of donors clustered into three experimental groups: low BMI group, weight loss group, and healthy control group. 10–11-week-old male GF mice were used as recipients. These animals underwent 5 hours of fasting before 0.2 mL of faecal material were administered on days one and three via oral gavage. FMT from the patient group with a lower BMI or poorer weight gain was associated with developing an augmented lean mass in the corresponding recipient animals.

4 | Discussion

This systematic literature review includes 13 publications on FMT in AN or undernutrition. Two case reports describe human-human FMT, one study protocol describes a human-

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human FMT trial, and 10 studies performed human-animal FMT. The FMT protocols of the individual studies differed strongly. Thus, it is too early to draw generalised conclusions. However, optimising and standardising FMT protocols in the future would offer significant advantages.

4.1 | Human-Human FMT

Firstly, human-human FMT protocols for treating AN must be methodically separated from human-animal protocols to understand AN's pathomechanisms.

Our search for human-human FMT protocols produced only case reports and a study protocol, limiting the ability to draw definitive conclusions. Nonetheless, these studies suggest initial evidence that human-human FMT can be effective, as both cases demonstrated (partial) success with changes in bacterial composition, and one may have also altered the phenotype in the recipient patient, including significant weight gain. Data on human-human FMT in patients with AN remains scarce, but the protocol proposed by Wilson et al. (2023) and other registered yet unpublished studies (one completed *NCT05834010*; three announced *NCT07143981*, *NCT06744751*, *NCT06593366*) may serve as a foundation for developing a standardised human protocol in the future.

4.2 | FMT in Other Clinical Contexts

In June 2025, a systematic review on FMT in several gastrointestinal, immunological, neuropsychiatric, and metabolic diseases was published, highlighting strengths and pointing out a lack in current human-to-animal FMT protocols (Ruszkowski et al. 2025). In this work, 13.3% of all included studies regarded mental, behavioural, or neurodevelopmental disorders, whereas 11.5% focused on endocrine, nutritional, or metabolic diseases. Due to the breadth of diseases covered, this review can also serve as a guide. Even though this review summarises a great variety of FMT studies in different diseases, the authors themselves criticised the divergent outcomes in different diseases, highlighting the need for a deeper look into crucial pathomechanisms of specific disorders. When it comes to AN, these (meta-)analyses are still missing (Ruszkowski et al. 2025).

When creating new study protocols, existing experience with successful transplants from other clinical conditions related to AN should be taken into account. FMT has already been extensively researched in obesity, a metabolic psychiatric disorder that is contrary to AN, and major depression, a psychiatric disorder and common comorbidity of AN (Proença et al. 2020; Wilson et al. 2025; Zhang et al. 2025; Chinna Meyyappan et al. 2020).

Firstly, these studies also present a great deal of variation in their protocols, duration, and analysed outcome parameters, highlighting the inherent challenges with FMT as an intervention.

In obesity, a meta-analysis of human-to-human FMT included six studies with various application methods and experimental durations. Contrary to AN studies, end points focused on metabolic parameters (e.g., insulin sensitivity) rather than engraftment efficiency (Proença et al. 2020). Of the studies that considered the phenotypic outcome, none presented a significant phenotypic change (Proença et al. 2020). By contrast, another trial involving obese participants, FMT via an oral capsule affected phenotypic parameters, including improved body composition and metabolic health (Wilson et al. 2025). These results illustrate how minor differences in FMT protocols can influence observed outcomes substantially, particularly regarding phenotypic effects. Hence, it is important to include other disease-specific endpoints.

Regarding major depression, a meta-analysis of 12 human studies identified short-term improvement in depressive symptoms following FMT from a healthy donor. In terms of application methods, endoscopic or enema administration produced better outcomes than oral intake via capsules, which have not been tested in AN (Zhang et al. 2025). Furthermore, a meta-analysis of 21 preclinical and clinical FMT studies compared animal and human models. Preclinical FMT transfers patient microbiota into healthy rodents, while clinical FMT uses healthy donors to treat patients, limiting comparability. Despite different directionality, both approaches can transmit psychiatric-like phenotypes, supporting the microbiome-brain interaction (Chinna Meyyappan et al. 2020).

4.3 | Recommendations for Future Faecal Microbiota Transplantation Research in AN

The following suggestions are now given for subsequent studies in human-to-animal models. As the two studies by Kooij et al. (2024), (2025) exhibited the same protocol for FMT, they are reported as one.

Considering the divergence in multiple crucial parameters within the studies included in this analysis, direct comparisons should only be made with caution. Differences in the nutritional status of the recipients (starved or ad libitum feeding), the frequency and duration of FMT, and the outcome parameters may interact with the results significantly. Careful and consistent documentation of the work is essential to establish links between variations in the study protocol and different outcomes. This allows studies conducted in different ways to be compared in terms of outcome parameters, quality and effectiveness, enabling the most suitable methodology to be identified. The diversity of presented data strengthens the importance of detailed documentation, including the recruitment criteria for donors and recipients, the selection of animal models, the purification and storage of samples, the faecal microbiota transplantation workflow, including dosage and frequency, as well as the potential to co-transfer other microorganisms (Figure 2). For the development of an improved protocol, conclusions can be drawn from the reported studies and previous analyses on FMT protocols (see Reviews by Ruszkowski et al. (2025); Gheorghe et al. (2021); Bokoliya et al. (2021)). Gheorghea et al. 2020 studied publications on FMT in several diseases regarding their protocol and experimental outcomes. This review also exhibits general recommendations on faecal sampling, purification, and storage, as well as parameters to evaluate FMT efficiency (Gheorghe et al. 2021). Nevertheless, in the context of AN, additional factors such as the nutritional status of donors and

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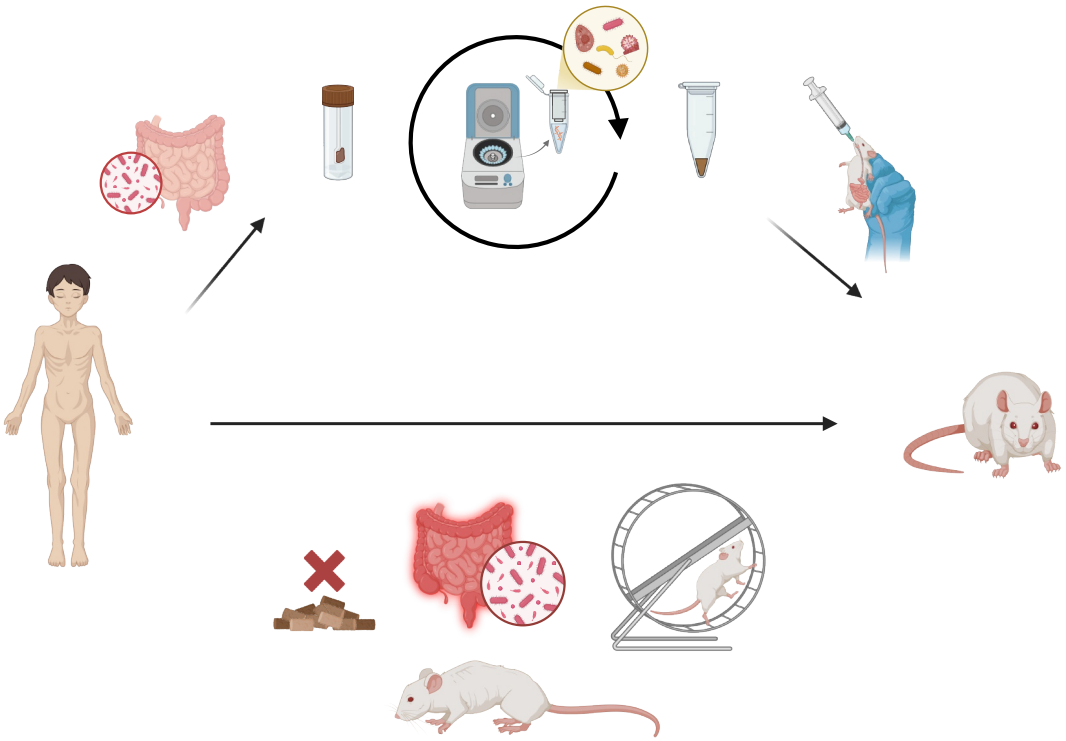

FIGURE 2 | Standardised study protocol for FMT from human patients with AN into healthy rodents. (From left to right) Donors are selected based on defined criteria, including the diagnosis of a typical AN according to DSM-5, female sex, adolescent age, and no intake of microbial disturbing substances before faecal donation, as well as no gastrointestinal disorder. After sample collection, faeces are processed and purified. 16s rRNA sequencing is performed to measure the microbial composition in faeces before transplants. 0.2–0.3 mL of weekly repeated FMT applications into recipient age and sex-matched rodents. Defined outcome parameters for FMT success: microbial alterations and overlapping of bacterial taxa from donor and recipients (16s rRNA sequencing), body weight loss, and reduced food intake. Additional assessment of secondary outcome parameters, such as brain cell composition alterations, behavioural changes, epigenetics, metabolic parameters, influence on physical activity, or inflammatory response. Created in BioRender. (2025) https://BioRender.com/gmwvc45. [Colour figure can be viewed at wileyonlinelibrary.com]

recipients are relevant. Hence, recommendations for designing future FMT studies in the context of AN will be provided based on the seven reviewed animal studies with AN donors, complemented by the three studies using underweight but non-AN donors, as they also represent physical symptoms and microbial alterations associated with malnutrition.

4.4 | Donors

The selection of a suitable donor is a fundamental aspect of effective FMT. In fact Gabriel-Segard et al. (2025) even criticised the lack of a precise donor selection in the literature. Comparing all animal studies presented, seven protocols required a (DSM-5) diagnosis of AN (Fan et al. 2023; Hata et al. 2019; Glenny et al. 2021; Kooij et al. 2024, 2025; Maschek et al. 2025; Gabriel-Segard et al. 2025), while two required underweight (Blanton et al. 2016; Fluitman et al. 2022), and one focused on the discordance of twin pairs regarding kwashikor (Smith et al. 2013). Concerning AN studies, including donors with DSM-5-defined typical AN might be beneficial due to their stronger somatic dysregulation compared to patients with atypical AN or underweight, especially when reduced bodyweight is used to measure transfer success. As a healthy control group (HC), all AN studies selected individuals without any history of mental or somatic disorders and a normal body weight. Five research groups specifically chose age- and sex-matched HCs and one chose sex-matched HCs to minimise confounders (Fan et al. 2023; Hata et al. 2019; Glenny et al. 2021; Kooij et al. 2024, 2025; Gabriel-Segard et al. 2025). Two underweight donor groups were twin pairs with one healthy co-twin as a control subject, ensuring no genetic influence on the outcome parameters (Smith et al. 2013; Blanton et al. 2016).

Furthermore, the microbiome can be influenced by various factors other than FMT. Five of the nine reviewed protocols excluded patients after using antibiotics (Fan et al. 2023; Hata et al. 2019; Glenny et al. 2021; Kooij et al. 2024, 2025; Gabriel-Segard et al. 2025; Fluitman et al. 2022), two additionally after using probiotics (Hata et al. 2019; Glenny et al. 2021), and one after antifungals (Fan et al. 2023). Patients with gastrointestinal diseases were excluded from five protocols (Hata et al. 2019; Glenny et al. 2021; Kooij et al. 2024, 2025; Maschek et al. 2025; Gabriel-Segard et al. 2025), while Fan et al. excluded all somatic diseases, and Fluitman et al. excluded active malignancies. Several studies have reported the impact of antibiotics on the microbiome, cognition, neurogenesis, and behaviour (Möhle et al. 2016; Gacias et al. 2016; Desbonnet et al. 2015). Among non-antibiotic drugs, antipsychotics in particular appear to influence the microbiome (Maier et al. 2018). Also, various gastrointestinal diseases are known to cause microbial alterations (DuPont et al. 2020). Therefore, it might be advisable to exclude participants who underwent antibiotic, antipsychotic, or probiotic treatment before the FMT study.

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There is no strong data on whether the donor's diet influences FMT transfer success in AN. However, recruiting patients shortly after or even before hospital admission ensures a microbial composition representative of the AN diet. Only Fan et al. (2023), Maschek et al. (2025), Kooij et al. (2025), and Gabriel-Segard et al. (2025) studies specifically avoided intersex transfer to eliminate gender-driven effects.

Furthermore, recent meta-analyses on FMT in infectious diseases discuss transplantation of single-donor versus multi-donor FMT. Results suggest a greater benefit-to-risk ratio and similar safety when performing multi-donor transplants compared to single-donor transplants (Levast et al. 2023; Laperrousaz et al. 2024), as performed via pooled faecal transfer by Gabriel-Segard et al. (2025). Nevertheless, a single donor approach can potentially better retain the ecological system of interrelated taxa, which might also be important in certain cases. In this context, studies on other clinical pictures reported a large impact of the donor's microbiome on transfer and engraftment efficacy, with 'phenotypic extremes' inducing disease-mimicking phenotypes in recipients more likely (Gheorghe et al. 2021). Yet, for example Kooij et al. (2024), (2025) did not find significant donor-related differences in microbial and phenotypical endpoints. Future research could explore donor selection based on extreme phenotypes or microbial traits, using pre-transplant faecal sequencing to identify microbiome profiles of interest. This approach may be relevant given the discussion about 'super-donors' and the increased abundance of certain taxa in patients with AN, despite the lack of a distinct AN-specific microbiome (Garcia and Gutierrez 2023; Prochazkova et al. 2019; Wilson et al. 2019; Shtossel et al. 2023).

Additionally, biomarkers identifying reliable AN (and healthy control) donors are needed. Apart from typical AN-symptoms linked to illness severity like weight-loss, illness duration, and psychopathology, also stool microbiome and SCFA, inflammatory parameters, Also or hormonal indicators could help identify suitable donor candidates (Käver et al. 2024; Voelz et al. 2024; Ruszkowski et al. 2025; Korten et al. 2025). Interestingly, recent results by Gabriel-Segard et al. (2025) revealed elevated levels of SCFAs in the AN transplant group compared to the control group.

In addition, the specific (not only bacterial) composition of the faecal material of an ideal donor should be identified in order to understand specific pathomechanisms determined by the presence or absence of certain bacteria, metabolites, viruses or other players.

Also, the recent review described gastrointestinal parameters, immune function, and metabolic markers (SCFAs) to be of scientific interest in a majority of the studies (Ruszkowski et al. 2025).

4.5 | Handling of Faecal Material

Concerning sample collection and the handling of faecal material, there are fewer disease-specific factors influencing these procedures. A standard operating procedure for sample collection, identification, and extraction is provided by the International Human Microbiome Standards project (human-microbiome.org).

An essential initial step before processing is determining whether to use fresh or frozen faecal material. Seven reviewed protocols administered frozen faeces (Fan et al. 2023; Glenny et al. 2021; Kooij et al. 2024, 2025; Gabriel-Segard et al. 2025; Smith et al. 2013; Blanton et al. 2016), and Hata et al. (2019) compared fresh and frozen material. They found greater overlapping of microbial features when using fresh material, which led the group to use this for their main study (Hata et al. 2019). However, using fresh faecal material for transfer experiments requires complex coordination, which can be challenging for long-term studies. Experiments modelling the chronic nature of AN might benefit from using frozen samples from a single donor to minimise variability across different fresh samples. In other diseases like *Clostridioides difficile* infections, no differences in bacterial richness, microbial composition, and diversity were identified when comparing FMT-treated mice that received frozen or fresh stool (Ericsson et al. 2017). When freezing is needed, adding a cryoprotective solution (e.g., glycerol) is highly recommended (Béal and Fonseca 2015) (Hirsch et al. 2015) (Keller et al. 2021) (Bokoliya et al. 2021; Burz et al. 2019; Cammarota et al. 2014). Given that thawing especially damages anaerobes, oxygen exposure should be minimised (Bokoliya et al. 2021; Papanicolas et al. 2019). Extended thawing for more than 10 minutes has been associated with greater microbial shifts (Gorzelak et al. 2015), while rapid thawing has shown the most favourable outcomes (Burz et al. 2019).

Processing was conducted under anaerobic conditions in almost all reviewed studies (Fan et al. 2023; Hata et al. 2019; Glenny et al. 2021; Kooij et al. 2024, 2025; Maschek et al. 2025; Gabriel-Segard et al. 2025; Smith et al. 2013; Blanton et al. 2016; Fluitman et al. 2022). As the human gut microbiome is dominated by oxygen-sensitive anaerobic bacteria, exposure to oxygen can decrease bacterial viability and the production of metabolites like SCFAs. Hence, maintaining anaerobic conditions during stool handling helps preserve microbial diversity, viability, and functional capacity, which are critical for the effectiveness of FMT (Bokoliya et al. 2021).

Three studies diluted samples in PBS only (Hata et al. 2019; Glenny et al. 2021; Smith et al. 2013), while two studies diluted in LYHBHI and glycerol, one in PBS with glycerol, one in glycerol, one in a maltodextrin-trehalose mixture and one in gut microbiota media (GMM) (Fan et al. 2023; Kooij et al. 2024, 2025; Maschek et al. 2025; Gabriel-Segard et al. 2025; Blanton et al. 2016; Fluitman et al. 2022). This aligns with general recommendations that samples should be homogenised and diluted in three to six times the volume of for example PBS or glycerol. Nevertheless, resulting FMT samples exhibit slightly inconsistent concentrations due to a naturally varying stool concentration and circadian fluctuation of bacterial taxa and metabolites, especially in females (Bokoliya et al. 2021; Heppner et al. 2024; Thaiss et al. 2014; Liang et al. 2015). Hence, a well-founded

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documentation of the defecation time for all donors might minimise confounders by correcting for time differences.

4.6 | Recipients

Equally crucial to donor recruitment and standardised sample handling is the recipient selection. To ensure a standardised report of recipient characteristics, ARRIVE guidelines for reporting of animal research should be considered (Percie du Sert et al. 2020). Only Maschek et al. mentioned the ARRIVE guidelines; however, six out of the nine other studies mostly met the 'Essential 10' items of the ARRIVE guidelines (Fan et al. 2023; Kooij et al. 2024; Gabriel-Segard et al. 2025; Smith et al. 2013; Blanton et al. 2016; Fluitman et al. 2022). Additionally, Kooij et al. (2025) mentioned the PREPARE guidelines for planning animal research in their 2025 article. Regarding rodent species, Kooij et al. stand out performing transplants on rats, while all other studies used mice. Previous studies have found that the rat microbiome is more similar to humans than the mouse microbiome and that rats capture more human microbial species following a single human stool transfer (Lleal et al. 2019; Wos-Oxley et al. 2012). However, since Fan et al., Hata et al. and Gabriel-Segard et al. (2025) all successfully induced an AN phenotype in mice after FMT, and transfer from undernourished donors into mice seems efficient (Smith et al. 2013; Blanton et al. 2016; Fluitman et al. 2022), using mice appears sufficient. Another difference between Kooij et al. and Maschek et al. and other protocols is the animal model: They utilised specific pathogen-free rodents and depleted the microbiome with antibiotics before transplantation. Meanwhile, the others performed experiments on germ-free rodents or, in the case of Hata et al., litters of transplanted GF mice. To determine a superior model, Roubalová et al. (2024) compared responses to the activity-based anorexia (ABA) model in female mice with divergent microbial compositions: Conventional, GF mice and antibiotic-treated mice. They found that antibiotic-treated, food-restricted animals present with less weight loss and physical activity than GF or conventional mice (Roubalová et al. 2024; Dohnalová et al. 2022). GF animals generally show impaired immune function, neuronal development, and behaviour, which may confound studies of FMT investigating neurological or behavioural outcomes (Wan et al. 2023; Kennedy et al. 2018; Luczynski et al. 2016). However, although to a lesser degree, antibiotic treatment has also been linked to long-lasting dysbiosis, dysregulated development, neurogenesis, or behaviour, especially in early life stages (Keogh et al. 2021; Fröhlich et al. 2016; Hao et al. 2020). Consequently, a superior model cannot be determined a priori. In their first publication, Kooij et al. reported no significant differences in bodyweight and activity between controls and antibiotic-treated, transplanted animals, in contrast to the GF studies, which observed significant effects (Fan et al. 2023; Hata et al. 2019; Kooij et al. 2024). Interestingly, in contrast to Roubalova et al., the antibiotic control group without transplantation in Kooij et al. (2025) presented with a loss of body weight, lower body temperature, and overall survival when including the ABA model in the FMT protocol, indicating the role of microbial dysbiosis within underweight.

When deciding on antibiotics to deplete the microbiome experimentally, successful depletion can be confirmed, for example via Gram staining, sequencing, or PCR, before faecal transfer (Kooij et al. 2025; Ericsson et al. 2017). Also, antibiotic control groups transplanting saline only can aid in distinguishing FMT-specific effects from those caused by antibiotics alone (Kooij et al. 2025). A cocktail of antibiotics seems effective in achieving microbial depletion (Kelly et al. 2016; Kooij et al. 2024), with a 7-day oral treatment suggested as sufficient without causing resistance (Staley et al. 2017). Furthermore, allowing an interval between antibiotic application and FMT could minimise residual antibiotic effects on the transferred bacteria (Bokoliya et al. 2021). Alternatively, vertical transfer of GF animals, as performed by Hata et al., can be considered to prevent early-life effects of microbial dysregulation, as litters are influenced by the maternal microbiome following parental FMT (Gheorghe et al. 2021).

Although there is limited literature on the effect of rodent strain on FMT, initial results indicate that mouse strain can influence FMT outcomes. BALB/c and C57BL/6 mice seem to differ in IgA-mediated microbial selection and baseline microbiota composition, highlighting strain-specific host-microbe interactions (Fransen et al. 2015; Stebegg et al. 2019). According to Ruszkowski et al. (2025), GF or antibiotic-treated C57BL/6 mice were predominantly used in FMT treatment of a variety of diseases.

Furthermore, Fan et al. compared FMT in food-reduced versus ad lib-fed animals, only finding an AN-phenotype in food-reduced animals. Thus, FMT alone might have limited capacity to induce AN-typical changes, with food reduction providing an additional driver. Contrastingly, even with starvation, Kooij et al. (2025) could not induce an AN-like phenotype in their recipients, highlighting the complex pathomechanisms of the development of AN. It must also be taken into account that Fan et al. and Kooij et al. employed different starvation protocols. In adolescent animals, the physiologically increasing food requirements increase the risk of a more severe caloric deprivation than intended in fixed starvation schemes. Providing 70% of baseline intake, as in Fan et al., likely resulted in a 'more pronounced' food-reduction, potentially contributing to the observed AN-phenotype. To account for growth, the target weight could be adjusted for height, similar to BMI in humans.

Fan et al. (2023); Maschek et al. (2025); Gabriel-Segard et al. (2025) reported sex-matched donors and recipients, while Glenny et al. (2021) matched the age, and Kooij et al. (2025) considered both sex and age. Generally, younger recipient mice seem to allow enhanced engraftment than older mice, potentially due to a more malleable microbiome (Le Roy et al. 2018). Nevertheless, age and sex should be matched as mismatches can influence various systemic endpoints. Studies showed that young-to-old FMT can reverse hallmarks of ageing, while old-to-young FMT can impair cognitive function (Parker et al. 2022; D'Amato et al. 2020). Meanwhile, cross-sex FMT seems to affect metabolism and hormonal status (Feješ et al. 2024). Also, feeding a humanised diet matching the transplanted human microbiome was conducted by Smith et al. (2013); Blanton et al. (2016). This could enhance engraftment and minimise possible confounders, though no data are currently available to support this in the context of AN (Moreno-Indias et al. 2020). In case of AN, it may also be

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relevant to adjust not only the nutritional composition but also the feeding pattern to reflect AN-like eating behaviour. Current AN animal models include a fasting period, which in acute starvation is typically interrupted by a feeding phase lasting 1–3 h or a single administration of a reduced amount of food. The exact composition of the food should be examined in more detail in follow-up studies and adapted to a typical AN diet in line with a humanised diet.

4.7 | Duration and Frequency

The exact dose and frequency of FMT application differed heavily among the reviewed protocols. Briefly, five groups administered FMT multiple times (Fan et al. 2023; Kooij et al. 2024, 2025; Maschek et al. 2025; Gabriel-Segard et al. 2025; Fluitman et al. 2022), and two used a single application (Smith et al. 2013; Blanton et al. 2016). Repeated FMT is generally recommended over single administrations (Choo and Rogers 2021), though consecutive-day transplants might disrupt microbial engraftment (Wrzosek et al. 2018). One or two FMTs per week have shown promising results in the reviewed studies (Fan et al. 2023; Kooij et al. 2024; Gabriel-Segard et al. 2025; Fluitman et al. 2022). All compared protocols used volumes between 0.2 and 0.5 mL, aligning with general guidelines of not exceeding 2% of bodyweight. Based on existing protocols and their outcomes, no recommendation on the duration of FMT treatment can be given.

Regarding the application of faecal material, published literature most commonly describes the use of oral gavage, allowing a quick and effective transfer (Ruszkowski et al. 2025). Nevertheless, this method holds significant risks (aspiration, pneumonia, injury) and other approaches, such as voluntary feeding, should be systematically compared regarding efficiency and safety (Bokoliya et al. 2021).

4.8 | Transfer Success

The assessment of consistent endpoints is central to evaluate transfer success and the development of an AN-specific phenotype. Microbial engraftment may serve as a key endpoint. All analysed protocols used 16S rRNA sequencing to study the bacterial composition of stool samples. Still, five groups used amplicon sequence variants (ASV) to study microbial DNA sequences (Fan et al. 2023; Glenny et al. 2021; Kooij et al. 2024, 2025; Gabriel-Segard et al. 2025; Fluitman et al. 2022), and three used operational taxonomic units (OTU) (Hata et al. 2019; Smith et al. 2013; Blanton et al. 2016). ASVs offer superior resolution and reproducibility, making them the preferred method in current microbiome research (Fasolo et al. 2024). Future analyses might also include shotgun metagenomics to further increase analytic precision. To measure microbial engraftment, microbial sequencing of donors at stool collection and recipients before and after FMT can be compared. Although all protocols address transplantation success, the methods for measuring engraftment are heterogeneous and often lack transparency. Glenny et al., Fan et al., and Kooij et al. quantified engraftment by calculating the percentage of overlapping ASVs between donors and recipients, providing a comparable method across studies.

Apart from bacterial taxa, only Fan et al. (2023) and Gabriel-Segard et al. (2025) reported data on co-transferring non-bacterial components, even though viruses, fungi, and archaea are known to be abundant in human faeces (Bojanova and Bordenstein 2016) (Chehoud et al. 2016). Due to previous studies revealing bidirectional viral-microbial interactions, transferring these components could be considered as potential contributors to the development of an AN-like phenotype in FMT recipients (Reyes et al. 2010).

In addition, it may be advisable to include secondary outcome parameters such as phenotypic alterations in body weight, food intake, and physical activity (Frintrop et al. 2018). According to the recent review on multiple diseases, most frequently, the impact of FMT on weight development and/or food intake was evaluated as a primary outcome (Ruszkowski et al. 2025). Including these parameters would enable comparable investigation of the direct effects of FMT on the microbiome and the impact of microbial changes on core aspects of AN. All examined studies reported bodyweight changes in recipients after FMT. Only five groups observed reduced bodyweight or weight gain in animals receiving FMT from underweight patients, suggesting these protocols were the most effective (Fan et al. 2023; Hata et al. 2019; Smith et al. 2013; Blanton et al. 2016; Fluitman et al. 2022). As a further step, other aspects of interest in the context of AN, such as the microbiome-gut-brain axis, epigenetic alterations, hypothalamic genes, and endocrine functions, could be considered (Frintrop et al. 2018; Trinh et al. 2021; Chowdhury et al. 2014; Scharner and Stengel 2020; Schwenzer et al. 2022). Examplarly, one study additionally reported alterations in liver and ovarian function of the animals after FMT from patients with AN, targeting a potential organ-cross-talk via unidentified pathways (Gabriel-Segard et al. 2025).

5 | Limitations and Outlook

The number of studies included in the systematic analysis was limited, highlighting the scientific value of future research on FMT in AN. However, this restricts the analysis and interpretation of previous study results. The studies included exhibit considerable heterogeneity not only in the actual FMT protocol but also in the general framework conditions. For instance, human-to-human transplantations can only be meaningfully compared with human-to-animal transfer to a limited extent. Finally, the inclusion of participants is inconsistent, resulting in a divergence of effect sizes across publications.

In summary, various parameters influence FMT, highlighting the need for detailed documentation of experimental protocols to enable interpretation of the studies and assessment of methodological strengths and weaknesses. Although heterogeneous protocols limit direct comparability across studies, comprehensive documentation will enable partial comparisons and facilitate the future development of an improved FMT protocol for AN. Based on the discussion of the different protocols and the greater transplantation success in Fan et al. and

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Hata et al., certain protocol aspects may be particularly influential. Given that the microbiome is highly fluctuating and responsive to environmental factors, potential confounders should be minimised. Regarding donors, this could concern excluding individuals with recent antibiotic, probiotic, or antipsychotic use and gastrointestinal diseases, as well as including age- and sex-matched healthy controls. General recommendations on handling faecal material may serve as a foundation, though fresh stool may be favourable in shorter studies and frozen stool in prolonged AN studies. While GF rodents are promising recipients, antibiotic-treated SPF rodents may offer a viable alternative to avoid GF-associated side effects. However, antibiotic-related effects must be carefully monitored and minimised and phenotype induction has not been achieved. Food reduction in recipients might be an important aspect of the protocol design, although one study managed phenotype induction without. Also, multiple applications of FMT, spaced by at least one day, might be the method of choice. To evaluate transfer success, overlapping ASVs between donors and recipients could be compared via 16S rRNA sequencing. Additionally, consistent reporting of phenotypic endpoints such as body weight, food intake, and physical activity enhances cross-study comparison, and including additional metabolic (energy conversion, faecal energy content, gut transit time, SCFA production) or hormonal endpoints (blood appetite satiety and sex hormones) could further improve understanding of FMT effects.

Author Contributions

Anna C. Thelen: conceptualization, acquisition and data analysis, writing – original draft, writing – review and editing, visualization **Nele M. Korten:** conceptualization, acquisition and data analysis, writing – original draft, writing – review and editing, visualization, **Larissa Blischke:** conceptualization, writing – review and editing, **Clara Voelz:** conceptualization, writing – review and editing, funding acquisition, **Cordian Beyer:** conceptualization, writing – review and editing, **Jochen Seitz:** conceptualization, writing – review and editing, **Stefanie Trinh:** conceptualization, writing – review and editing, funding acquisition.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Table S1: Search terms for systematic search.

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