An Exploration of Gut Hormone Therapy to Treat Infertility Caused by Type 2 Diabetes

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Abstract

BACKGROUND: Type II Diabetes Mellitus is a common disease associated with multiple debilitating symptoms, including reduced fertility in women of reproductive age. Gut hormone therapies have shown promise in improving fertility in these patients.

OBJECTIVES:

1. To identify the relationship between the human gut microbiota and the successful functioning of the female reproductive system.
2. To explore treatments to improve bacteria culture in the gut, and to examine if these improvements affect fertility in female patients with Type II Diabetes.

METHODS: A systematic search was undertaken; studies were collected from PubMed and The Diabetology and Metabolic Syndrome Journal. Searches were performed between January 2023 and March 2023. Studies focused on female patients suffering simultaneously from Type II Diabetes Mellitus and fertility complications. Ten papers that met criteria were appraised and included.

RESULTS: There is evidence to suggest a causative relationship between the gut microbiome and reproductive functioning. Infertile patients had increased abundance of the phylum Verrucomicrobia and Phascolarctobacterium in the gastrointestinal tract, and decreased amounts of genera Stenotrophomonas, Streptococcus, and Roseburia. These abnormalities were associated with depleted circulating oestrogen concentrations, irregular menstrual cycling, and hyperandrogenism.

Evidence authenticates the use of probiotics and hormone therapy in treatment of Type II Diabetes and its associated symptoms. Supplements studied included metformin, GIP agonists, and GLP-1 receptors. Across studies, patients showed significant improvements in Type II Diabetes management following treatment.

CONCLUSION: Alterations to the composition of the gut microbiome are associated with improvements in glycaemic control as well as improvements in fertility in female patients with Type II Diabetes Mellitus.

Introduction

Type II Diabetes Mellitus is an impactful disease that currently affects 537 million adults globally (Diabetes Ireland, n.d.). The disease is characterised by the pancreas producing an insufficient quantity of insulin, or the body's inability to utilise insulin (Diabetes Ireland, n.d.). Complications associated with Type II Diabetes include kidney disease, neuropathy, and infertility (Diabetes Ireland, n.d.; Diabetes.co.uk, 2023). Previously, the majority of female patients with Type II Diabetes were postmenopausal, which severely limited research surrounding reproductive effects of the disease (Livshits and Seidman, 2009). However, with rapidly increasing Type II Diabetes rates, greater proportions of younger patients are suffering from this disease. The current prevalence of Type II Diabetes in women of reproductive ages ranges from 3% to 7% (Williams and Kreider, 2021), with the World Health Organization defining "reproductive age" as 15-49 years old (World Health Organization, n.d.). The reduction in the mean age of Type II

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Diabetes Mellitus patients is presenting new challenges in the disease's management. Adverse effects on fertility in female patients is a growing and substantial concern. Available studies show women with Type II Diabetes have a higher rate of both infertility and miscarriage than the general population (Mattsson et al., 2021). Women suffering from Type II Diabetes furthermore have a higher prevalence of oligomenorrhea, irregular menses, and diminished ovarian reserve, and are at higher risk for pregnancy loss than their undiagnosed counterparts (Mattsson et al., 2021).

Bariatric surgery is the leading option to improve fertility in Type II Diabetes patients (Cheah et al., 2022). The term, "bariatric surgery" encompasses a number of operations that promote weight loss by altering the digestive system (Mayo Clinic, 2023). Bariatric surgery is associated with a reduction in insulin resistance, hyperandrogenism, menstrual irregularity, and ovulatory dysfunction (Lee et al., 2020). There are strict requirements to undergo bariatric surgery, including a patient BMI greater than 40 kg/m2 (NHS, 2024). The overarching benefit of this surgery on fertility is the restoration of normal reproductive hormone levels (Moxthe et al., 2020). The gut microbiota interacts with a number of reproductive hormones including oestrogen and testosterone (Qi et al., 2021); new oral supplementation therapies have the potential to mitigate the effects of Type II Diabetes on female fertility in women who are unable or unwilling to undergo bariatric surgery. While available research is minimal, promising results have already appeared in clinical trials, chart reviews, and systematic literature reviews.

Objectives

The objective of this literature review is to systematically examine scientific databases to identify and analyse published scientific literature pertaining to:

1. The relationship between the human gut microbiota and the successful functioning of the female reproductive system regarding successful implantation and delivery.
2. The therapeutic use of hormonal medications and dietary probiotics to improve the culture of bacteria in the gut, and the effect these therapies have on fertility in female patients with Type II Diabetes.

Methodology

SEARCH STRATEGY

An electronic database search was conducted using PubMed and The Diabetes and Metabolic Syndrome Journal. Search strategy was devised to yield case-control studies, retrospective chart reviews, and systematic literature reviews that addressed the objectives of this review.

The following search strategy was used for PubMed:

I. ((Gut Microbiota) OR (Gut Microbiome) OR (GIP) OR (GLP-1) OR (Gut Hormones) OR (Probiotics))

AND

II. ((Infertility) OR (Reproductive Function) OR (Menstrual Irregularities) OR (Glycaemic Control) OR (Reproduction) OR (Oestrogen))

Filters applied: Clinical Trial, Meta-Analysis, Randomised Controlled Trial, Systematic Review, Female

Using the above key words without applying filters initially yielded 755 results. Results were filtered to exclusively include studies conducted between 2010 and 2022 and studies available in their free full text, yielding 335 records. This temporal limitation was applied to prioritise the inclusion of recent and significant studies. Additionally, gender and age filters were applied to narrow studies to females between 13-49 years of age, yielding 234 studies. Subsequently, a title screening process was conducted, involving the examination of titles and abstracts to eliminate clearly irrelevant material. This screening led to the exclusion of an additional 207 studies, leaving 34 records for further appraisal. From these, 24 records were excluded due to failing to meet the predefined inclusion/exclusion criteria or not appropriately addressing the stated objectives. Detailed inclusion/exclusion criteria are presented in Table 1. 8 records in total were selected for review and appraisal.

The following search strategy was used for The Diabetes and Metabolic Syndrome Journal:

I. "Probiotic"

AND
II. “Type II Diabetes”
AND
III. “Hormone”
AND
IV. “Management” OR “Treatment” OR “Therapy”

Using the above key words yielded 8 records. 4 of these records were duplicates of the systematic search conducted with PubMed. The remaining four studies were screened and two were selected for review and appraisal. The study selection process is illustrated in a PRISMA Flow Diagram in Figure 1.

SELECTION CRITERIA

Table 1: inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Studies available in English	Studies using exclusively male populations
Studies conducted between 2010 - 2022 inclusive	Narrative literature reviews, editorials, or periodicals
Case-control studies, retrospective chart reviews, and systematic literature reviews	Studies exploring gut hormone therapy to treat infertility caused by Type I Diabetes Mellitus or obesity, without specific reference to Type II Diabetes Mellitus
Peer-reviewed studies published in academic journals	Studies unavailable in English
Human trials and animal trials	Studies without full text availability
Studies exploring the link between Type II Diabetes Mellitus and infertility, with reference to treatment methods	Studies that exclusively addressed delay of menarche as a marker of fertility, without addressing presentation in later life
Studies exploring treatment methods to infertility, with reference to the involvement of Type II Diabetes Mellitus	

SELECTION PROCESS

When evaluating case-control studies, both human and animal trials were included in the final review. This is due to the contemporary nature of research in this field. Initial attempts to exclusively include trials with human subjects severely limited results.

To expand the scope of accessed articles while ensuring relevance, two search engines were explored. The two articles yielded from the search of The Diabetes and Metabolic Syndrome Journal were not published in PubMed. Due to the specialised nature of The Diabetes and Metabolic Syndrome Journal, the search strategy employed for PubMed had to be modified to yield relevant results from The Diabetes and Metabolic Syndrome Journal.

Due to the range of key terms associated with this area of research, narrowing search terms without excluding relevant literature proved challenging. To ameliorate this, three records were selected from the references section of records obtained from this systematic search. These studies addressed the objectives of this systematic review and met all relevant selection criteria.

All records were appraised prior to inclusion to ensure the quality of the studies. Analysis of all records was performed using The Critical Appraisal Skills Programme (CASP) Checklist for their respective genre of publication. CASP Analysis of the included articles is detailed in Table 4 (Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Salles, Cioffi and Ferreira, 2020; Jensterle et al., 2019; Shyangdan et al., 2010; Rittiphairoj et al., 2020), Table 5 (Komiya et al., 2020; Khalili et al., 2019; Khan et al., 2022; Rosenstock et al., 2021), and Table 6 (Christ and Falcone, 2018).

Results

Ten records were selected and appraised, including four case-control studies, five systematic review studies, and one retrospective chart review. Locations of studies included Japan, the United States, and Ireland. Of the case-control studies, sample sizes ranged from 36 to 478. A number of key themes emerged: significant differences existed between the gut microbiota of female patients with Type II Diabetes Mellitus and the control population. Studies furthermore demonstrated that restoration of the gut microbiota to standard conditions aided in primary management of T2DM, as well as management of symptoms, including infertility. A summation of included studies is detailed in Table 3. Abbreviations used in Table 3 are detailed in Table 2.

Objective 1: To explore the relationship between the human gut microbiota and the successful functioning of the female reproductive system in regard to successful implantation and delivery.

The relationship between bacteria population in the human gastrointestinal tract and infertility rates is primarily investigated in five of the included studies (Komiya et al., 2020; Khan et al., 2022; Christ and Falcone, 2018; Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Jensterle et al., 2019). Significant differences were found between the gut microbiome makeup of

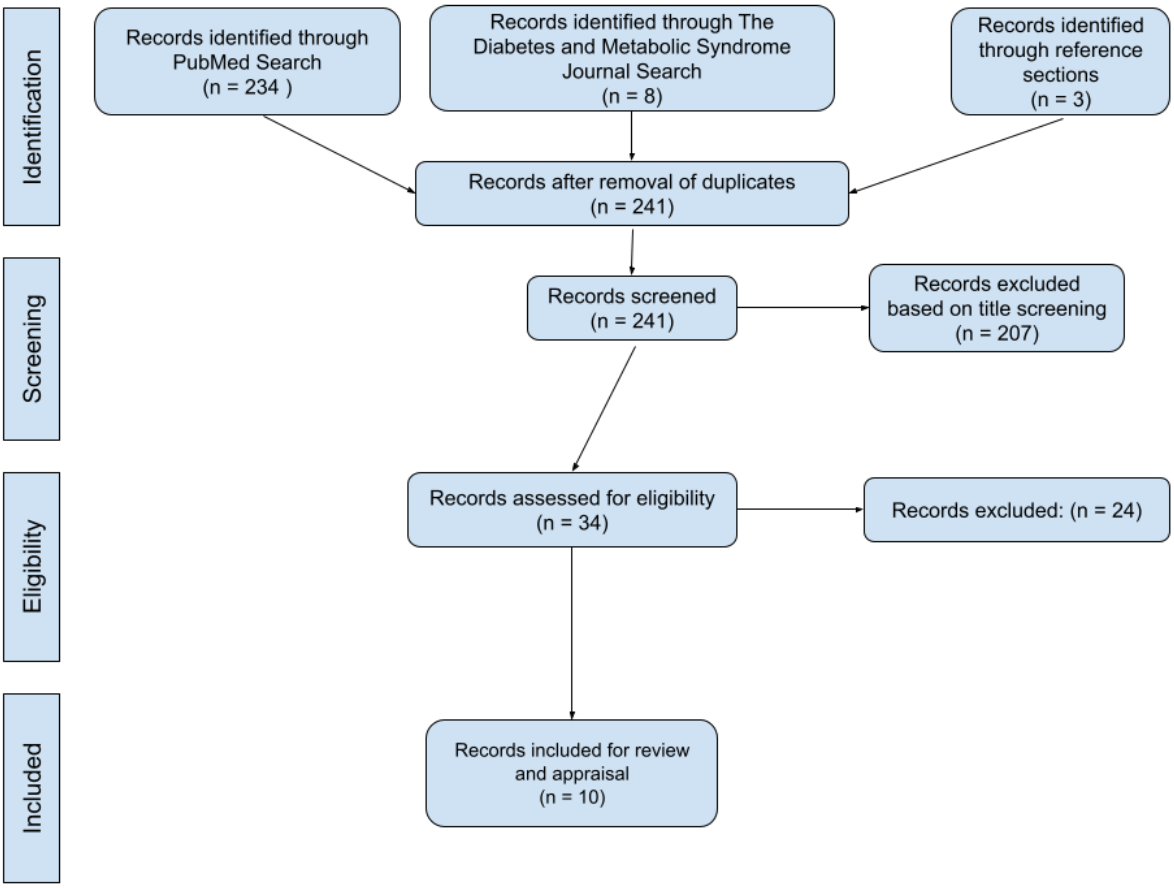

Figure 1: Flow chart illustrating study selection process.

Table 2: List of abbreviations present in Table 3

Abbreviation	**Full meaning**
BMI	Body Mass Index
PHGG	Partially Hydrolyzed Guar Gum
T1D	Type I Diabetes
T2DM	Type II Diabetes Mellitus
CFU	Colony Forming Unit
L. Casei	Lactobacillus Casei
fetuin-A	Alpha-2-HS-Glycoprotein
$SIRT_1$	Nicotinamide Adenosine Dinucleotide (NAD)-Dependent Deacetylase Sirtuin-1
HbA1c	Glycohemoglobin
PYY	Peptide Tyrosine
GIP	Glucose-Dependent Insulinotropic Polypeptide
GLP-1	Glucagon-Like Peptide 1
GLP-1R	Glucagon-Like Peptide 1 Receptor
PCOS	Polycystic Ovary Syndrome
FBG	Fasting Blood Sugar

Table 3: Summaries of articles selected for inclusion.

Author, date, location, title	**Objective**	**Study Type, Population, sample size**	**Methodology**	**Key Findings**	**Strengths and weaknesses**
Komiya et al. (2020), Japan Characterising the gut microbiota in females with infertility and preliminary results of a water-soluble dietary fibre intervention study	To contrast the gut microbiota in infertile female patients to that in fertile control subjects. To examine the effect of prebiotic partially hydrolyzed guar gum supplementation on the outcomes of fertilisation attempts in these infertile patients.	Case-control study Population: 18 women with infertility and 18 fertile controls. No significant variation in BMI, age, or diet. Sample size = 36	Prior to experiment, faecal samples were obtained, and a microbiome analysis was performed. Observed species and differences in microbiomes between control and experimental subjects were noted. 12 of the 18 infertile subjects received PHGG supplementation while undergoing embryo transfer.	Not insignificant differences were found between the gut microbiomes of the control and experimental groups prior to experimentation. PHGG supplementation aided to homogenise the gut microbiota of infertile patients to that of the control group. 58.3% of infertile participants became pregnant and carried to term.	<u>Strengths</u>: Adequate elimination of secondary factors between participants Sampling technique was standardised <u>Weaknesses</u>: Conflict of interest: funding from EA Pharma Co. LTD to support this study Small sample size
Khalili et al. (2019), Tabriz, Islamic Republic of Islam The Effects of Lactobacillus casei on Glycemic Response, Serum Sirtuin1 and Fetuin-A Levels in Patients with Type 2 Diabetes Mellitus: A Randomized Controlled Trial	To evaluate the effect of Lactobacillus Casei 01 dietary supplementation on diet, body weight, and glycemic control in patients with T2DM.	Case-control study Population: patients with T2DM, 30-50 years of age, BMI < 35 kg/m^2, patients were diagnosed with T2DM for at least one year. Exclusion criteria: patients with thyroid disorders, immunodeficiency diseases, and patients who had used alternative probiotic products within previous two months prior to testing. Sample size = 40	40 patients with T2DM were separated into two groups. One group received a daily probiotic containing 10^8 CFU/ml of L. casei 01 for 8 weeks. The secondary control group consumed placebo capsules containing maltodextrin for 8 weeks. Participants' dietary intake, anthropometric measurements, and hormonal levels were assessed at the beginning and the end of this eight-week period.	Patients consuming probiotic L. casei 01 supplements significantly reduced caloric intake compared to placebo. At the end of the 8-week period, the experimental group had improved fetuin-A levels, $SIRT_1$ levels, and glycaemic response compared to the control group.	<u>Strengths</u>: Double-blind, placebo controlled Sampling technique was standardised <u>Weaknesses</u>: Experiment fails to directly address effects of treatment on the reproductive system, only analyses effects on hormone levels

Table 3 continued

Khan et al. (2022) Ulster, United Kingdom Evidence for Involvement of GIP and GLP-1 Receptors and the Gut-Gonadal Axis in Regulating Female Reproductive Function in Mice	To evaluate the role of GIP and GLP-1 receptors in reproductive functioning. To examine how the presence or absence of these receptors affects oestrous cycling in mice.	Case-control study 14-week-old female mice bred at Ulster University Animal Unit. Body weight, non-fasted blood glucose levels, and insulin levels of control mice and experimental mice showed no significant variation. Sample size = N/A	Vaginal sampling was conducted to obtain information regarding oestrous cycling in female mice prior to experimentation. Samples were collected and examined over a twenty-day period. GIPR -/- Mice, GLP-1 Receptor -/- Mice, and Wild Type Mice underwent 3 breeding periods of 21 days. Following the 1st breeding period, all mice were treated with oral metformin prior to the subsequent two breeding periods.	Female mice deficient in GIP and GLP-1 gut hormones had significantly different oestrous cycling compared to control mice. Fewer of these deficient mice were capable of breeding with wild type male mice across three breeding cycles. Mice capable of breeding had significantly smaller litters than the wild type of control mice. Supplementation with oral metformin significantly improved litter size in female mice. Supplementation was not associated with improvements in pregnancy outcomes.	Strengths: Strong correlation between gut hormone levels and reproductive functioning Sampling technique was standardised Weaknesses: Animal-based study; limited extrapolation to human species No provided sample size, only percentages given in results. Challenging to assess validity of results
Christ et al. (2018), Ohio, United States Bariatric Surgery Improves Hyperandrogenism, Menstrual Irregularities, and Metabolic Dysfunction Among Women with Polycystic Ovary Syndrome (PCOS)	To characterise the ability of bariatric surgery to improve fertility in patients with PCOS.	Retrospective chart review 930 women who had undergone bariatric surgery from 2009-2014 inclusive. 44 women with PCOS and 63 controls were chosen.	Evaluations were done of pre-operative and post-operative menstrual regularity, ovarian volume, and BMI measurements in bariatric surgery patients. Further analysis of reproductive hormones, lipid imbalance, and blood sugar levels was performed.	Bariatric surgery led to significant reduction in androgen levels, hyperandrogenism, and irregular menstrual cycling. Study suggests a strong correlation between bariatric surgery and improved fertility amongst female patients.	Strengths: Large sample size Weaknesses: Secondary variable: unable to distinguish if improved fertility is due to weight loss or hormonal influences

Table 3 continued

Rosenstock et al. (2021), multicentre Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with Type II Diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial	To examine the use of GIP hormones and GLP-1 Receptors in the treatment of T2DM and its secondary effects. To evaluate the side effects of gut hormone therapy, and to question if it is comparable to dietary and exercise regime changes.	Placebo-control study Adult participants with T2DM. HbA1c ≥ 7·0% to ≤ 9·5%. BMI ≥ 23 kg/m^2. Participants had a stable weight (no change ≥ 5%) during the previous 3 months. Participants agreed to not alter diet or exercise habits during the study with the intent of reducing body weight. Exclusion criteria: included patients with T1D and use of any oral antihyperglycemic medication for 3 months prior to screening. N = 478	Parallel group trials were conducted in four countries across 52 research centres. Following a 3-week screening period, Patients underwent 40 weeks of tirzepatide treatment, followed by a 4-week follow-up period. Patients were given tirzepatide in escalating doses, 15mg dosage was achieved at 20 weeks. Patients were randomly assigned to receive a placebo, or a once weekly oral dose of 15mg tirzepatide.	Participants receiving hormone therapy had significantly improved glycaemic control compared to the placebo group. Experimental group also experienced a reduction in body weight and had no increased risk of hypoglycaemia.	<u>Strengths</u>: random sampling Large sample size Double-blind, placebo controlled Patients were thoroughly examined before and after receiving supplementation <u>Weaknesses</u>: Study does not distinguish effects on female patients from male patients
Baker et al. (2017), Arizona, United States Oestrogen-gut microbiome axis: Physiological and clinical implications	To contemplate a number of oestrogen-modulated diseases, and how they are impacted by the gut microbiota. To examine treatment options for reproductive complications caused by metabolic diseases, including T2DM.	A systematic review Inclusion criteria: search terms included “Oestrogen and Gut Microbiome”, “Metabolic Syndrome”, and “Infertility”. No limitations were applied to publication dates.	Search using PubMed and Google Scholar databases to collect scientific literature. All included studies were manually examined for relevance to the topic.	Decreased microbial diversity in the gut microbiome is strongly associated with decreased circulating oestrogen concentrations. Lowered circulating oestrogen concentrations inhibit fertility. Oral supplementation with probiotics is shown to reduce the effects of multiple metabolic syndromes, including T2DM.	<u>Strengths</u>: Extensive range of sources provided <u>Weaknesses</u>: No limitations on publication dates, possible outdated material

Table 3 continued

Salles et al. (2020), São Paulo, Brazil Probiotics supplementation and insulin resistance: a systematic review	To research the role of probiotics on markers of insulin resistance in both human and animal trials.	Systematic Review 34 probiotic intervention trials included. Inclusion Criteria: Original research articles published January 1990 - January 2020 inclusive. Search terms included "Probiotics", "Gastrointestinal Microbiome", and "Type II Diabetes".	Review based on PRISMA guidelines Two independent reviewers analysed records that met inclusion/exclusion criteria. Double entry was used to ensure accuracy of all data.	In 79% of included articles, probiotic intervention was correlated with significant beneficial alterations to insulin resistance markers. In these trials additional improvements in inflammation and gut microbiota composition were associated with probiotic supplementation. In 15% of included articles, one of the defined markers of insulin resistance improved upon probiotic supplementation. In two remaining trials, no change between control and study was detected.	<u>Strengths</u>: Objective methodology and assurances of accurate information Comparisons were made between various probiotic supplementations, probiotic supplementation under varying time periods, and probiotics compared to antidiabetic agents. Increases relevance of findings <u>Weaknesses</u>: Lack of direct research related to reproductive functioning Review contained insufficient human trials to effectively advocate for probiotic treatment
Jensterle et al. (2019) Ljubljana, Slovenia The role of glucagon-like peptide-1 in reproduction: from physiology to therapeutic perspective	To perform an in-depth examination of the relationship between GLP-1 receptor agonists and reproductive functioning. To consider therapeutic uses of GLP-1 receptor agonists to treat infertility, especially as it is related to T2DM and obesity.	Systematic Review Inclusion criteria: search terms included "GLP-1", "GLP-1R" combined with terms including "Fertility" and "Reproductive axis", no limitations were placed on publication years. All articles were screened for relevance.	Series of PubMed data searches. Identified 983 potentially relevant pieces. Through considerate screening, final review included 6 observational studies, 24 interventional reports, 4 case reports, 1 systematic review, and 2 narrative reviews. Material was supplemented by authors' knowledge and research experience.	GLP-1 hormones seem to have anti-inflammatory and anti-fibrotic effects in the endometrium. Increased levels of these hormones mitigate damage to the endometrium associated with T2DM and obesity. Ovulation rate and menstrual frequency are consistently improved in studies where patients are treated with GLP-1 receptor agonists including exenatide and liraglutide.	<u>Strengths</u>: A highly experienced reviewer was consulted during the screening process to choose relevant studies for inclusion <u>Weaknesses</u>: Multiple underdeveloped concepts due to lack of research in contemporary areas No limitations on publication years applied

Table 3 continued

Shyangdan et al. (2010) Aberdeen, Scotland Glucagon-like peptide analogues for type 2 diabetes mellitus: systematic review and meta-analysis	To provide evidence for the effectiveness of GLP-1 agonists in treatment and management of T2DM. To contrast the performance of GLP-1 agonists as a medication with common oral glucose-lowering drugs.	Systematic Review 28 randomised control trials were included. Studies compared GLP-1 agonists to placebos or other glucose-lowering agents. Patient population: patients with T2DM suffering from inadequate glucose control on a single oral agent or on dual therapy.	Scientific literature databases, including Medline, Embase, and the Cochrane Library and Web of Science, were searched. Three authors examined potential studies for relevance. Differences in opinion were resolved by a third party. Inclusion criteria: randomised control trials of patients with T2DM, studies conducted in full, studies conducted over a minimum duration of 8 weeks. Exclusion criteria: GLP-1 agonists used as the singular form of therapy in patients with T2DM.	All GLP-1 agonists reduced HbA1c by about 1% compared to placebo. Different agonists showed different levels of effectiveness in reducing HbA1c. Exenatide and liraglutide showed the greatest reductions in HbA1c and the greatest proportion of weight loss in patients with T2DM across trials. Most common adverse effects of GLP-1 agonists were nausea and vomiting during the early experimental period.	Strengths: Only included studies comparing GLP-1 agonists to other treatment options Thorough selection process of included studies Weaknesses: No direct link on reproductive functioning examined
Rittiphairoj et al. (2021), Maryland, United States Probiotics Contribute to Glycaemic Control in Patients with Type II Diabetes Mellitus: A Systematic Review and Meta-Analysis	To assess the effectiveness of probiotics as a management option for patients with T2DM over short- and long-term time frames. To explore differing effects of variables on probiotic treatment; variables included secondary, simultaneous, treatment plans, such as insulin therapy.	Systematic Review 26 trials were included in meta-analysis (1947 participants) Inclusion criteria: included studies that compared probiotics to placebo, comparative probiotics, or no intervention. Exclusion criteria: included studies that provided insufficient data and studies that did not address T2DM or prediabetes patients.	A search was performed using the scientific databases PubMed, Embase, and Cochrane. Trials conducted between January 2011 and February 2019 were included in the final paper. All included articles were randomised controlled trials performed with participants with prediabetes or T2DM. Two reviewers screened potential studies and assessed risk of bias using “Cochrane Risk of Bias 2”.	Probiotics reduced FBG more than the placebo/no intervention group with an average difference of –12.99 mg/dL in the short-term and –2.99 mg/dL in the long-term. There is evidence for reduced HbA1c both in short-term and long-term studies under probiotic supplementation. Effects were stronger in participants not undergoing insulin therapy.	Strengths: Steps were taken to reduce bias, including Cochrane Risk of Bias 2 Systematic review was registered with International Prospective Register of Systematic Reviews and a pre-established protocol was followed when conducting the review Large sample size Weaknesses: Unclear distinguishing between effects of various strains of probiotics.

Table 4: CASP Checklist Findings for Systematic Reviews

	Is a clearly focused question addressed ?	Are all included papers appropriate ?	Are all relevant studies included ?	Was the author's assessment of study quality sufficiently rigorous?	Was the author's method of combining results from studies valid?	What are the results of the systematic review?	Are the results sufficiently precise?	Can results be applied to the target population ?	Were all important outcomes considered ?	Do the benefits of this review outweigh the costs?
Baker et al. (2017)	Yes	Yes	Yes	No	Yes	See Table 3	Yes	Yes	Yes	Yes
Salles et al. (2020)	Yes	Yes	Yes	Yes	Yes	See Table 3	Yes	No	Yes	Yes
Jensterle et al. (2019)	Yes	Yes	Yes	No	Yes	See Table 3	Yes	Yes	No	Yes
Shyangdan et al. (2010)	Yes	Yes	Yes	Yes	Yes	See Table 3	Yes	Yes	Yes	Yes
Rittiphairoj et al. (2021)	Yes	Yes	Yes	Yes	Yes	See Table 3	No	Yes	Yes	Yes

Table 5: CASP Checklist Findings for Case-Control Studies

	Does the study address a clear objective?	Is appro-priate meth-odology used to assess the objective?	Were cases selected in an accep-table manner ?	Were controls selected in an accept-able manner?	Were measures taken to minimise bias?	Were second-ary variables between groups controlled?	Have authors included confoun-ding factors in their analysis ?	How large was the treatment effect?	How precise was the estimate of treatment effect?	Overall , are the results of the study trust-worthy ?	Can results be applied to the target popula - tion?	Do the results of this study correlate to other available evidence?
Komiya et al. (2020)	Yes	Yes	Yes	Yes	Yes	Yes	No	See Table 3	See Table 3	Yes	Yes	Yes
Khalili et al. (2019)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	See Table 3	See Table 3	Yes	No	Yes
Khan et al. (2022)	Yes	Yes	Yes	Yes	Yes	Yes	No	See Table 3	See Table 3	Yes	No	Yes
Rosenstock et al. (2021)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	See Table 3	See Table 3	Yes	Yes	Yes

Table 6: CASP Checklist Findings for Cohort Studies

	Does the study address a clearly focused issue?	Was the Cohort recruited in an acceptable manner?	Was exposure measured to minimise bias?	Were outcomes measured to minimise bias?	Have confound-ing factors been identified and analysed?	Was the follow up of subjects sufficiently long and accurate?	What are the results of this study?	How precise are the results of this study?	Overall, are the results of the study trustworthy?	Can results be applied to the target population?	Do the results of this study correlate to other available evidence?
Christ et al. (2018)	Yes	Yes	Yes	Yes	No	Yes	See Table 3	See Table 3	Yes	No	Yes

female patients with normal and impaired reproductive function. Depleted circulating oestrogen concentrations, irregular menstrual cycling, hyperandrogenism, and endometrium tissue damage were all used as markers of diminished fertility.

Komiya found infertile patients had increased abundance of the phylum Verrucomicrobia, the genera Unclassified [Barnesiellaceae], and Phascolarctobacterium. The gut microbiome of infertile women is furthermore more likely to contain a decreased amount of the genera Stenotrophomonas, Streptococcus, and Roseburia (Komiya et al., 2020). Baker's systematic review found increased diversity is related to improved fertility via increased circulating oestrogen concentrations (Baker, Al-Nakkash and Herbst-Kralovetz, 2017).

Khan's case-control study explored the effects of GIP and GLP-1 Hormones on reproductive functioning. This trial found mice with diminished amounts of GIP receptors and GLP-1 receptors had significantly ($p < 0.05$ and $p < 0.01$) deranged oestrous cycling compared to control mice. These mice furthermore produced significantly fewer litters than wild type mice, and litters produced were notably smaller ($p < 0.001$ - $p < 0.05$) than control litters. However, there were no differences in pregnancy outcomes between control and experimental mice (Khan et al., 2022). Jensterle corroborated these findings, using endometrium inflammation and fibrosis as principal measures of infertility (Jensterle et al., 2019).

It has been well established in alternative studies that bariatric surgery alters hormone concentrations in the gut microbiome (Ulker and Yildiran, 2019). Christ explored the effects of bariatric surgery on fertility rates in previously infertile patients. Surgery led to significant reduction in androgen levels, hyperandrogenism, and irregular menstrual cycling (Christ and Falcone, 2018).

Objective 2: To consider the therapeutic use of hormonal medications and dietary probiotics to improve the culture of bacteria in the gut, and to explore the effect these therapies have on symptom management in patients with Type II Diabetes, with a special focus on fertility.

Nine of the included studies addressed treatment options for Type II Diabetes Mellitus and associated infertility, with focus on the human gut microbiome (Komiya et al., 2020; Khalili et al., 2019; Khan et al., 2022; Rosenstock et al., 2021; Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Salles, Cioffi and Ferreira, 2020; Jensterle et al., 2019; Shyangdan et al., 2010; Rittiphairoj et al., 2020).

Through dietary fibre supplementation, Komiya's case-control study shows a normal gut microbiome may be re-established in patients with T2DM. Following supplementation, infertile patients underwent embryo transfer, where 58.3% had successful pregnancies. Successful patients demonstrated a significant decrease in Paraprevotella and Blautia levels and an increase in the abundance of Bifidobacterium (Komiya et al., 2020).

Khalili studied effects of Lactobacillus casei 01 supplementation on symptoms in T2DM patients. Following treatment, patients had significantly decreased serum fetuin-A level, insulin concentrations, insulin resistance, and fasting blood sugar. These patients furthermore had increased serum SIRT1 levels (Khalili et al., 2019). These results were corroborated by Rittiphairoj's systematic review (Rittiphairoj et al., 2020). Lactobacillus was additionally studied in Salles's systematic review. Salles's results corroborated Khalli's

findings, with patients receiving supplementation showing improved lipid profile, inflammatory and oxidative markers, short-chain fatty acid production and gut microbiota composition (Salles, Cioffi and Ferreira, 2020). Finally, Rittiphairoj examined Lactobacillus as a treatment option for T2DM patients. Supplementation resulted in reduced fasting blood glucose compared to the placebo group with an average difference of –12.99 mg/dL in the short-term and –2.99 mg/dL in the long-term (Rittiphairoj et al., 2020).

Two included studies explored the effect of metformin, a drug treatment used to restore hormone levels in the body, on infertility caused by T2DM (Khan et al., 2022; Baker, Al-Nakkash and Herbst-Kralovetz, 2017). In mice trials conducted by Khan, metformin increased litter size (approximate 100% increase in litter size) (Khan et al., 2022). Baker's study furthermore showed Metformin alters the gut microbiome by increasing Akkermansia levels (Baker, Al-Nakkash and Herbst-Kralovetz, 2017).

A number of included studies explored the effects of GIP and GLP-1 receptors and agonists on glycaemic control and reproductive functioning in T2DM patients. Rosenstock explored this phenomenon through tirzepadide supplementation over a 40-week trial period. Patients receiving 15mg supplementation experienced a reduction in mean HBA1c by 2.07% (compared to placebo group HBA1c, which showed an increase of 0.04%). Furthermore, 31-52% of patients on tirzepatide achieved HbA1c of less than 5·7%, compared to 1% of patients receiving placebo supplementation (Rosenstock et al., 2021). Shyangdan corroborated these findings; their systematic review showed a range of GLP-1 agonists reduced HbA1c by about 1% compared to placebo administration in T2DM patients (Shyangdan et al., 2010). Jensterle's systematic review had similar findings; patients treated with GLP-1 receptors showed improved ovulation rate and menstrual frequency (Jensterle et al., 2019). Specifically, both Jensterle's and Shyangdan's systematic reviews examined the effects of exenatide and liraglutide GLP-1 receptor agonists on general T2DM management (Shyangdan et al., 2010), as well as direct effects on reproductive functioning (Jensterle et al., 2019). Both articles found significant improvements in markers of T2DM, as well as symptom management (Jensterle et al., 2019; Shyangdan et al., 2010).

Discussion

This systematic review consisting of ten studies aims to analyse the relationship between the gut microbiome and Type II Diabetes, with a focus on treatment options of female infertility through manipulation of this relationship. There is evidence suggesting a correlation between the gut microbiome and the reproductive capacity of female patients with T2DM (Komiya et al., 2020; Khan et al., 2022; Christ and Falcone, 2018; Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Jensterle et al., 2019). Furthermore, a number of successful trials demonstrated an association between various gut therapies and improvements to reproductive capabilities (Komiya et al., 2020; Khalili et al., 2019; Khan et al., 2022; Rosenstock et al., 2021; Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Salles, Cioffi and Ferreira, 2020; Jensterle et al., 2019; Shyangdan et al., 2010; Rittiphairoj et al., 2020).

STRENGTHS OF REVIEW

Studies (Komiya et al., 2020; Khalili et al., 2019) made efforts to eliminate possible confounders. BMI, age, and years since diagnosis of T2DM were consistent between intervention and control groups. While this limits application of studies to a wider population, it largely eliminates secondary variables that could influence findings.

All included studies were conducted in a rigorous manner, with specific efforts being made to reduce bias. Sampling techniques were standardised across studies (Komiya et al., 2020; Khalili et al., 2019; Khan et al., 2022; Rosenstock et al., 2021). Placebo-controlled studies utilised double-blind testing procedure (Khalili et al., 2019; Rosenstock et al., 2021). Systematic reviews were based on strict guidelines, including PRISMA guidelines (Salles, Cioffi and Ferreira, 2020), and multiple independent reviewers screened included records to reduce possible bias (Salles, Cioffi and Ferreira, 2020; Jensterle et al., 2019; Shyangdan et al., 2010; Rittiphairoj et al., 2020). Shyangden's and Rittiphairoj's systematic reviews utilised Cochrane Collaboration's tool for risk assessment of bias (Shyangdan et al., 2010; Rittiphairoj et al., 2020).

Selection criteria of this systematic review was carefully chosen to yield a range of relevant material.

Appropriate use of key terms, filters, and inclusion and exclusion criteria was strictly adhered to. Efforts were made to reduce risk of bias, including a CASP analysis of included systematic reviews (Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Salles, Cioffi and Ferreira, 2020; Jensterle et al., 2019; Shyangdan et al., 2010; Rittiphairoj et al., 2020), case-control studies (Komiya et al., 2020; Khalili et al., 2019; Khan et al., 2022; Rosenstock et al., 2021), and retrospective chart reviews (Christ and Falcone, 2018).

LIMITATIONS OF REVIEW

This systematic review does not address the effect of BMI on treatment outcomes. Studies (Komiya et al., 2020; Khalili et al., 2019; Rosenstock et al., 2021; Rittiphairoj et al., 2020) used exclusively female patients of a healthy BMI. All chosen subjects had similar BMI (Komiya et al., 2020: fertile control BMI 20.78 ± 2.39, infertile patient BMI 21.41 ± 3.34). (Khalili et al., 2019: BMI < 35 kg/m2). (Rosenstock et al., 2021: Control BMI = 31.7, experimental BMI = 31.5), (Rittiphairoj et al., 2020: Control BMI 29.14 ± 0.78, Intervention BMI: 28.95 ± 0.67). This eliminates the secondary factor of weight on glycaemic control and fertility. However, it severely limits the applicability of these treatments on a wider population. Currently, 90% of adult patients with T2DM are overweight or obese. (Public Health England, 2014). One of the objectives of this systematic review is to evaluate treatment options for infertility caused by T2DM. Further research is required to establish the effect of obesity on treatment outcome.

The findings of some included studies addressed the relationship between metabolic syndromes, including T2DM, and the gut microbiome, without explicit reference to effects on reproductive functioning (Khalili et al., 2019; Rosenstock et al., 2021; Salles, Cioffi and Ferreira, 2020; Shyangdan et al., 2010; Rittiphairoj et al., 2020). There are alternative included studies (Komiya et al., 2020; Khan et al., 2022; Christ and Falcone, 2018; Baker, Al-Nakkash and Herbst-Kralovetz, 2017; Jensterle et al., 2019) and established scientific literature (Moxthe et al., 2020; Qi et al., 2021) that demonstrate a relationship between the gut microbiome and the female reproductive system. However, it is still extrapolation to assume probiotic and hormonal treatments that improved T2DM markers would additionally improve fertility in these patients. There are limited applications of these studies for fertility treatments for patients.

A mice-based study was included in this report (Khan et al., 2022), as well as a systematic literature review that focused on animal-based trials (Salles, Cioffi and Ferreira, 2020). Due to the novel nature of this research, inclusion of these studies was vital to provide an encompassing image of research in this field. However, there are severe limitations in applications of these results to a human population.

This systematic review was conducted by one author; this presents a risk of bias in selection and appraisal of included records. Furthermore, this systematic review was constrained by its inclusion criteria, which only included articles available in their full, free text. This inherently limits the breadth of articles included in this paper. A systematic review including restricted articles may yield additional or divergent results. To supplement the systematic review process, handsearching was undertaken. This involved scrutinising the reference lists of the included articles to identify additional case-control studies, retrospective chart reviews, and systematic literature reviews that were not captured in the original search. However, the use of handsearching compromises the integrity of this systematic review. This underscores the importance of employing flexible key terms throughout a systematic search process to ensure a comprehensive review.

Conclusion

Alterations to the hormonal and bacteria composition of the gut microbiome are associated with improvements in glycaemic control and fertility in patients. Therapeutic use of hormonal and bacterial supplementation may improve infertility caused by Type II Diabetes Mellitus in female patients. However, this is still a novel area of research, and there is a notable lack of human-based clinical trials available examining the effects of hormonal and bacterial supplementation on Type II Diabetes Mellitus and the disease's symptoms. Further research is required to clarify the link between metabolic diseases, the gut microbiome, and reproductive functioning. Once these relationships are less ambiguous, more significant progress can be made in management of this disease.

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