GVK Biosciences Private Limited

Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study

Regulatory Sponsor :	Navchetana Kendra
Study Product :	**FLORAS DROP**
Protocol Number :	CT4510

SIGNATURE(S)

GVK BIOSCIENCES PRIVATE LIMITED INDIA

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1. INTRODUCTION

Study Summary

Title	*Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study.*
Protocol Number	CT4510
Methodology	This study is a quantitative, descriptive sub-study based on unpublished data collected in two RCT´s examining the effect of acupuncture in colic. Screening lists for infants who were not included and background data for included infants from a questionnaire were analyzed.
Study Duration	ONE MONTH
Study Center(s)	GVK bioscience Private Limited
Objectives	Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study.
Number of Subjects	144 infants studied
Main Inclusion Criteria	Inclusion criteria for both studies were healthy infants, not older than eight weeks, who according to a daily baseline registration fulfilled the criteria for colic: crying/fussing more than three hours/ day at least three days per week. At least one of the parents had to speak Swedish.
Study Product	**FLORAS DROP**
Dose, Route	**ORALLY**

1. 1 BACKGROUND

Infantile colic affects 10-17% of infants in the Western world (Canivet et al 2002; Lucassen, 2010). Colic is often defined as crying or fussing for at least three hours per day, three days per week in otherwise healthy infants under three months of age (Wessel et al 1954).

Although infantile colic is a benign condition with spontaneous recovery it causes substantial distress to the parents as well as the infant, which can affect essential early interaction and family relations. Excessive crying is associated with depression in mothers during the postpartum period. and has been associated with an elevated risk of child abuse in multiple studies.

et al 200. Infantile colic is a frequent cause of pediatric consultations at Child Health Centers (CHC). It is of great importance that health professionals do not dismiss the condition as harmless and temporary, but rather respond to the parents´ mental and physical exhaustion with respect and proficiency. Nurses working at the CHC have therefor an important but difficult role to support these parents since there is still no known cure and several treatments are lacking scientific evidence.

Pathogeneses

Although the etiology is unknown there have been multiple theories for the cause of infantile colic and unanimity is still lacking in its treatment. Theories about inadequate interaction between the parents and the infant as a cause of colic have been considered controversial (Savino, 2007). Another possible explanation is intolerance to cows' milk protein. Approximately 5-20% of the infants are relieved of their symptoms when excluding cow's milk protein from the breastfeeding mother's diet and/ or the infant's formula for five days (Iacovou et al 2012). A common theory has been the lack of normal gastrointestinal function due to the infants nervous or digestive system being immature.

This theory has led to scientists studying the effect of simethicone to help reduce gas (Savino, 2007) and the effect of probiotics (Lactobacillus Reuteri) to help normalize the composition of the intestinal microbial environment (Savino et al., 2005; Lehtonen et al 1994). Complementary factors that could increase the risk for infantile colic might be associated with maternal smoking, infant being a firstborn, increased maternal age and atopic eczema.

1.2 INVESTIGATIONAL AGENT

Simeticone is used to relieve the painful symptoms of too much gas in the stomach and intestines. Oral ant flatulent agent, available as a single agent or in combination with antacids or antidiarrheal. Simeticone-coated cellulose suspension is used to enhance upper GI visibility in ultrasound images and is not used as an anti flatulent.

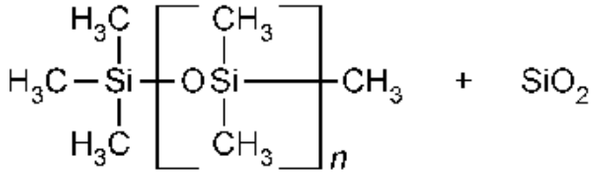
ACTIVE INGREDIENT *:* Simeticone

CHEMICAL NAME : dioxosilane; trimethyl (trimethylsilyloxy) silane

MOLECULAR FORMULA: $(C_2H_6OSi)_n \cdot (SiO_2)_m$

222.462 g/mol

STRUCTURAL FORMULA:



1.3 PRECLINICAL DATA

None stated.

1.4 Clinical Pharmacology

Pharmacodynamics

Simeticone acts by decreasing the surface tension of gas bubbles, thus facilitating their coalescence and expulsion as flatus or belching. It also prevents the formation and accumulation of mucus-enclosed pockets of gas in digestive tract. Simeticone also facilitates the passage of gas through bowel lumen and allows patients to excrete a greater volume of gas at one time, thereby reducing the number of flatus events.

Mechanism of action

Decreases the surface tension of gas bubbles thereby disperses and prevents gas pockets in the GI system.

Absorption

Not absorbed following oral administration.

Volume of distribution

Simeticone is pharmacologically inert and is not absorbed from the gastrointestinal tract.

Protein binding

Simeticone is pharmacologically inert and is not absorbed from the gastrointestinal tract.

Metabolism

The drug is not absorbed and it is excreted in the feces unchanged.

Route of elimination

Excreted unchanged in feces.

Half life

The drug is not absorbed.

Clearance

Excreted unchanged in feces.

Toxicity

LD50 (oral, rat): 4070 mg/Kg.

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Clinical particulars

Therapeutic indications

Anti-flatulent defaming agent for the symptomatic relief of flatulence, wind pains, bloating, abdominal distension and other symptoms associated with intestinal gas.

Posology and method of administration

Oral

Adults, the elderly and children:

0.5 -1 ml of drops taken 3 or 4 times daily or as required for relief

Contraindications

Hypersensitivity to any of the ingredients.

Special warnings and precautions for use

None stated.

Interaction with other medicinal products and other forms of interaction

None reported.

Fertility, pregnancy and lactation

As simeticone is not absorbed, it is not anticipated that Floras will have any adverse effects on pregnancy and lactation. However, as with all drugs, caution should be exercised in these conditions.

Effects on ability to drive and use machines

None stated.

Undesirable effects

As simeticone is not absorbed from the gastro-intestinal tract, adverse effects attributable to the active ingredient would not be expected.

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the Yellow Card Scheme at:

Overdose

No cases of overdose have been reported. Theoretically, constipation may occur. Treat with fluids and keep under observation.

Pharmacological properties

Pharmacodynamic properties

Floras contains activated simeticone, a chemically inert gastric de-foaming agent which alters the elasticity of interfaces of mucous-embedded bubbles in the gastro-intestinal tract. The gas bubbles are thus broken or coalesced and in this form, the gas is more easily eliminated through belching or passing flatus.

Pharmacokinetic properties

Activated simeticone is not absorbed from the gastrointestinal tract and does not interfere with gastric secretion or absorption of nutrients. Following oral administration, it is excreted unchanged in the faeces.

Preclinical safety data

None stated.

1.4 CLINICAL DATA TO DATE

Clinical symptoms were monitored daily. Blood cell counts were obtained at least twice a week, and biochemical parameters were measured at least once a week. Blood culture, serum endotoxin, β-d-glucan, and chest radiographs were obtained before starting antibacterial therapy and in the case of a sustained or worsened pattern of fever.

1.5 DOSE RATIONALE AND RISK/BENEFITS

Dose Rationale

0 to <2 years: 20 mg orally 4 times a day.

2 to 12 years: 40 mg orally 4 times a day. For the 40 mg strips, allow 1 strip to dissolve on the tongue as needed after meals and at bedtime. Do not exceed 6 strips in 24 hours except under the advice and supervision of a physician.

12 to 18 years: 40 to 125 mg orally after meals and at bedtime as needed, not to exceed 500 mg/24 hours.

Potential Risks

SIDE EFFECTS: There are no reports of any side effects due to this medication. However, tell the doctor if your child experiences any unpleasant effects while taking this medication. A very serious allergic reaction to this product is rare. However, seek immediate medical attention if you notice any of the following symptoms of a serious allergic reaction: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.This is not a complete list of possible side effects.

Potential Benefits

Anti-flatulent defoaming agent for the symptomatic relief of flatulence, wind pains, bloating, abdominal distension and other symptoms associated with intestinal gas.

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Risk Benefit Ratio

If you have an **allergy** to Simeticone or any other part of Simeticone suspension.
If you are allergic to any drugs like this one, any other drugs, foods, or other substances.

Tell your doctor about the allergy and what signs you had, like rash; **hives**; itching; shortness of breath; **wheezing**; cough; swelling of face, lips, tongue, or throat; or any other signs.

This medicine may interact with other drugs or health problems.
Tell your doctor and pharmacist about all of your drugs (prescription or OTC, natural products, **vitamins**) and health problems. You must check to make sure that it is safe for you to take Simeticone suspension with all of your drugs and health problems. Do not start, stop, or change the dose of any drug without checking with your doctor.

2 STUDY OBJECTIVES

Primary Objective:

Adverse events [Time Frame: 1 month.] [Designated as safety issue: Yes]

Enrollment:	144 infants studied
Study Start Date:	26 April 2012
Study Completion Date:	25 may 2012
Primary Completion Date:	25 may 2012 (Final data collection date for primary outcome measure)

Secondary Objective:

Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study

3 STUDY DESIGN

3.1 GENERAL DESIGN

This study is a quantitative, descriptive sub-study based on unpublished data collected in two RCT´s examining the effect of acupuncture in colic. Screening lists for infants who were not included and background data for included infants from a questionnaire were analyzed.

3.2 PRIMARY STUDY ENDPOINTS

The primary outcome in the present sub-study was the reasons for which screened infants were not included in the two main trials. A secondary outcome was the frequencies of parents using simethicone and probiotics and their reported effect of the treatment.

3.3 SECONDARY STUDY ENDPOINTS

Other secondary outcomes were the association between treatment with simethicone and probiotics and background characteristics and the difference in frequency of treatment with simethicone and probiotics over time between the two main studies.

3.4 PRIMARY SAFETY ENDPOINTS

Primary outcomes were evaluated by mother’s opinion about responses to the treatment, number of daily episodes of colic, and time spent crying, measured by a chronometer. Secondary outcomes were number of milk regurgitation, vomiting, diarrhea, constipation, and drowsiness.

4 SUBJECT SELECTIONS AND WITHDRAWAL

4.1 INCLUSION CRITERIA

Inclusion criteria for both studies were healthy infants, not older than eight weeks, who according to a daily baseline registration fulfilled the criteria for colic: crying/fussing more than three hours/ day at least three days per week. At least one of the parents had to speak Swedish.

4.2 EXCLUSION CRITERIA

Exclusion criteria were infants born before gestational week 37, insufficient weight gain, having tried acupuncture or taking prescribed medication. Simethicone and/ or probiotics were allowed.

4.3 SUBJECT RECRUITMENT AND SCREENING

Inclusion criteria for both studies were healthy infants, not older than eight weeks, who according to a daily baseline registration fulfilled the criteria for colic: crying/fussing more than three hours/ day at least three days per week. At least one of the parents had to speak Swedish.

Exclusion criteria were infants born before gestational week 37, insufficient weight gain, having tried acupuncture or taking prescribed medication. Simethicone and/ or probiotics were allowed. In the first trial parents to 210 infants registered their infants crying and fussing in a diary during at least three days. Of these infants, 90 fulfilled the criteria for colic and were included. In the second trial, ACU-COL (Landgren et al., 2015), 426 infants were initially screened for participation in the study and after daily registration of crying and fussing in a diary during a baseline week, 152 infants met the criteria and were included in the study (Landgren & Hallström, in manuscript).

Sample size

The power analysis for the first trial (Landgren et al., 2010) for detecting a significant difference in crying between the intervention and control group was calculated to 40 infants per group. For ACU-COL the sample size calculation, conducted by an independent statistician at the center for clinical research and development (R&D, Region Skåne), found that a total of 144 infants were needed to detect a significant difference in crying between infants who got acupuncture and infants who did not (Landgren et al., 2015).

Outcomes

The primary outcome in the present sub-study was the reasons for which screened infants were not included in the two main trials. A secondary outcome was the frequencies of parents using simethicone and probiotics and their reported effect of the treatment.

Other secondary outcomes were the association between treatment with simethicone and probiotics and background characteristics and the difference in frequency of treatment with simethicone and probiotics over time between the two main studies.

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Laboratory and physical exam screening

A blood sample will be drawn from each subject and the following investigations carried out:

TESTS	NORMAL RANGE	UNIT
WBC	4.8 - 10.8	$\times 10^3/\mu l$
RBC	4.7 - 6.1 (M)	$\times 10^6/\mu l$
HGB	14 - 18 (M)	g/dl
HCT	42 - 52 (M)	%
MCV	80 - 94 (M)	f l
MCH	27 - 32	p g
MCHC	32 - 37	g /dl
RDW	11.5 - 14.5	%
PLT	150 - 400	$\times 10^3/\mu l$
NEUT	40.0 - 74.0	%
LYMP	19.0 - 48.0	%
MONO	3.4 - 9.0	%
EOS	0.0 - 7.0	%
BASO	0.0 - 1.5	%

All hematological parameters will be assessed using a Technician H-1 system, or equivalent.

Blood sample will be stained and examined for:

- Differential white cell count

- Red cell morphology

A blood sample will be taken for assay for the following:

TESTS	NORMAL RANGE	UNIT
BILIRUBIN	2 - 20	µmol/l
ALK. PHOS	98 - 279	IU/I
AST	< 37	IU/I
CREATININE	50 - 130	µmol/l

These will be assayed using a Hitachi 737/ 704 system or equivalent.

Assessment

Specimens of the morning sputum will be collected once before commencement of therapy, and after one year treatment.

The occurrence of symptoms will be recorded pre-treatment, and at the end of therapy.

Clinical laboratory tests (hematology and blood chemistry) will be performed at the same time. Appropriate aerobic and anaerobic cultures will be required for all patients at the time of the initial procedure within 24 hours of enrollment for in vitro susceptibility in the local microbiology laboratory using standard disk diffusion or MIC test using NCCLS guidelines and either approved breakpoints.

Documentation of Screening

Screening assessments will be performed to determine subject eligibility criteria, which will be documented on a case report form.

The PI will confirm and sign off on the inclusion and exclusion criteria on a case report form (CRF) prior to the patient formally included in the study or given study medication.

4.4 EARLY WITHDRAWAL OF SUBJECTS

4.4.1 WHEN AND HOW TO WITHDRAW SUBJECTS

Exclusion criteria were infants born before gestational week 37, insufficient weight gain, having tried acupuncture or taking prescribed medication. Simethicone and/ or probiotics were allowed.

5 STUDY DRUG

INDICATION

Anti-flatulent defoaming agent for the symptomatic relief of flatulence, wind pains, bloating, abdominal distension and other symptoms associated with intestinal gas.

5.1 DESCRIPTION

Simeticone is used to relieve the painful symptoms of too much gas in the stomach and intestines. Oral ant flatulent agent, available as a single agent or in combination with antacids or antidiarrheal. Simeticone-coated cellulose suspension is used to enhance upper GI visibility in ultrasound images and is not used as an anti flatulent.

ACTIVE INGREDIENT *:* Simeticone

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MOLECULAR FORMULA: $(C_2H_6OSi)_n \cdot (SiO_2)_m$

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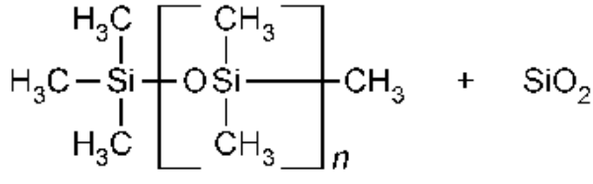
STRUCTURAL FORMULA:



5.2 TREATMENT REGIMEN

Treatment is often recommended from parents' subjective description of the infants' behavior without measuring the infants crying, and advice given by the CHC nurse often lack structure and follow up (Landgren et al., 2012).

Counselling parents by giving them specific instructions on how to adapt their interaction with the infant showed good results in two studies (Keefe et al 2005; Dihigo, 1998) while another study did not find that the infants crying reduced more than compared with evaluation and support from the CHC nurse and paediatrician alone. The use of herbal tea might have effect when treating colic but given the lack of standardization of components in the herbal products, unknown optimal dosage and the risk of interference with the infant's normal food intake parents are advised to use these products with caution (Roberts et al 2004). Sugar solution can be used for its analgesic effect in infants and has therefore been suggested as a possible method to also reduce discomfort in infants with colic.

Although there are limited studies conducted there have been some positive results when compared to placebo. Spinal manipulations as treatment for colic are performed by chiropractors. This treatment has however shown varied results in studies conducted in Scandinavia;

Olafsdottir et al 2001; Wiberg et al 1999). Acupuncture has a pain-inhibiting effect and has been proven to reduce crying and gastrointestinal symptoms in infants with colic according to Landgren et al (2010) and Reinthal et al., 2008. Skjeie et al (2013) however did not find any statistically significant effect of acupuncture as treatment for colic in infants.

The anticholinergic drug Dicyclomine showed significant improvement when used for treating colic (Lucassen et al., 1998) but was withdrawn from the Swedish market due to serious side effects.

5.3 METHOD FOR ASSIGNING SUBJECTS TO TREATMENT GROUPS

This study is a quantitative, descriptive sub-study based on unpublished data collected in two RCT´s examining the effect of acupuncture in colic.

Screening lists for infants who were not included and background data for included infants from a questionnaire were analyzed.

5.4 PREPARATION AND ADMINISTRATION OF STUDY DRUG

Both the drugs included in the trials will be used as supplied, with instructions of preparation and oral administration duly provided. Study drugs are administered through orally in drop dosage form only.

5.4.1 PREPARATION OF STUDY DRUG

United States Patent 01 Ffice 3,767,794 Patented Oct. 23, 1973 ABSTRACT OF THE DISCLOSURE An antifoaming product, which retains its antifoaming activity in the presence of anti-acids and other drugs, is prepared by suspending up to 20% by weight of an antifoaming organo-polysiloxane, such as Simeticone, in molten sorbitol, cooling the suspension to harden it and thereafter reducing it to a powder of a particle size larger than about 115 mesh. The new antifoaming compositions may be used in therapeutically active amounts in the preparation of compositions for the relief of gastrointestinal distress caused by flatulency.

This invention relates to stable antiform compositions which are particularly useful in the preparation of antacids and other preparations for the relief of gastrointestinal distress. The compositions also provide for a convenient method of disbursing and dispersing antifoam compositions in solid form.

A material which has been given the non-proprietary name of Simeticone has been in use for more than ten years as an antifoaming agent for the relief of frothy bloat and related conditions in ruminants and for the treatment of gastrointestinal disorders in humans. This material is a composition consisting essentially of dimethylpolysiloxane and 4% to 4 /2% by weight of a silica aerogel. For example, the silicone fluide of the composition, as produced by Dow-Corning

Chemical Company, has a molecular weight of between 14,000 and 21,000, a silicon content of 37.3 to 38.5%, a viscosity at 25 C. of from 300 to 600 centistokes (cs.), a specific gravity at 25 C. of from 0.965 to 0.970, and a refractive index, n of 1.403 $0.002.

One form of the product is sold under the trade name DC Antifoam A. Another form of the same material is an emulsion referred to in the trade as DC Antifoam AF. These are products of the Dow-Corning Chemical Company. Similar materials are available from other sources. These antifoaming agents and other polysiloxane compositions, and their use in the treatment of gastrointestinal disorders, are described in US. Pat. Nos. 2,635,981, issued to Austin et al., 2,934,472, issued to May, 2,951,011, issued to Feinstone, and 3,422,189, issued to Rider.

As noted by Rezak, J. Pharm. Sci. 55, 538-9 (1966), the foam-depressing action of these silicone preparations is inactivated to some extent when they are placed in contact with many commonly used anti-acid materials. Loss of their antifoam activity is accelerated at elevated temperatures. Some of the most commonly used anti-acid materials, such as aluminum hydroxide, magnesium carbonate and magnesium trisilicate, are particularly harmful to the antifoaming activity of Simeticone.

To illustrate the inactivation of Simeticone in several typical anti-acid preparations, the following products were prepared. In each case the powders were weighed, placed in a mortar and triturated, and stored in tightly-closed glass bottles.

EXAMPLE A Gm. Sodium Carboxymethylcellulose 0.3 Magnesium trisilicate 0.2 Simeticone 20 EXAMPLE B Gm. Magnesium trisilicate 0.5 Aluminum hydroxide dried gel 0.25 Simeticone 20 EXAMPLE C Gm. Sodium bicarbonate 0.275 Calcium carbonate 0.15 Magnesium carbonate 0.1 Magnesium trisilicate 0.07 Simeticone Similar anti-acid products were prepared in which the Simeticone was replaced with 0.1 gram of the antifoam product of the present invention containing 20 mg. of Simeticone. The antifoaming performance of the six different products was determined in the following manner:

Thirty grams of a commercially available surfactant, an alkyl phenoxy polyethoxy ethanol, was dispersed in 3 liters of 0.1 N hydrochloric acid and milliliters of this solution was placed in clean 8-ounce bottles with each of the six products described above. The bottles were closed and inverted ten times rapidly. The length of time in seconds for the foam to collapse was determined with a stopwatch. This procedure was repeated ten times, and the average time for the foam to

collapse was determined. When the six products were tested in this manner, the following results were observed:

After 1 week at- Two commercially available anti-acid products, one said to contain magnesium hydroxide, 200 mg., aluminum hydroxide, 200 mg. Simeticone, 200 mg, and the other containing unstated amounts of magnesium carbonate, magnesium hydroxide, aluminum hydroxide and 25 mg. of Simeticone, were tested for their antifoaming activity. The tablets were triturated in a mortar and placed in an 8-ounce bottle with 100 ml. of 0.1 N hydrochloric acid containing the same surfactant as used in the experiments described above. These bottles were placed in a 37 C. water bath for two hours (to simulate body temperature and acidity of the stomach). At the end of this time the bottles were inverted ten times to develop a foam. In each case the foam had not collapsed in three minutes, and it was considered that neither product possessed satisfactory antifoam activity. Obviously the anti-foaming component of these preparations, Simethicone, had been deactivated since the tablets had been prepared.

The instability of the antifoaming properties of Simeticone in the presence of conventional anti-acid agents, and the desirability of having such tablets with antifoaming activity which is stable over a long period of time led to the present invention. Various attempts were made to protect the Simeticone from contact with the anti-acids with which it might be mixed. Successful efforts were as follows:

EXAMPLE 1 100 grams of sorbitol and 67 grams of DC Antifoam AF emulsion were mixed and heated to 125 C. with mixing. Heating was discontinued, but stirring was continued until the temperature of the mixture was about 50 C. The molten material was then poured onto a stainless steel tray to form a thin film. Upon hardening, the thin film was placed in a freezer until it was cold. It was then reduced to particles which would pass through a mesh screen. A commercially available anti-acid preparation of the following composition was used in this experiment:

Aluminum hydroxide (supplied as dried aluminum hydroxide gel) 180.0 Magnesium hydroxide 170.0 Methylcellulose 50.0 Dicyclomine hydrochloride 2.5

To a granulation of the above composition there was added an equivalent of 20 mg. of the Simeticone, as the preparation just described, and the mixture was compressed into a tablet. When tested for its antifoaming activity by the procedure described above, it was found that the foam was depressed within two seconds, and this activity remained after storage of the tablets for one week at room temperature and also at 56 C.

To a granulation of the above composition there was added the equivalent of 20 mg. of the Simeticone, without protection, and the mixture was compressed into a tablet. When tested for its antifoaming activity by the procedure described above, it wa found that the foam was not depressed after 180 seconds, which indicated that the simethicone was inactivated by the anti-acids of the composition.

The Simeticone was enrobed in the sorbitol by other methods, as is demonstrated by the following examples.

EXAMPLE 2 Crystals of sorbitol were heated to 130 C. to obtain molten sorbitol. Fifty grams of Simeticone (DC Antifoam A), was stirred into 950 grams of the molten sorbitol and stirring was continued while the mass cooled to 70 to 75 C. The material was then poured onto a metal candy plate and allowed to harden. It was ground and passed through a No. 12 screen. This material maintained its antifoaming activity when mixed with various anti-acid materials.

EXAMPLE 3 In the event it is desired to use commercial solutions of sorbitol, instead of solid sorbitol, one may heat the sorbitol solution to remove the water and then cool it to 130 C., mix it with the Simeticone, cool it until it hardens and then grind it to a suitable degree of fineness.

Protected Simeticone prepared by the methods illustrated in Examples 2 and 3 was added to the anti-acid preparation described in Example 1. Again, when tested for its antifoaming activity by the procedure described earlier, comparable results to those described for the material in Example 1 were found.

During the numerous experiments that were conducted it was found that it was possible to incorporate up to 20% by weight of Simeticone in molten sorbitol, and thereafter cool, harden and grind the material to form an antifoaming composition that is stable in the presence of antacids. 20% of Simeticone in sorbitol seems to be an upper practical limit. When Simeticone was incorporated in a concentration of 30% into molten sorbitol, the mass did not harden. Furthermore, Simeticone is not protected as well from anti-acids when the antifoam product contained 20% of Simeticone in sorbitol, as where the product contained a lower amount of Simeticone. For example, when anti-acid tablets containing aluminum hydroxide and magnesium hydroxide were prepared and stored for three weeks at 45 C. it required 3.2 seconds for the foam to collapse where the anti-foaming products consisted of 20% Simeticone and sorbitol. When the antifoaming agent contained 10% of Simeticone and sorbitol the foam collapsed in 4.8 seconds.

When the antifoaming composition consisted of 5% of Simeticone enrobed in of sorbitol, in accordance with the present invention, the foam collapsed in 3.2 seconds.

These latter tablets were found to be stable for three months at 37 C., collapsing the foam in 3.2 seconds, whereas the 20% Simeticone product required 60 seconds to collapse the foam.

The practical range of concentration of Simeticone in sorbitol is about 0.1 to 20% by weight. The lower limit has been established on the basis of 20 mg. of Simeticone being the therapeutic dose. Twenty (20) grams of sorbitol antifoam powder, containing 0.1% Simeticone, would be required to deliver 20 mg. of Simeticone.

This amount of sorbitol is close to a laxative dose. With regard to the upper limit, experience has shown that at a level of 20% Simeticone the enrobing agent does not provide sufficient protection from the anti-acids. The optimum concentration appears to be 5% to 10%, depending upon the volume of antifoam powder that can be tolerated in the formulation.

The granules of the sorbitol-Simeticone anti-foaming product of the present invention should be greater than about mesh. If the material is ground finer than this the Simeticone will not be as well-protected from the antacids as is desirable. Preferably the hardened sorbitol/ Simeticone product should be ground to pass a 12-mesh screen, but be retained on a l5-mesh screen. Powders so prepared have a discrete fluid-granular character and do not tend to cake. They are easily mixed with other conventional components of anti-acid tablets, such as aluminum and magnesium hydroxides, magnesium trisilicate, bismuth sub carbonate, calcium carbonate, phenobarbital, anticholinergics, enzymes, belladonna and the like, binders and excipients, such as sugar, lactose, dextrose, starch, talc and other pharmaceutically acceptable, non-toxic powders and lubricants which are also compatible with the antifoam agent of the present invention.

What is claimed is:

1. A method of preparing a composition effective in depressing foam in aqueous systems which comprises melting sorbitol and uniformly dispersing therein 0.1 to 20% by weight of the sorbitol, a non-toxic foam-depressing Simeticone, cooling the mass to harden it, and grinding the solid product to particle sizes that will pass through a 12- mesh screen but not through a 115-mesh screen.

2. The antifoam composition prepared by the process of claim 1.

3. The antifoam composition of claim 2 containing Simeticone in the antifoaming composition in an amount of from 5 to 10% by weight of the sorbitol.

4. A method of preparing an antifoaming antacid composition which comprises melting sorbitol and dispersing from 0.1 to 20% by weight of Simeticone in the molten sorbitol, allowing the molten mass to harden, grinding the hardened product and recovering from the ground material those particles which pass through a 12-mesh screen but not through a 115-mesh screen and incorporating said recovered particles into a non-toxic pharmaceutically acceptable antacid.

5.5 SUBJECT COMPLIANCE MONITORING

Inclusion criteria for both studies were healthy infants, not older than eight weeks, who according to a daily baseline registration fulfilled the criteria for colic: crying/fussing more than three hours/ day at least three days per week. At least one of the parents had to speak Swedish.

Exclusion criteria were infants born before gestational week 37, insufficient weight gain, having tried acupuncture or taking prescribed medication. Simethicone and/ or probiotics were allowed. In the first trial (Landgren et al., 2010) parents to 210 infants registered their infants crying and fussing in a diary during at least three days. Of these infants, 90 fulfilled the criteria for colic and were included. In the second trial, ACU-COL (Landgren et al., 2015), 426 infants were initially screened for participation in the study and after daily registration of crying and fussing in a diary during a baseline week, 152 infants met the criteria and were included in the study (Landgren & Hallström, in manuscript).

Table 1. Reasons noted on the screening list for infants not being included after the baseline week (Landgren et al., 2010; Landgren & Hallström, submitted).

	RCT 1	RCT 2
	Infants (n=120)	Infants (n=269)
Crying and fussing < 3 hours/day, n (%)	63 (52,5)	119 (44,2)
Did not want to participate, n (%)	16 (13,3)	54 (20,0)
Did not answer phone calls/email at the end of the baseline week, n (%)	0 (0,0)	20 (7,4)
Premature	1 (0,8)	8 (2,9)
Parents chose to take the infant to a private acupuncturist	3 (2,5)	17 (6,3)
Parents chose to take the infant to a homeopath	1 (0,8)	0 (0,0)
Parents chose to take the infant to a chiropractor	0 (0,0)	1 (0,3)
Participation in the trial/registering in the diary required too much time/ energy, n (%)	6 (5,0)	17 (6,3)
Symptoms improved when formula was introduced, n (%)	2 (1,6)	0 (0,0)
Spontaneous remission or remission due to cows milk free diet, n (%)	8 (6,6)	26 (9,6)
Treated with dicyclomine	6 (5,0)	0 (0,0)
The father did not want the infant to participate	1 (0,8)	2 (0,7)
The infant was hospitalized	1 (0,8)	0 (0,0)
The mother was hospitalized	0 (0,0)	1 (0,3)
The breastfeeding mother could not manage a cow´s milk protein free diet for 5 days	0 (0,0)	1 (0,3)
The infant fulfilled the criteria but no acupuncturist was available at the study center	0 (0,0)	3 (1,1)
Missing	13 (10,8)	0 (0,0)

Table 2. Treatment with simethicone and probiotics in the first (Landren et al., 2010) and the second (Landgren & Hallström., submitted) RCT.

	RCT 1		RCT 2	
Frequency and effect of treatments	Infants (n=81)	Missing n (%)	Infants (n=147)	Missing (n (%)
Simethicone				
Treated with simethicone, n (%)	75 (92,6)	-	111 (76,0)	1 (0,7)
Reported "effect" of treatment with simethicone, n (%)	2 (2,7)	-	6 (5,4)	-
Reported "some effect" of treatment with simethicone, n (%)	25 (33,3)	-	29 (26,1)	-
Reported "no effect " of treatment with simethicone, n (%)	48 (64)	-	76 (68,5)	-
Probiotics				
Treated with probiotics, n (%)	12 (14,8)	-	126 (86,3)	1 (0,7)
Reported "effect" of treatment with probiotics, n (%)	0 (0)	-	4 (3,2)	-
Reported "some effect" of treatment with probiotics, n (%)	2 (16,7)	-	28 (22,2)	-
Reported "no effect " of treatment with probiotics, n (%)	10 (83,3)	-	94 (74,6)	-

Table 3. Descriptive data from the first (Landgren et al., 2010) and the second (Landgren & Hallström, in manuscript) RCT.

	RCT 1		RCT 2	
Background characteristics	Infants (n=81)	Missing n (%)	Infants (n=147)	Missing n (%)
Family				
Born in Sweden, mother, n (%)	76 (93,8)	-	125 (85)	-
Born in Sweden, father, n (%)	72 (88,9)	-	125 (85)	-
University education, both parents, n (%)	26 (32,1)	-	52 (35,4)	2 (1,4)
University education, one parent, n (%)	33 (40,7)	-	50 (34,5)	2 (1,4)
Smoking, mother, n (%)	6 (7,4)	-	3 (2,1)	1 (0,7)
Smoking, father, n (%)	5 (6,2)	-	15 (10,3)	2 (1,4)
Parent and/or sibling with food intolerance/allergy, n (%)	60 (74,1)	-	77 (52,7)	1 (0,7)
Parent and/or sibling who had asthma, n (%)	16 (19,8)	-	34 (23,3)	1 (0,7)
Parent and/or sibling who had eczema, n (%)	25 (30,9)	-	35 (24,0)	1 (0,7)
Parent and/or sibling who had a bowel disease, n (%)	22 (27,2)	-	29 (19,9)	1 (0,7)
Parent and/or sibling who had had infantile colic, n (%)	45 (55,6)	-	81 (55,5)	1 (0,7)
Parent and/or sibling had infantile colic; "don't know", n (%)	3 (3,7)	-	14 (9,6)	1 (0,7)
Pregnancy and delivery				
Pregnancy was overall free from complications, n (%)	35 (43,2)	-	106 (72,1)	-
Pregnancy was overall a positive experience, n (%)	63 (77,8)	-	114 (77,6)	-
Prescription drugs were used during or after pregnancy, n (%)	44 (54,3)	-	84 (57,5)	1 (0,7)
Normal delivery, n (%)	48 (59,3)	-	103 (70,5)	1 (0,7)
Infant				
Firstborn, n (%)	42 (52,5)	1 (1,2)	57 (38,8)	-
Infants gender, male, n (%)	41 (50,6)	-	80 (54,4)	-
Healthy appetite, n (%)	79 (97,5)	-	142 (97,3)	1 (0,7)
Exclusive breastfeeding, n (%)	57 (70,4)	-	85 (58,2)	1 (0,7)
Excessive gastrointenstinal gas, n (%)	76 (93,8)	-	136 (93,8)	2 (1,4)
Repeated vomiting, n (%)	43 (53,1)	-	65 (44,5)	1 (0,7)
Skin rash, n (%)	47 (58)	-	52 (36,4)	4 (2,7)
Alternative treatment for colic				
Treatment with alterative options; herbal tea, suger solution, chiropractor or other treatment, n (%)	-	-	30 (20,6)	1 (0,7)
Effect of alternative treatment, n (%)	-	-	10 (34,5)	1 (0,7)
Some effect of alternative treatment, n (%)	-	-	8 (27,6)	1 (0,7)
No effect of alternative treatment, n (%)	-	-	11 (37,9)	1 (0,7)

5.6 DISPENSING OF STUDY DRUG

The study drugs will be dispensed as a kit to the investigator or their designated study staff. The investigator or designated study staff will then dispense the appropriate amount of study drug from the study kit to the research subject assigned to that kit.

6 STUDY PROCEDURES

6.1 POST RANDOMIZATION TREATMENT

During the visit at the end of therapy and follow up after 6 month, the following measures will be obtained:

1. Collect unused study drug
2. Vital signs and weight
3. Chest auscultation, TPR, abdominal palpation (for any signs of hepatomegaly, hepatic tenderness), palpation for lymph nodes, signs for anemia
4. AE recorded by SAFTEE

After the end of treatment and during the follow-up visit at the end of 6 month, following tests will be conducted:

1) Complete Blood Count
2) ESR
3) Liver Enzyme tests SGPT, SGOT, Serum bilirubin, alkaline phosphatase; creatinine, BUN
4) X-Ray chest postero-anterior view
5) Urine routine and microscopic test
6) Sputum test for bacterial culture
7) ECG (12-lead)

6.2 END OF MEDICATION EVALUATION

This evaluation will be scheduled 6 month after the completion of the 21 days study drug treatment period.

6.3 FOLLOW-UP EVALUATION

A follow-up visit will be scheduled after the 6 month days of the end of the study period. During the follow-up visit, clinical signs will be evaluated.

7 STATISTICAL PLANS

7.1 STATISTICAL METHODS

The statistical software package SPSS™ version 22 (SPSS Inc., Chicago, IL) was used for calculations. Initially the descriptive statistics were calculated. The Kolmogorov Smirnoff's test was used to determine if the variables were normally distributed. Since all the variables were not normally distributed the data were analyzed with a non parametric Chi square test using crosstabs for significance testing and Phi for determining the strength of the correlations. Phi was chosen instead of Cramer's V since the analysis only demanded a two-by-two contingency table, which was used to describe the association between the variables as well as the difference in use of simethicone and probiotics over time. P-values <0.05 were considered statistically significant. For determining the strength of the correlations between parents use of probiotics or simethicone and their reported experience of the infants appetite the Spearman rank correlation test were used since this variable contained more than two available answers where the parents could rank appetite on a three level scale.

7.2 DATA MANAGEMENT

When a parent sought treatment for colic and wanted to participate in the study they were noted on a screening list and instructed to register the infant's behavior in a diary. The project leader called the parents three to seven days later.

Infants who according to the diary cried > 3 hours/day were then included. The project leader made a short note on the screening list for the rest of the infants on why they were not included; these notes were analyzed in this study. Data on the use of simethicone and probiotics and background data were collected from a structured questionnaire that parents had to answer at the first visit to the study center after inclusion.

The questionnaire consisted of 43 questions, including 30 main topics and 13 follow-up questions with different set of answers. One question regarding alternative treatments for colic were added to the questionnaire in the ACU-COL study. To describe the use and perceived effect of simethicone and probiotics two questions were analyzed in the present study. These questions asked whether parents treated, or had treated, their infant with simethicone or probiotics.

If the answer was "yes" parents were asked if simethicone/probiotics had had effect. This question could be answered with "yes", "no" or "some effect". To investigate the possible associations between the use of simethicone/ probiotics and demographic background data collected in the same questionnaire the associations between these variables were analyzed. This data included demographic information as well as information about the parents education, smoking, food intolerance, allergies, asthma, eczema or bowel diseases in the family, other family members having had infantile colic, complications during pregnancy or delivery and the mother's intake of prescribed medication during pregnancy and after the delivery. It also contained information about the infants feeding, gas, vomiting, rashes, and the use of alternative treatment as herbal tea, sugar solution or chiropractic treatment.

8 SAFETYAND ADVERSE EVENTS

8.1 DEFINITIONS

Adverse events, regardless of whether they appeared to be related to the use of the study medication, were carefully recorded throughout the study. Causal relationships between the study drugs and adverse events were analyzed using six stages: definitive, probable, possible, unlikely, not related, and not assessable.

Adverse events were considered related to the study drug if the stage was definitive, probable, or possible. The severity of the adverse events was classified according to the Navchetana Kendra.

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Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined a,.i & Period from the initiation of any study procedures to the end of the study treatment for issue it study, the study treatment follow-up is defined as 6 month following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that as per the subject's understanding might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

The sponsor should also be notified if the investigator should become aware of the development of premenstrual syndrome or of a congenital anomaly in a subsequently conceived off spring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
The abnormality suggests a disease and/or organ toxicity.

The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization or Prolonged Hospitalization

Any adverse event that results in prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Neither the condition, nor prolonged hospitalization is reported as an adverse event in the following circumstances:

Prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition.

- Prolonged hospitalization required allowing efficacy measurement for the study.
- Prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 RECORDING OF ADVERSE EVENTS

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 MEDICAL MONITORING

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.4 PROTECTION OF SUBJECTS

Complementing the safety measures noted above, additional procedures will be followed to protect the safety of the research subjects. Potential Subjects will be screened for medical illnesses that would preclude the use of **FLORAS DROP** used in the trial, as mentioned in the exclusion criteria.

9 DATA HANDLING AND RECORD KEEPING

9.1 CONFIDENTIALITY

Information about study subjects will be kept confidential and managed according to the requirements of the Health Information Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

The personal health information (PHI) to be collected from subjects in this study who will have access to that information and why

Who will use or disclose that information

To whom the data may be disclosed and the reasons for this disclosure

The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 SOURCE DOCUMENTS

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 CASE REPORT FORMS

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained.

If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated.

9.4 RECORDS RETENTION

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country.

10 STUDY AUDITING AND INSPECTING

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance and quality assurance offices.

11. ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines). This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC), in agreement with local legal prescriptions, for formal approval of the study conduct.

The decision of the EC concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

11.1 CONSENT PROCEDURES

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.

The formal consent of a subject, using the EC-approved consent form, must be obtained before that subject is submitted to any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

The PI or study physician will obtain informed consent before any study procedures occur, explaining all procedures in detail in an individual session.

The explanation will include; detailed information about the study drug, the rationale for why it is being studied, frequency of dosing, and length of treatment, potential side effects and risks, safeguards and emergency procedures.

The collection of all lab specimens will be described in detail, as will the number and frequency of the visits.

Subjects will be assured that their participation is voluntary and that withdrawal from the study would not jeopardize current or future treatment.

All subjects will be informed of potential risks and benefits involved in the study, including side effects of the study drug as well as other drugs used in the trial.

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ETHICS

Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

Independent Ethics Committee Approval

The study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). Patients can only be included in the study after obtaining written informed consent from both parents. Both parents will receive verbal and written information on all aspects of the study, including possible risks and benefits. The right of a parent to refuse participation without a given reason will be respected. The parents will remain free to withdraw their child at any time from the study without consequences for further treatment. The study protocol has been reviewed and approved by the Ethics Committee of the University of United States, Alabama Clinical Research Associates Huntsville and the local Ethics Committee of each participating hospital.

The complies with the requirement of FDA 21 CFR part 50 (protection of human subject) and 56 Institutional Review Board. These are the principal that govern the assuring that the right and welfare are protection in the Belmont Report: Ethical principal and Guidelines for the protection of Human Subject and Research, of the National Commission for the protection of human subject of biochemical and behavioral research and the declaration of Helsinki.

Ethical Conduct of the Study

The study was conducted according to the U. S. Code of federal regulation Guidelines for food clinical practice (Code of federal Regulation (21CFR), part 50, 56, 312,and 314), the international conference on harmonization (ICH) guidelines for good clinical practice ICH guidelines E6), the declaration of Helsinki on the ethical conduct of medical research Edinburgh, Scotland,2002),and the Belmont Report.

Patient Information and Consent

Information as to the objective procedure, risk, benefits restrictions and requirement of the study was presented to all subjects before the start of the study. The subjects were encouraged to ask question, which were fully answered. All subject signified their willingness to participate in this study by reading, signing and dating the approved consent from (Revision 1) Copies were provided to each subject, signed dated and witnessed informed consent forms are on file at sample copy of the subject consent form (Revision 1) is provide in 16.1.3

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Ethics Committee
(R. M. SHAH)

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DECLARATION OF PRINICIPLE OF HELSINKI (1964)

I. Basic principles

1. Clinical research must conform to the moral and scientific principles that justify medical research and should be based on laboratory and animal experiments or other scientifically established facts.
2. Clinical research should be conducted only by scientifically qualified persons and under the supervision of a qualified medical person.
3. Clinical research cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
4. Every clinical research project should be preceded by careful assessment of inherent risks in comparison to foreseeable benefits to the subject or to others.
5. Special caution should be exercised by the doctor in performing clinical research in which the personality of the subject is liable to be altered by drugs or experimental procedure.

II. Clinical Research combined with professional care

1. In the treatment of the sick persons, the doctor must be free to use a new therapeutic measure, if in the doctor's judgment it offers hope of saving life, re-establishing health, or alleviating suffering.
2. If at all possible, consistent with patient psychology, the doctor should obtain the patient's freely given consent after the patient has been given a full explanation. In case of legal incapacity, consent should also be procured from the legal guardian; in case of physical incapacity the permission of the legal guardian replaces that of the patient.
3. The doctor can combine clinical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that clinical research is justified by its therapeutic value for the patient.

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III. No therapeutic clinical research

1. In the purely scientific application of clinical research carried out on a human being, it is the duty of the doctor to remain the protector of the life and health of that person on whom clinical research is being carried out.
2. The nature, the purpose and the risk of clinical research must be explained to the subject by the doctor.
3. Clinical research on a human being can not be undertaken without that person's consent after being informed; if the person is legally incompetent the consent of the legal guardian should be procured.
4. The object of clinical research should be in such a mental, physical and legal state as to be able to exercise fully the power of choice.
5. Consent should, as a rule, be obtained in writing. However, the responsibility for clinical research always remains with the research worker; it never falls on the subject even after consent is obtained.
6. The investigator must respect the right of each individual to safeguard his/her personal integrity, especially if the subject is in a dependent relationship to the investigator.
7. At any time during the course of clinical research the subject or the subject's guardian should be free to withdraw permission for research to be continued.
8. The investigator or the investigation team should discontinue the research if in their judgment, it may, if continued be harmful to the individual.

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R. M. Shah

Ethics Committee
(R. M. SHAH)

GVK Biosciences Private Limited

INFORMED CONSENT FORM

Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study.

Name of Patient:

Date of Birth: Age in Years Gender (M/F)

Address:

Telephone or contact no.

1. Purpose of the trial

Almost every infant in the first trial (Landgren et al., 2010) had been treated with simethicone and even though the frequency decreased from 93 to 76% in the second trial (Landgren et al., submitted) the majority of infants still got treated with simethicone. Only a few reported "effect" and about one third reported "some effect" in both trials. The use of probiotics increased significantly between the two trials but despite the remarkable increase there were still 83.3% in the first trial and 74.6% in the second trial who reported "no effect" from the treatment.

Association with background characteristics

Descriptive data and background characteristics from both RCT´s (Landgren et al., 2010, Landgren & Hallström, submitted) are reported in table 3.

There was a significant association between the use of simethicone and parents having more than one child (p=0.018) as well as treatment with simethicone and previous experience of colic in the family (p=0.013). There was also a difference in the reported use of simethicone after having had a normal or a complicated delivery (p=0.015), where the majority of infants being treated with simethicone had been delivered without complications.

The use of probiotics had a significant association with reported food allergies and/or food intolerances amongst siblings and/or parents (p=0.019). There was also a significant association between higher frequency of complications during the pregnancy and a more frequent use of probiotics (p=0.002) as well as having tried probiotics and exclusive breastfeeding (p=0.031). The results also indicate an association between higher use of probiotics in families where parents and/or siblings had fewer problems with eczema (p=0.049) as well as having tried probiotics and the infant having less problems with skin rashes (p=0.035). All background characteristics where analyzed for association between the frequency of treatment with simethicone and use of probiotics. There were no other significant associations found amongst the remaining background characteristics.

3. Risks

A very serious allergic reaction to this product is rare. However, seek immediate medical attention if you notice any of the following symptoms of a serious allergic reaction: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

4. Benefits

Out of the 210 infants whose parents completed daily registrations in the diary in the first trial (Landgren et al., 2010) 90 were included and 120 were not. In the second trial, ACU-COL (Landgren & Hallström, submitted), 426 infants were screened for participation of which 157 were randomized and 269 were not included. Notes made by the project leader for the reasons why infants were not included were categorized (Table 1). For some infants more than one reason was listed (e.g. ”Don’t want to be randomized” and “Is going to a private acupuncturist”) and for some infants in the first RCT the reason is missing.

The category ”Participation in the trial/ registering in the diary requires more time/energy than I have” contains four families living on islands which required lengthy travelling times and several who remarked they were too stressed, among them one single mother with two more children. Two families also referred to issues with the mothers’ psychiatric health. In the category”Don´t want to participate” many referred to ”Don´t want to be randomized”. One did not want to leave the child alone with the acupuncturist for five minutes.

5. Alternative treatments

These drugs will be continued as per the advice of the consulting physician.

However, you MUST NOT take any heart failure without consulting the doctor in-charge, because of the possibility of drug interactions, which might affect you adversely.

If you wish the Doctor in-charge will explain the benefits and risks of receiving the treatment with any (or all) of these cervical dysplasia.

6. Confidentiality of the records

Your medical records that are related to this trial will be maintained in confidentiality. The sponsor may examine your medical records, as long as your names cannot be identified from these records.

Your records from this trial will be submitted by sponsor to the food and drug administration or other regulatory agencies, but your name will not be able to be identified from such records. No identity of specific patient in this trial will be disclosed in any public reports or publications.

The FDA has the right upon proper judicial order to review pertinent medical records and other data with your name identified. They require by the law, however, handling this information in a confidential manner.

7. If problem develops

If any serious problems develop you will receive prompt and appropriate medical attention. It is agreed that the facilities of Hospital will be made available to you. Reasonable medical treatment will be free when provided through the facilities of Hospital/Institution. Financial compensation is not available for medical treatment elsewhere, loss of work, or other expenses.

8. Obtaining additional information

You are encouraged to ask any questions that occur to you at this time or at any time during your participation in the trial.

You will be given a copy of this agreement for your own information.

9. Basis of participation

You are free to withdraw your consent to participate in this trial at any trial, if you choose to do so, your rights to present or future medical care by doctor in-charge or at Hospital will not be affected.

10. Signature

I have read the above information and have had an opportunity to ask any question and all of my questions have been answered. I consent to take part in the study of the treatment for response and the Floras minimum inhibitory concentration (MIC) of the infecting isolate.

I fully understand that its use in humans is limited and that though its safety and effectiveness is established, there is a risk of adverse reaction to the cervical dysplasia.

I have been given a copy of this consent form.

GVK Biosciences Private Limited

10. Signature

I have read the above information and have had an opportunity to ask any question and all of my questions have been answered. I consent to take part in the study of the treatment for **premenstrual syndrome .**

I fully understand that its use in humans is limited and that though its safety and effectiveness is established, there is a risk of adverse reaction to the medicine.

I have been given a copy of this consent form.

Signature mukesh kumar (Patient) 26 April 2012 Date

Signature (Patient or Legal Guardian) 26 April 2012 Date

I the undersigned have fully explained the relevant details of this trial to the patient named above and/or the person authorized to consent for the patient.

I am qualified to perform this role.

SIGNATURE(S)

Signature Print (Investigator) 26 April 2012 Date Name Dr P. K. Mishra

Signature Print (Witness) 26 April 2012 Date Name A. K. Rai

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Clinical Evaluation of Efficacy and Safety

FLORAS DROP

IN THE TREATMENT OF

Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study

SPONSOR:

Navchetana Kendra™
Manufacture, Importer, Exporter & Supplier of Herbal Products

E-138/A, Ground Floor Shastri Nagar
Delhi-110052 India

Study Product: **(FLORAS DROP)**

Protocol Number: CT4510

GVK Biosciences Private Limited

Investigators' Declaration

We, the undersigned, have read and understood this protocol and hereby agree to conduct the study in accordance with this protocol and to comply with all the requirements regarding the obligations of investigators and all other pertinent requirements of the ICH `Guidance on Good Clinical Practice'.

We agree to comply with all relevant SOPs required for the conduct of this study. We further agree to ensure that all associates assisting in the conduct of this study are informed regarding their obligations.

INVESTIGATOR: **Dr. Vivek Kumar**
GVK Biosciences Pvt ltd

OR SPONSOR'S RESPONSIBLE
MEDICAL OFFICER

SIGNATURE(S)

AFFILIATION: **Dr. Vivek Kumar**
GVK Biosciences Pvt ltd

GVK BIOSCIENCES PRIVATE LIMITED INDIA

Date: 27th May 2012

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TABLE OF CONTENT

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List of abbreviations:

Abbreviation	Full term
CRF	Case report form
BUN	Blood Urea Nitrogen
ECG	Electrocardiogram
HIV	Human Immunodeficiency Virus
ICF	Informed consent form
SGOT (AST)	Serum Glutamate Oxaloacetate Transaminase (Aspartate aminotransferase)
SGPT (ALP)	Serum Glutamate Pyruvate Transaminase (Alanine aminotransferase)
ESR	Erythrocytes Sedimentation Rate
CBC	Complete Blood Count
MIC	Minimum inhibitory concentration
IAI	Intraabdominal infections

ETHICS

The study and any amendments were reviewed by an Independent Ethics Committee.
The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

Patient Information and Consent

Informed consent was obtained in relation to patient enrollment (at the time of allocation, and prescreening).
The sample of the patient consent form used is attached along with.

Introduction

Infantile colic affects 10-17% of infants in the Western world (Canivet et al 2002; Lucassen, 2010). Colic is often defined as crying or fussing for at least three hours per day, three days per week in otherwise healthy infants under three months of age (Wessel et al 1954).
Although infantile colic is a benign condition with spontaneous recovery it causes substantial distress to the parents as well as the infant, which can affect essential early interaction and family relations.

Excessive crying is associated with depression in mothers during the postpartum period (Howell et al 2009; Miller et al 1993; Murray et al 1996; Vik et al., 2009) and has been associated with an elevated risk of child abuse in multiple studies (Barr, 2014; Talvik et al 2008; Barr et al 2006; Reijneveld et al., 2004; van der Wal et al 1998). Infantile colic is a frequent cause of pediatric consultations at Child Health Centers (CHC).

It is of great importance that health professionals do not dismiss the condition as harmless and temporary, but rather respond to the parents´ mental and physical exhaustion with respect and proficiency. Nurses working at the CHC have therefor an important but difficult role to support these parents since there is still no known cure and several treatments are lacking scientific evidence (Landgren et al 2012).

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STUDY OBJECTIVES

Primary Objective:

Adverse events [Time Frame: 1 month.] [Designated as safety issue: Yes]

Enrollment:	144 infants studied
Study Start Date:	26 April 2012
Study Completion Date:	25 may 2012
Primary Completion Date:	25 may 2012 (Final data collection date for primary outcome measure)

Secondary Objective:

Screening for infantile colic and parents experiences of simethicone and probiotics: a quantitative, descriptive sub-study

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INVESTIGATIONAL PLAN

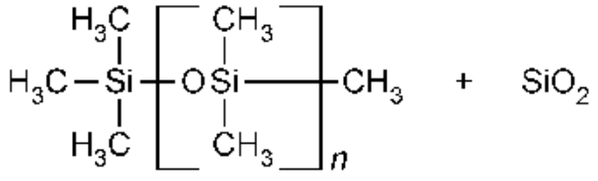
Simeticone is used to relieve the painful symptoms of too much gas in the stomach and intestines. Oral ant flatulent agent, available as a single agent or in combination with antacids or antidiarrheal. Simeticone-coated cellulose suspension is used to enhance upper GI visibility in ultrasound images and is not used as an anti flatulent.

ACTIVE INGREDIENT *:* Simeticone

CHEMICAL NAME : dioxosilane; trimethyl (trimethylsilyloxy) silane

MOLECULAR FORMULA: $(C_2H_6OSi)_n \cdot (SiO_2)_m$
222.462 g/mol

STRUCTURAL FORMULA:



Inclusion Criteria

Inclusion criteria for both studies were healthy infants, not older than eight weeks, who according to a daily baseline registration fulfilled the criteria for colic: crying/fussing more than three hours/ day at least three days per week. At least one of the parents had to speak Swedish.

Exclusion Criteria

Exclusion criteria were infants born before gestational week 37, insufficient weight gain, having tried acupuncture or taking prescribed medication. Simethicone and/ or probiotics were allowed.

Treatments Administered

Treatment is often recommended from parents' subjective description of the infants' behavior without measuring the infants crying, and advice given by the CHC nurse often lack structure and follow up (Landgren et al., 2012).

Counselling parents by giving them specific instructions on how to adapt their interaction with the infant showed good results in two studies (Keefe et al 2005; Dihigo, 1998) while another study did not find that the infants crying reduced more than compared with evaluation and support from the CHC nurse and paediatrician alone (Parkin et al 1993). The use of herbal tea might have effect when treating colic but given the lack of standardization of components in the herbal products, unknown optimal dosage and the risk of interference with the infant's normal food intake parents are advised to use these products with caution (Roberts et al 2004). Sugar solution can be used for its analgesic effect in infants and has therefore been suggested as a possible method to also reduce discomfort in infants with colic.

Although there are limited studies conducted there have been some positive results when compared to placebo (Perry et al 2011). Spinal manipulations as treatment for colic are performed by chiropractors. This treatment has however shown varied results in studies conducted in Scandinavia (Wiberg & Wiberg, 2010;

Olafsdottir et al 2001; Wiberg et al 1999). Acupuncture has a pain-inhibiting effect and has been proven to reduce crying and gastrointestinal symptoms in infants with colic according to

Landgren et al (2010) and Reinthal et al., 2008. Skjeie et al (2013) however did not find any statistically significant effect of acupuncture as treatment for colic in infants.

The anticholinergic drug Dicyclomine showed significant improvement when used for treating colic (Lucassen et al., 1998) but was withdrawn from the Swedish market due to serious side effects.

Removal of Patients from Therapy or Assessment

Exclusion criteria were infants born before gestational week 37, insufficient weight gain, having tried acupuncture or taking prescribed medication. Simethicone and/ or probiotics were allowed.

Drug Susceptibility Tests

Not Applicable

Primary Efficacy Parameters

The primary outcome in the present sub-study was the reasons for which screened infants were not included in the two main trials. A secondary outcome was the frequencies of parents using simethicone and probiotics and their reported effect of the treatment.

Safety variables

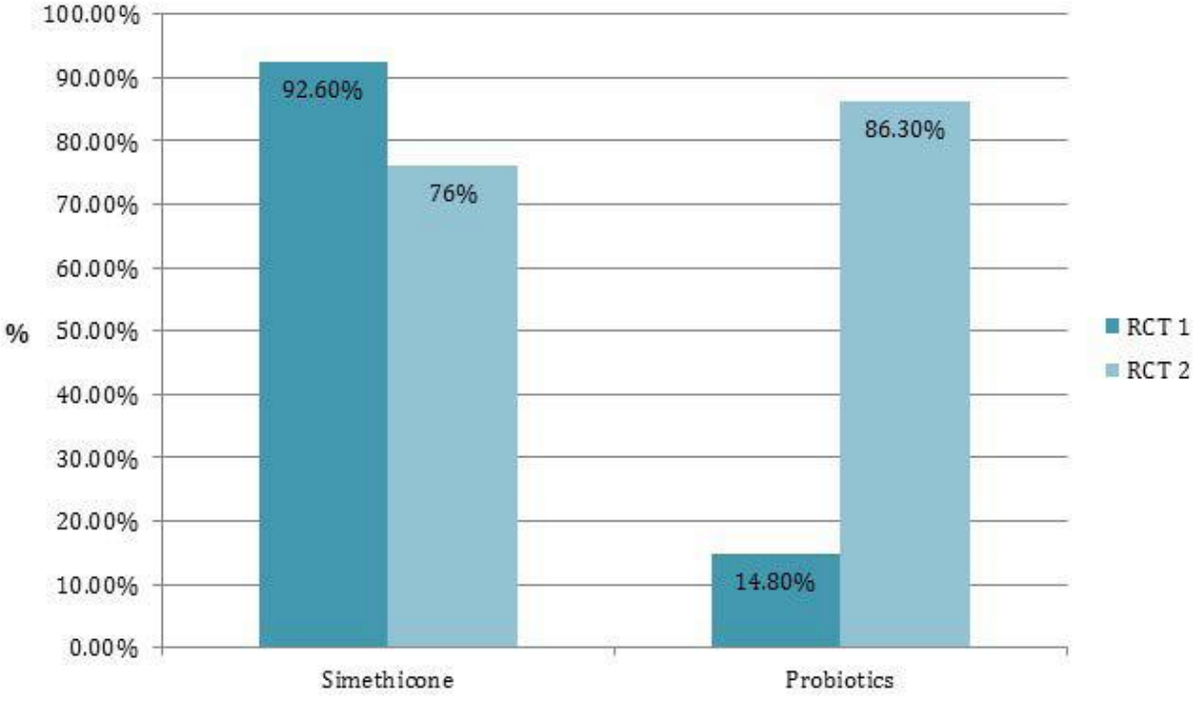
The strength of this study was the possibility to analyze unpublished data from two different RCT's, which allowed a comparison over time and a greater sample size. Furthermore, data from all the infants with symptoms of colic that were screened for participation in the study but were not included could be categorized which gave a valuable and unusual insight to how these parents and infants may be helped by using a diary for both registration of crying and for evaluating the effects of given advice and treatments. It is also a strength that this study used data from two different trials where the location of the study centers were chosen to provide a wide range of the population, incorporating both larger and smaller cities with cultural and socioeconomic differences. The participants should therefore be reasonably representative of the general population. All the infants in the two trials that had been treated with simethicone or probiotics still fulfilled the criteria for colic at inclusion.

This is a limitation as there might be infants who received the same medication with sufficient effect and therefor never applied for participation. Although this is a risk for both treatments it is however unlikely for simethicone since there is a lack of positive results in earlier RCTs that where specifically designed to evaluate the effect of simethicone when treating colic (Lucassen, 2010)

Statistical Analysis

The statistical software package SPSS™ version 22 (SPSS Inc., Chicago, IL) was used for calculations. Initially the descriptive statistics were calculated. The Kolmogorov Smirnoff's test was used to determine if the variables were normally distributed. Since all the variables were not normally distributed the data were analyzed with a non parametric Chi square test using crosstabs for significance testing and Phi for determining the strength of the correlations. Phi was chosen instead of Cramer's V since the analysis only demanded a two-by-two contingency table, which was used to describe the association between the variables as well as the difference in use of simethicone and probiotics over time. P-values <0.05 were considered statistically significant. For determining the strength of the correlations between parents use of probiotics or simethicone and their reported experience of the infants appetite the Spearman rank correlation test were used since this variable contained more than two available answers where the parents could rank appetite on a three level scale.

Table 4. Differences in frequency of treatment with simethicone and probiotics between the first (Landgren et al., 2010) and the second (Landgren & Hallström, in manuscript) RCT.



EFFICACY EVALUATION

Descriptive data and background characteristics from both RCT´s (Landgren et al., 2010, Landgren & Hallström, submitted) are reported in table 3. There was a significant association between the use of simethicone and parents having more than one child (p=0.018) as well as treatment with simethicone and previous experience of colic in the family (p=0.013).

There was also a difference in the reported use of simethicone after having had a normal or a complicated delivery (p=0.015), where the majority of infants being treated with simethicone had been delivered without complications. The use of probiotics had a significant association with reported food allergies and/or food intolerances amongst siblings and/or parents (p=0.019). There was also a significant association between higher frequency of complications during the pregnancy and a more frequent use of probiotics (p=0.002) as well as having tried probiotics and exclusive breastfeeding (p=0.031).

The results also indicate an association between higher use of probiotics in families where parents and/or siblings had fewer problems with eczema (p=0.049) as well as having tried probiotics and the infant having less problems with skin rashes (p=0.035).

All background characteristics where analyzed for association between the frequency of treatment with simethicone and use of probiotics. There were no other significant associations found amongst the remaining background characteristics.

Primary Objective Outcome

All patients provided signed informed consent. In accordance with the Declaration of Helsinki, International Conference on Harmonization - Good Clinical Practice (ICH-GCP) – guidelines and applicable local laws. The Ethics Committee of the GVK Bioscience private limited approved the protocol.

Efficacy and safety Conclusions

It is valuable for paediatricians and nurses working at the CHC to know that the majority of parents treat their infant with simethicone and/or probiotics despite weak evidence to support this in infantile colic. The CHC nurse is the primary contact between these parents and the health care system and they are required to base their recommendations on scientific evidence or methods that has been well proven to have effect from years of experience. This foundation helps built trust between the parents seeking advice and their CHC nurse and ensures the safety and quality of care. Since there is no scientific evidence for the effect of simethicone in infantile colic, simethicone should not be recommended by the CHC nurse when giving advice on treating colic in infants. There is a need for more RCTs that could compare the effect of probiotics to placebo when treating infantile colic before CHC nurses can recommend probiotics based on scientific evidence.

Parents also wish that advice given by their CHC nurse are more structured and systematically evaluated. The CHC nurse has therefore a great responsibility to not only give advice based on up-to-date scientific evidence but to also adapt how the advice is being given and evaluated.

By using a diary where parents report the infants crying both the parents and the CHC nurse get valuable information about

Adverse Reactions Experienced in both the treatment groups

As dimeticone is not absorbed from the gastro-intestinal tract, adverse effects attributable to the active ingredient would not be expected.

DISCUSSION AND OVERALL CONCLUSIONS

There were two main reasons for infants who were screened for participation in the two trials not to be included. When the crying was actually measured in a diary, about half of the infants were reported to cry < 3 hours per day and therefore did not fulfill the criteria for colic. Besides, several infants reduced their symptoms of colic spontaneously when introducing a cows' milk protein free diet. It is interesting that so many parents who claimed their infant had colic reported normal crying when they used a diary. This indicates that the diary is a valuable tool for nurses working at CHCs when discussing normal crying and fussing with this group of parents. All parents who had not already tried a period of five days where the infant was not exposed to cows' milk protein neither via breast milk nor formula was instructed to try this intervention during baseline. Thus there might be a substantial overlap between the categories "Crying and fussing < 3 hours/ day" and "Spontaneous remission or remission due to cows' milk free diet". It is noteworthy that so many of the infants with excessive crying calmed down to normal crying when they were no longer exposed to cows' milk protein. As five days was enough to normalize the crying in a large group of infants, this seems to be a safe, inexpensive and simple intervention that nurses could encourage parents to try as an early intervention. In these trials where more than 600 parents were instructed to try this diet only one mother was reported not to manage a cows' milk protein free diet for five days.

The result also showed a difference in the use of the anticholinergic drug Dicyclomine between the two trials. In the first trial, collecting data 2005 – 2007, six infants started with dicyclomine during baseline, and four more dropped out as parents started to give the infant Dicyclomine (Landgren et al., 2010). In the ACUCOL trial conducted eight years later the use of dicyclomine had been reduced to zero. This reflects changed recommendations from the authorities due to reported side effects from this medication. Another difference is that more parents in the second trial chose to take the infant to an acupuncturist outside the study center to avoid being randomized to the control group. This choice was possible during ACU-COL´s data collection period since acupuncture for colic at that time was available in more clinics. Even though almost every infant in the first trial had been treated with simethicone only 2.7% of the parents reported effect.

The number of infants being treated with simethicone had decreased in the second trial where 5.4% reported having effect of the treatment. Probiotics were introduced on the market while the first trial was being conducted which can explain the increased use of probiotics from only 14.8% in the first trial to 86.3% in the second trial.

While no one reported perceived effect of probiotics in the first trial, only 3.2% reported effect in the second trial despite the excessive increase. It is noteworthy that despite the lack of research that supports the use of simethicone when treating infantile colic (Lucassen, 2010; Hall et al., 2011; Savino et al., 2014) it is still one of the most common recommended treatments in Sweden (Landgren et al., 2012).

The use of simethicone reduced between the two trials but more than three quarters of the infants was still given simethicone. About one third of these reported “effect” or “some effect” of treatment with simethicone which is comparable to using placebo (Metcalf et al., 1994) and the infants still fulfilled the criteria for colic in spite of the medication. While the use of simethicone was significantly reduced, the treatment with probiotics increased remarkably which could be a reflection of both the introduction of the product on the market during the first trail and the increasing research on treatment with probiotics that are showing positive results. These studies have however mainly been conducted on infants that are being exclusively breastfed which still leaves a need for research on infants being fed with formula before probiotics can be recommended to the general population (Urbanska & Szajewska, 2014). This might also explain the association between the use of probiotics and exclusive breastfeeding

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