

DOES PROBIOTIC AND PREBIOTIC TREATMENT IN IVF CYCLES OF INFERTILE WOMEN WITH INTESTINAL DYSBIOSIS, AFFECT PREGNANCY RATE?

Irollo A.M¹, Gangale M.F.¹, Tartaglione A.¹, Criscuolo C.², Aiello R.¹, Stortini E.¹, Infante V.¹

¹Center for Reproductive Medicine Chianciano Salute via C. Marchesi 73 Chianciano Terme (SI);

²OmniaSalute, viale di Villa Massimo 8, Roma.

Abstract. Background: Several findings show how as the intestinal microbiota regulates some important metabolic and physiological body functions, as production of toxic or useful substances, induction of inflammatory processes and interaction with immune system.

Alterations of the intestinal microbiota can occur by changes in composition (dysbiosis), function, or microbiota-host interactions and they can be directly correlated with several diseases as bacterial vaginosis (BV).

Objectives: the aim of this study is to understand if there is a correlation between acute intestinal dysbiosis condition and the failure in IVF freeze embryo transfer(FET)cycles, and if a prebiotic and probiotic treatment can improve the success rate. Search strategy: we investigate about the incidence of pregnancy rate in acute-severe dysbiotic patients and prebiotics-probiotics treated patients undergoing to a FET cycle.

Selection criteria: 53 patient with acute or severe intestinal dysbiosis undergoing IVF cycle was recruited and randomized in two group. Group A (n=29) (control group) and Group B (n=24) have to transfer two freeze embryos each. Group B was treated with prebiotic and probiotic for at least two months to reduce the dysbiosis condition from acute-severe to mild-moderate level.

Main results: After FET in the group A there have been 5 ongoing pregnancy and 3 born baby, in the group B (treated group) there was been 15 pregnancy(p<0,05) and 12 born baby (p<0,05)

Conclusion: Our result show that a treatment whit probiotic and prebiotic is able to increase pregnancy rate in IVF cycles of infertile women affected by acute or severe intestinal dysbiosis, by modulation of some crucial mechanisms involved in embryo implantation.

Key word: microbiota, IVF, Pregnancy rate, bacteria, infertility

INTRODUCTION

The studies to improve the embryo implantation, led to discovery of a very important correlation between fertility and microbiota. (1)

The researchers focused their interest on the vaginal microbiota, a set of bacteria that colonize the vagina. (2)

In the physiological conditions, the vaginal environment is predominately colonized by lactobacilli, GRAM-positive bacteria, able to produce lactic acid, with an associated decreasing of the vaginal pH which prevent the growth of pathogenic bacteria.

The presence and concentration of lactobacilli is influenced by estrogen levels and change cyclically throughout a woman's life. During women's fertile life, the higher levels of estrogens, increase the thickness of

the vaginal epithelium and the glycogen levels, thus enriching the microbiota of lactobacilli. This condition protects the woman from the infections of the reproductive tract.

A change of the vaginal microbiota (vaginal dysbiosis), from Lactobacillus dominated environment, to a more heterogeneous environment with anaerobic bacteria, such as Gardnerella vaginalis and Atopobium vaginae, is observed in a common genital disorder named bacterial vaginosis (BV). (3)

BV is a common genital disorder, often sub clinic, with a prevalence of approximately 19% in the infertile population.

Few studies have been conducted in infertile women, and some have suggested a negative impact on fertility as a consequence of the presence of BV. In particular was observed a lower pregnancy rate in women with

Correspondence to:

Infante V. OmniaSalute, viale di Villa Massimo 8, Roma
email: atlkenzo@gmail.com, phone: 3395611037

vaginal dysbiosis than in those with a prevalence of lactobacilli (9% vs.40%).(3)

OBJECT OF STUDY

In addition to hormonal changes, vaginal microbiota is influenced by changes in intestinal microbiota. In the women affected by vaginal microbiota disorders, intestinal dysbiosis and chronic intestinal disease are present, which contribute to chronic inflammatory process of pelvic organs, as well as increased risk of adverse pregnancy outcomes. (4)

This evidence put a common-base link to hypothesize an important role of intestinal microbiota in the embryo-implant mechanism of infertile woman.

In accordance with our hypothesis 53 patients were recruited, between 30 and 35 years old, genetically healthy, affected by tubal infertility with no male factor, and undergoing frozen embryo transfer (F.E.T.) after at least two fresh embryo transfer and implantation failures. The screening was performed by bacterial DNA extraction (real-time PCR) on fecal sample searching for: Firmicutes, Bacteroidetes, Bacteriodes, Prevotella, Lactobacillus acidophilus, C. Perfringens, Helicobacter pylorii, Enterococcus, E.coli, Bifidobacterium, Streptococcus, C. albicans, Enterobacteriaceae and Staphylococcus aureus.

The PCR screening test uses five levels for dysbiosis (based on Dysbiosis index (5) (see Tab.1)

Tab 1. Dysbiosis panels

Level 1: no dysbiosis	Level 4: acute dysbiosis
Level 2: mild dysbiosis	Level 5: severe dysbiosis
Level 3: moderate dysbiosis	

All patients showed intestinal dysbiosis from acute to severe level.

all of them showed a healthy uterine cavity after hysteroscopy. The same post-FET therapy was observed by everyone (cardioaspirin, prontosgest, seleparina and deltacortene 5 mg/day).

These patients were randomly assigned to 2 separate groups.

GROUP A: 29 patients were assigned to control group: no probiotics treatment

GROUP B: 24 patients receiving therapy with probiotics and prebiotics (syngut and serplus 2 capsules/die each) for at least two months before the "frozen embryo-transfer", to allow an improvement of dysbiosis patterns.

Only five patients of group B, affected by the severe dysbiosis, were treated with hydrocolonotherapy and bacteria repopulation through an insufflation of probiotic mixed in a physiological saline (five VSL3 [explain what is VSL3] bags into a 250 ml of sodium chloride

solution). In the next two months, they assume oral probiotics-prebiotics therapy as previously showed. After at least two months of therapy, all treated patients repeated PCR screening test for dysbiosis.

All the patients underwent a frozen embryo transfer (2 good quality embryos for each patient, except 2 patients for each group to which 2 blastocysts were transferred. The F.E.T was performed with the same uterine preparation and by ultrasound-guided procedure.

STATISTICS

Statistical analysis was carried out with the Yate's chi squared test.

RESULTS

Our results reported the following data:

After probiotic treatment, all treated patients (Group B) have repeated the PCR bacteria screening with these results:

all acute and severe panel (level 4-5), showed a regression up to "mild" to "moderate" panel (level 2-3).

The ongoing pregnancies in the group A (n=29), were 5 (17,2%) while there were 15(62,5%) in group B(n=24). These data showed an improvement of 45% of pregnancy rate (see TAB 2)

The born babies rate was 3 vs 12 (see TAB 2)

Tab 2. Number of Pregnancies and birth babies obtained in the two groups after frozen embryo transfer (F.E.T.)

Therapy	Specimens	On going pregnancies (p=0,0447)*	Live birth babies (p=0,037)*
Not treated	Group A (n=29)	5/29	3
Treated	Group B (n=24)	15/24	12

*=Significative

DISCUSSION

The intestine is the most densely populated organ in the human body, although other parts, such as the skin, vaginal mucosa, or respiratory tract, also harbor specific microbiota. This microbial community regulates some important metabolic and physiological functions of the host, and drives the maturation of the immune system in early life, contributing to its homeostasis during life. Alterations of the intestinal microbiota can occur by

changes in composition (dysbiosis), function, or microbiota-host interactions and they can be directly correlated with several diseases. (6)

The intestinal microbiota performs the following important functions inside our body:

a) Production of toxic or useful substances:

Microbiota represents a second digestive organ; in fact, bacteria with their enzymes can produce useful or toxic metabolites.

In physiological conditions, probiotics bacteria metabolize organic fats favoring the energy production. For example, the pyruvic acid metabolism, produces useful substances in the Krebs cycle and stimulates the ATP production by mitochondria. In conditions of dysbiosis, saprophytic (increased in concentration) or pathogenic bacteria convert pyruvic acid into toxic metabolites (phenolic acid and alcohol) depriving the Krebs cycle substrate and consequently the energy cells.

In dysbiosis status were also observed a surplus of proteolytic bacteria and an increase of decarboxylation process of amino acids into biogenic amines which consequent toxic and inflammatory effects (2). In particular, increased conversion of histidine into histamine, stimulates the uterus smooth muscle and induces an allergic state; Tyrosine is transformed into tyramine with a consequent vasoconstrictive effect; the phenylalanine change into phenylethylamine causing a hypertensive effect; the tryptophan is turned into skatole or 3-methylindole and subtracted from the production of leptin; the lysine is transformed into cadaverine with mutagenic effects.

b) Induction of an inflammatory process

In dysbiosis status, there is an evident increase of the lipopolysaccharide (LPS), known as component of the external membrane of GRAM negative bacteria (*Escherichia coli*, *Shigella*, *Neisseria*, *Salmonella* sp). (7) The LPS induces the production of TNF-ALFA, IL6, PAF and other cytokines that activate an inflammatory process by prostaglandin production.

Furthermore, these conditions, activate the complement, thrombotic phenomena, and alteration of insulin sensitivity.

C) Interaction with the immune system

In the intestinal mucosa, between the enterocytes, there are some cells called M-CELL which are part of the MALT (lymphoid tissue associated with mucous membranes); (8). These cells have the function of sampling the antigens and discriminate self from "non-self" maintaining the immune tolerance.

In a dysbiosis status this protective film is missing; self or non-self antigens can penetrate through the intestinal mucosa stimulating the lymphocytes with consequent activation of the antibody and cell mediate immune response.

In this way self-antibodies can attack human antigens with consequent damages or autoimmune diseases.

CONCLUSION

The intestinal microbiota dysregulation, could have negative effects in the human fertility through the following mechanisms:

- induction of a toxic state capable of injuring the embryonic cells;
- reduction of metabolites useful for fertilization and embryo implantation;
- increase of an inflammatory condition caused by the presence of cytokines that interfere with embryo ability to implant;
- Stimulation of the immune response versus embryonic antigens.

Our results show that a probiotics and prebiotics treatment is able to increase pregnancy rate in IVF cycles of infertile women affected by acute or severe intestinal dysbiosis, probably by modulating some crucial mechanisms involved in embryo implantation.

Further studies should be useful to investigate on main involved bacterial strains on the implantation failure.

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