FFECT OF PROBIOTICS ON MICROBIAL FLORA IN PREGNAN WOMEN WITH BACTERIAL VAGINOSIS

Vasundhara D*, B Dinesh Kumar, Hemalatha R@

ICMR - National Institute of Nutrition, Hyderabad

*SRF –UGC & Investigator; @ Director, NIN & PI

ck ground…..

female lower genital tract is an ecological niche –aerobic and anaerobic microorganisms co-exist

dynamic balance.

erial Vaginosis (BV): affects 10 to 30% of women (Geeta gupta et.al.,2013; Hemalatha et.al., 201

ata et.al.,2010; Neeraja et al., 2009; Madhivanan et.al., 2008)

is a polymicrobial vaginal condition – alteration of *Lactobacillus* predominant normal vaginal flo

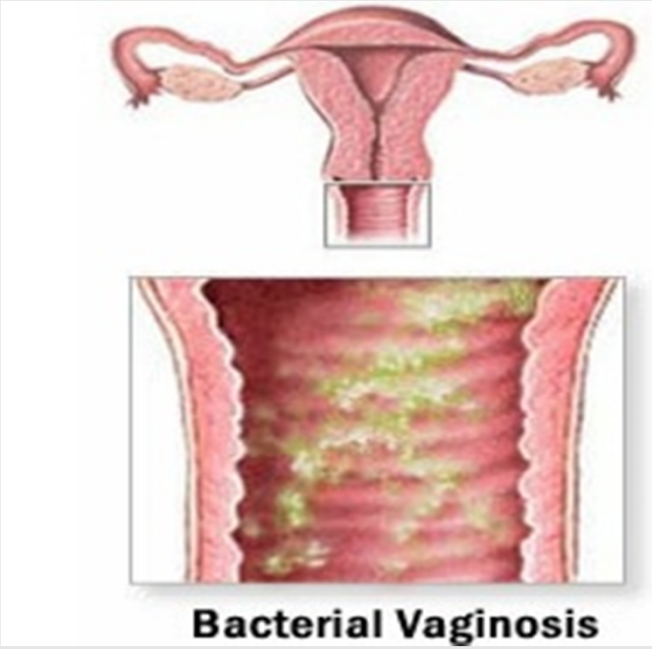
an environment dominated by anaerobes, *Gardnerella vaginalis, Mobiluncus,Prevotella, Bacteriodes*

nd *Mycoplasma species.*

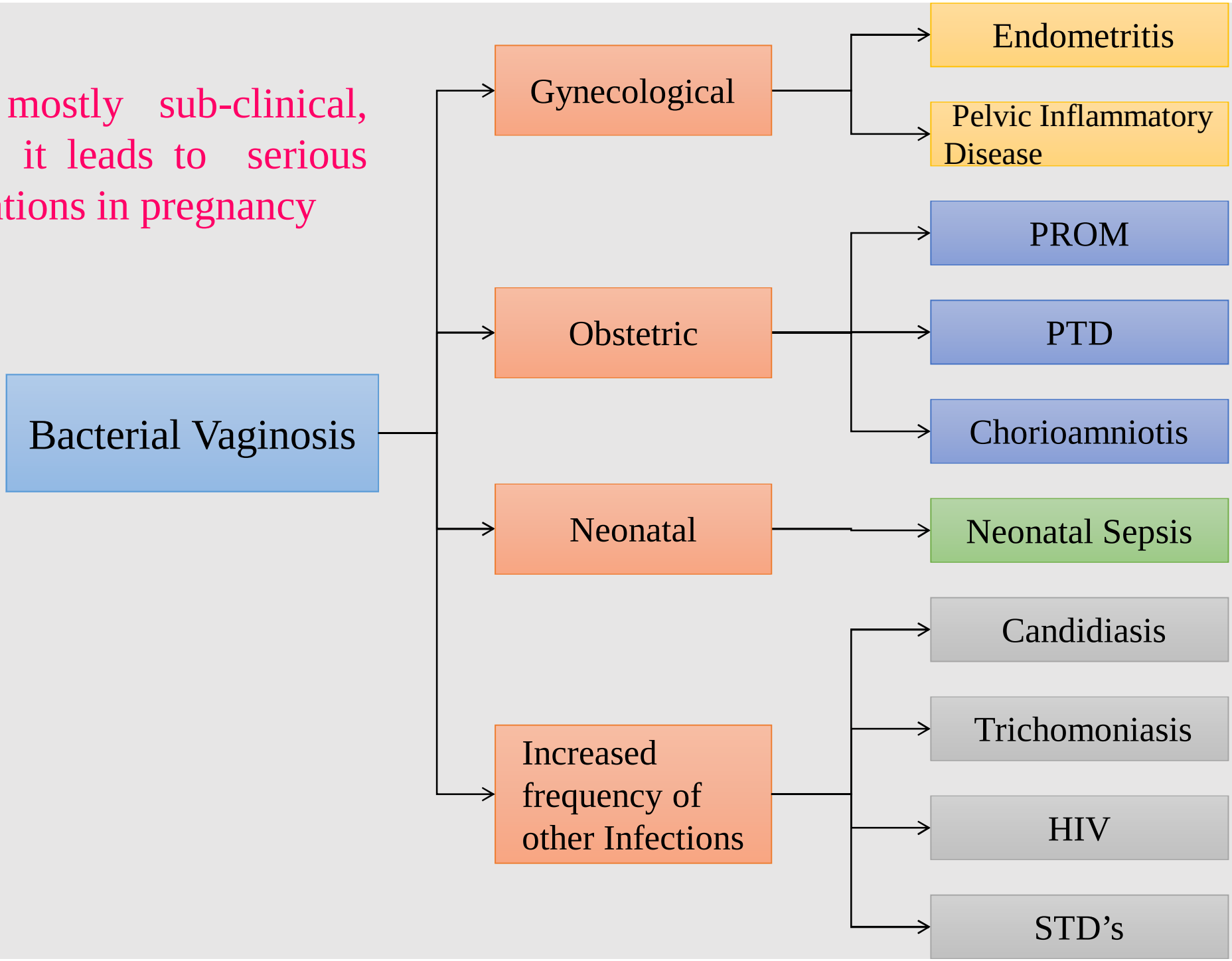
is the most common infection in women of child bearing

and incidence during pregnancy is difficult to determine

e up to 40% women are asymptomatic (Susan Van et al. ,2008).



y is mostly sub-clinical, wever, it leads to serious mplications in pregnancy



vailable treatment:

Metronidazole (tablet or vaginal gel)is the most common and preferred antibiotic treatment for BV

Clindamycin (tablet or cream)

Tinidazole tablet

de effects:

ndigenous gut flora gets disturbed due to oral antibiotic intake along with nausea

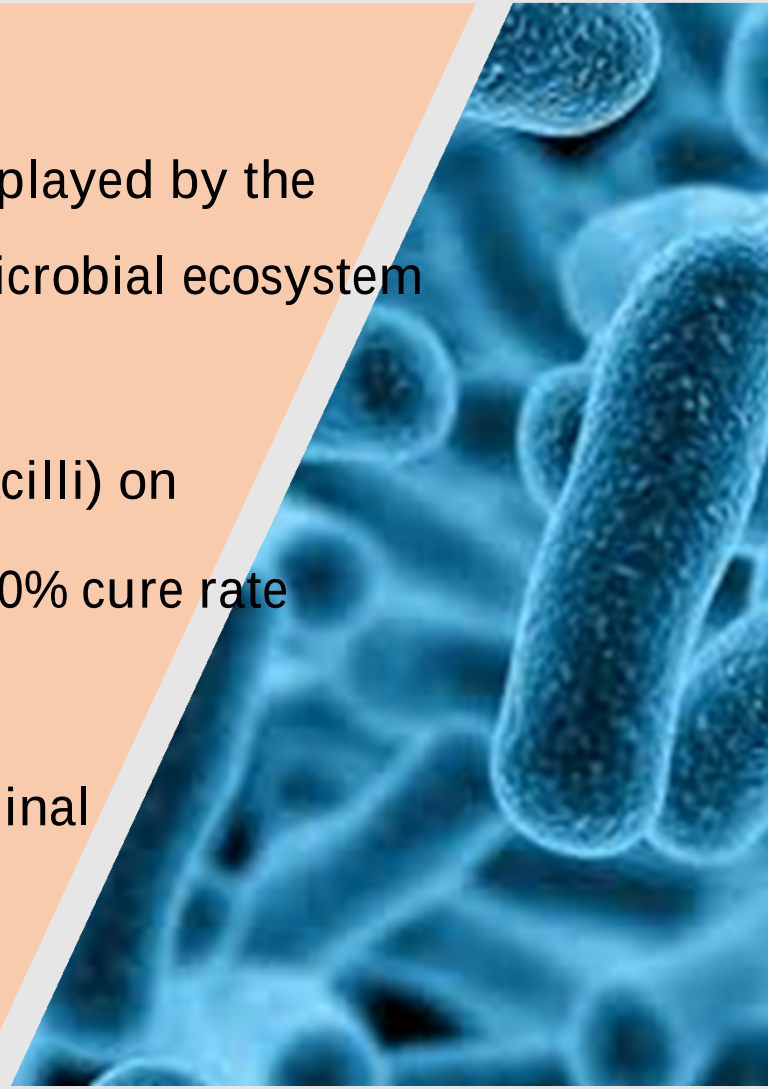
Healthy bacteria is effected by local antibiotic applications

Antibiotics are not advised in first trimester of pregnancy

Failure of antibiotics to change host receptivity to pathogen recurrences

Relapse of BV is very frequent (30-50%). Replacing the pathogenic bacteria with beneficial may aid in quicker recovery & lesser relapse.

obiotics are "Live microorganisms which when administered in adequate amounts confer a hea
benefit on the host" as defined by FAO/WHO. They have been recently studied a lot in prevent
and treating vaginal infections. Lactobacilli are the commonest organisms used as Probiotics



ne rationale for use of Probiotics in women is based on the role played by the
ginal healthy microbiota and the need for restoration of this microbial ecosystem
ter infections

study on effect of probioitcs (vaginal tablets containing lactobacilli) on
ginal health and pro-inflammatory cytokines showed nearly 80% cure rate
emalatha et.all,2012)

here are studies showing the positive effect of probiotics as vaginal
aries in pregnant women (Samuli et.al.,2001; Nishijima,2005)

Clinical trials on probiotics use for treatment of bacterial vaginosis (BV).

uthors	Size	Type of study/ duration	Intervention	BV cure rate
nukam *et al.*, 006b	40	R, OB, AC 30 days	Daily vaginal capsule containing *L. rhamnosus* GR-1 (109 CFU) and *L. reuteri* RC-14 (109 CFU) or 0.75% metronidazole gel b.i.d. for 5 days	65% compared to 33% metronidazole (P = 0.056)
Iastromarino *et al.*, 009	34	R, DB, PC 3 week	Daily vaginal tablet containing ≥109 CFU of *L. brevis* CD2, *L. salivarius* FV2, and *L. plantarum* FV9 for 7 days	50% compared to 6% control (P = 0.017)
arent *et al.*, 996	32	R, PC 4 week	1-2 daily vaginal tablet containing *L. acidophilus* ≥107 CFU and 0.03 mg estriol for 6 days	88% compared to 22% control (P <0.05)
Iallén *et al.*, 992	57	R, DB, PC 20-40 days	Vaginal suppository containing *L. acidophilus* 10^{8-9} CFU or placebo b.i.d. for 6 days	21% compared to 0% control (P = NS)

= randomized; DB = double blind; PC = placebo controlled; OB = observer blind. AC = active controlled. CFU = colony forming units.

Paola Mastromarino, Beatrice Vitali *et al.* , 2013 NEW MICROBIOLOGICA, 36, 229-238, 2013

obiotics when used locally are effective in BV cure. However, the effects are transient and curs within 3 to 4 weeks. Therefore, in the current study we used oral probiotics.

pothesis

ence supplementation of probiotics to pregnant women will treat and prevent relapse of Bacte aginosis. Probiotics help to attain healthy vaginal flora.

ective:

study the effect of oral probiotics supplementation on Bacterial vaginosis (BV) cure and relapse egnant women.

his study is first of kind where oral supplementation of probiotics was given for pregnant omen with BV

HICAL APPROVALS

itutional Ethical Committee**(IEC)** has approved the study, **EC NIN no: CR1/I/2014**

gs Controller General India **(DCGI)** approval obtained, **DCGI no: CT-/180/2012**

ical Trails Registry – India **(CTRI)** registered on 29th January 2013.

RI Registration No: **CTRI/2013/01/003337**

hania Medical college Ethics Scientific committee approval was obtained.

MPLE SIZE

ssuming 88% cure from BV in the probiotic and antibiotic treated group and 40% cure in placeb
d antibiotic treated group (Kingsley et.al.,2006, Microbes and Infection:1450-54) 50 women / gr
required to get significance at 5% with 80% power. Expecting 25% attrition, 70 women will be
cruited per group i.e.140 women with BV will be randomized into probiotic and placebo groups
ut all of them are treated with local antibiotic, Clindamycin).

lusion Criteria

gnant women in 26-28 weeks of gestation

lusion Criteria

vical incompetence (Circlage in current gestation)

/ infected and Hbs AG

ltiple gestation

temic arterial hypertension under medication

onic asthma requiring intermittent therapy

ent not willing to comply with the clinical study instructions

existing Thyroid disease

tational Diabetes / preeclampsia

tudy design

andomized, double blind, placebo controlled study conducted at Government Maternity Hospital

upplementation

Probiotic - ***Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14** with the concentration of $2X10^8$ CFU / capsule . (The strains were initially isolated from vagina of healthy women by Dr. Gregor Reid & Dr. Andrew Bruce).

Placebo

eening of the subjects is done by using **Nugent's score** of the Vaginal smears

Species	None (0)	<1	1-4	5-30	>30
ctobacillus rphotypes	4	3	2	1	0
vaginalis	0	1	2	3	4
obilincus p	0	1	1	2	2

The count of all species was taken as total score

Total Score	Conditi
0-3	Norma Flora
4-6	Interme e
7-10	Indicati of BV

nsel's criteria was also followed as the diagnostic measure

omogenous white discharge

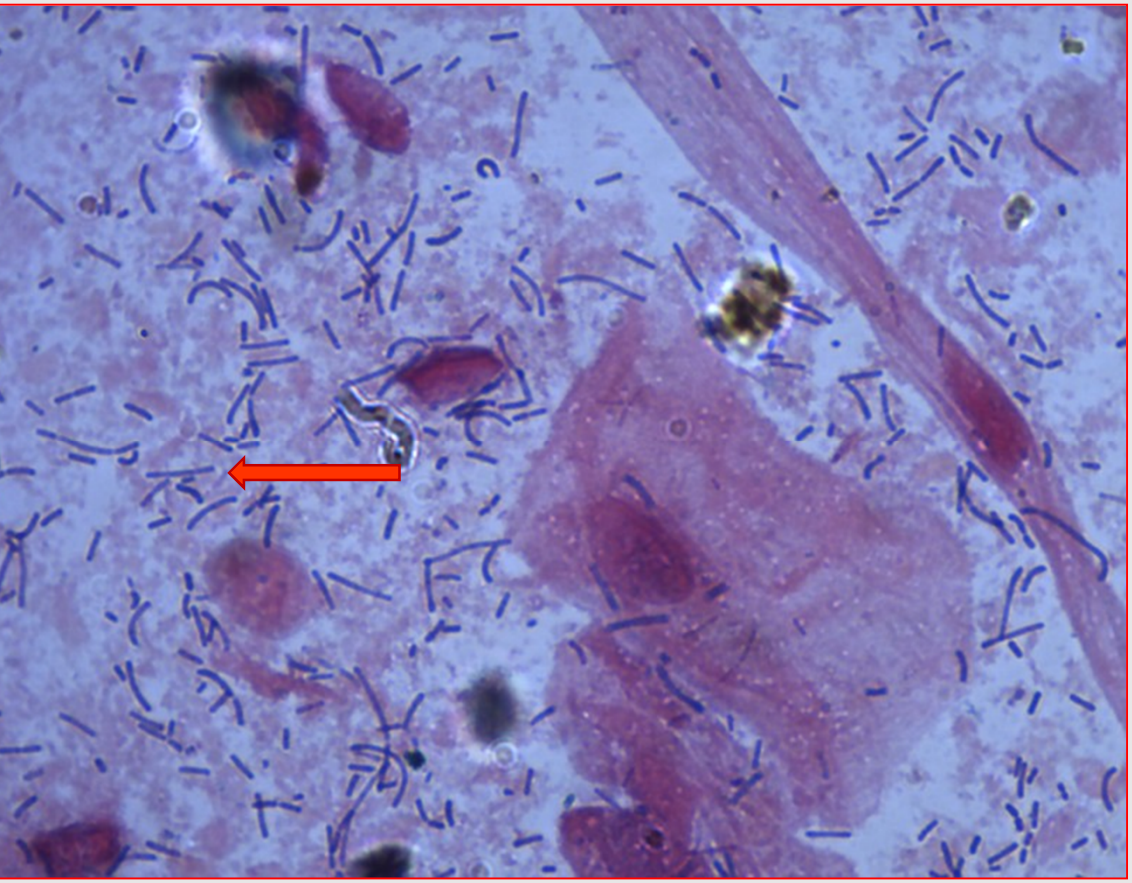
mine or fishy odor when added KOH

H more than 4.5

esence of clue cells

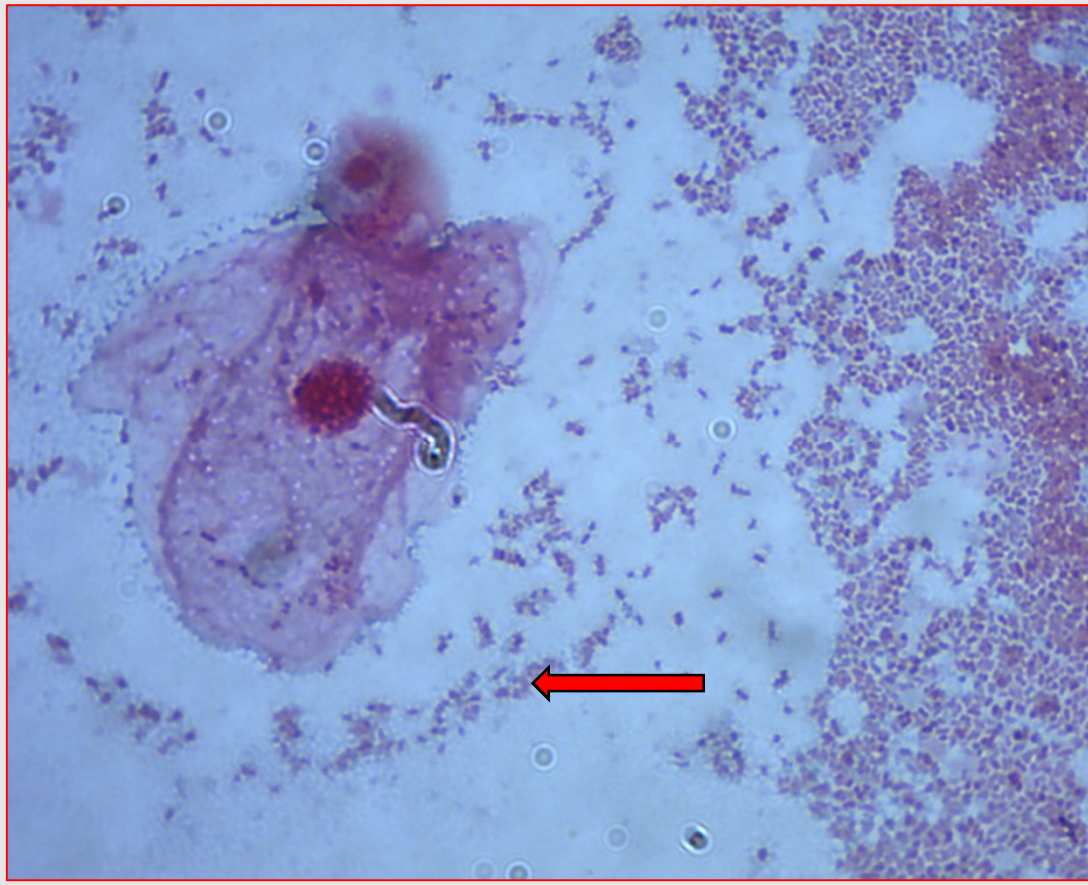
Image 1- Vaginal smear examination

Normal smear



➡ **Healthy microbiota- *Lactobacilli***

Infective smear



➡ **Epithelial cell whose borders are obscur**
gram variable, pleomorphic pathogenic bacte
called 'Clue cell'

Pregnant women in their initial days of third trimester
356 women screened for BV

Nugent's score > 3 and Amsel's criteria positive = 140
Anthropometry and blood sample were collected,Vaginal and fecal samples were collected for analysis and 140 pregnant women were randomized

Visit 1

Probiotic arm: 70 women Antibiotic for seven days + Probiotic supplementation until delivery

Placebo arm: 70 women Antibiotic for seven days + Placebo supplementation until delivery

Visit 2

ata could
ollected
ubjects
igration

30 days: Vaginal samples for BV screening (n=64). Vaginal samples for DNA isolation and Real-Time PCR analysis & Microbiome analysis

30 days: Vaginal samples for BV screening (67). Vaginal samples for DNA isolation and Real-Time PCR analysis & Microbiome analysis

Visit 4

Within 3-5th days of delivery, Neonates' anthropometry

Within 3-5th days of delivery, Neonates' anthropometry

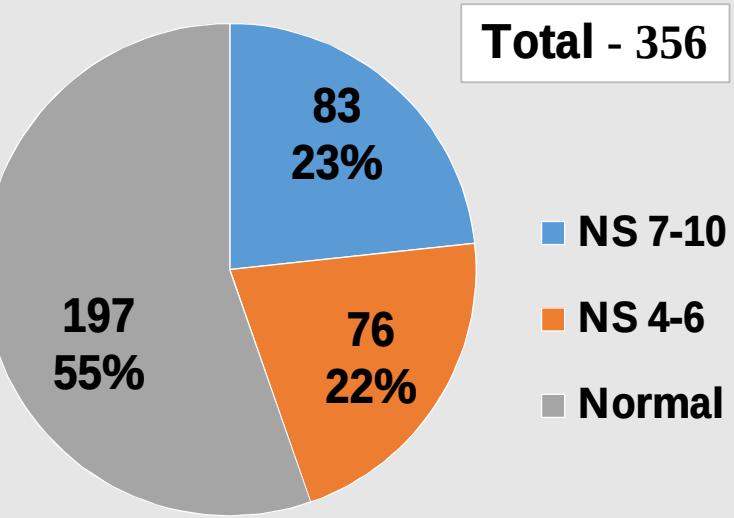
Data could
collected
4 subjects
migration

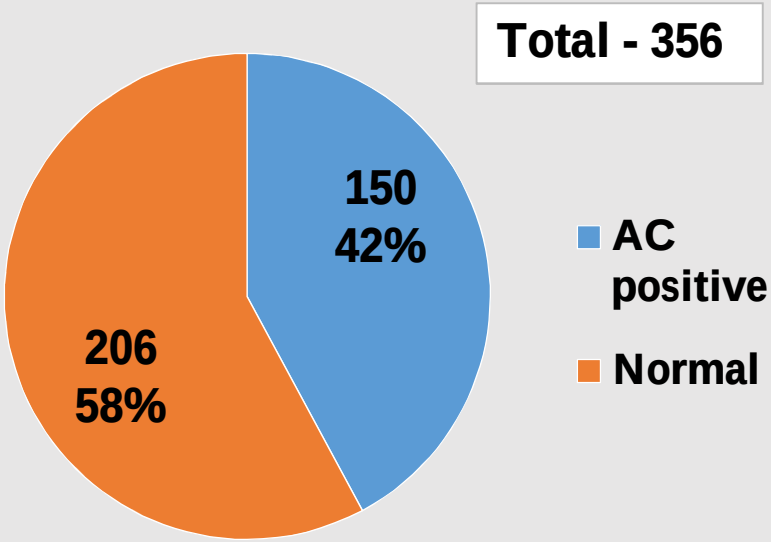
45 days after delivery vaginal samples were collected for BV screening (n=25)

45 days after delivery vaginal samples were collected for BV screening (n=31)

Results

Incidence of BV in the screened subjects





-Nugent Score, AC- Amsel's Criteria

ed on Nugent's score, of the 356 pregnant women, 23.3 % (83) had BV, 21.3% (76) had intermedi
a and 55.3% (197) had normal vaginal flora (Figure 1). Based on Amsel's criteria, 42.1% (150) ha
and the rest were normal 57.8% (206). Of these pregnant women, 42% (150) were positive for BV
h the methods (Nugent's score and Amsel's criteria).

Table 1: Demographic and Clinical profile of the subjects

Parameter	Probiotic group	Placebo group	Total	p.valu
Age (years)	22.50±2.34 (70)	22.57±2.65 (70)	22.54±2.49 (140)	0.866
Height (cms)	153±5.2 (70)	153±5.19 (70)	153±5.19 (140)	0.881
Weight (kgs)	54.21±9.33 (70)	54.53±7.97 (70)	54.3±8.6 (140)	0.853
BMI	23.14±3.73 (70)	23.31±3.23 (70)	23.2±3.47 (140)	0.918
Hemoglobin (gm/dl)	10.12±1.36 (70)	10.29±1.45 (70)	10.2±1.4 (140)	0.466
Gestational age at recruitment	28.6±1.27(70)	27.8±1.82(70)	28.57±1.36(140)	0.872
Gestational age at delivery (weeks)	39.8±1.92 (68)	38.97±1.31 (68)	38.9±1.6 (136)	0.327
Birth weight	2.73±0.39 (67)	2.62±0.5 (69)	2.67±0.43 (136)	0.985
New born baby's length (cms)	48.79±1.7 (56)	47.25±2.3 (60)	47.9±1.8 (116)	0.083
Chest circumference (cms)	31.3±1.6 (56)	31.1±1.7 (60)	31.2±1.7 (116)	0.410
Head circumference (cms)	33±1.35 (56)	32.8±1.36 (60)	32.9±1.7 (116)	0.335
Mid upper arm circumference (cms)	9.58±0.84(56)	9.550.74±(60)	9.560.79±(116)	0.828
Low birth weight	13%	15%	20.7%	0.30
Pre-term births	5.0%	6.0%	5.8%	0.63

Results continued…

he demographic details of the subjects were similar in both the groups

n the anthropometric measurements, length of the baby was observed to be higher in robiotic group compared to placebo group but was not statistically significant

he Nugent's scoring during the post-natal follow-up significantly decreased in probioti roup compared to placebo showing reduction of infection

ased on Amsel's criteria, during both the follow-ups probiotic group showed a significa eduction of infection

resence of "Clue cells" which is considered as the manifestation of the infection also educed during post-natal follow-up in probiotic group

eal-Time PCR analysis showed the dynamics of the microbial flora by increase of *actobacilli* species quantity and in particular the supplemented strains

Results continued…

nterestingly the other healthy species like ***L.gasseri*** and *L.jensenii* increased in robiotic group

s expected, ***G.vaginalis*** decreased in probiotic group compaerd to placebo grou

Iners remained to be in similar quantities in both the groups

Interpretations….

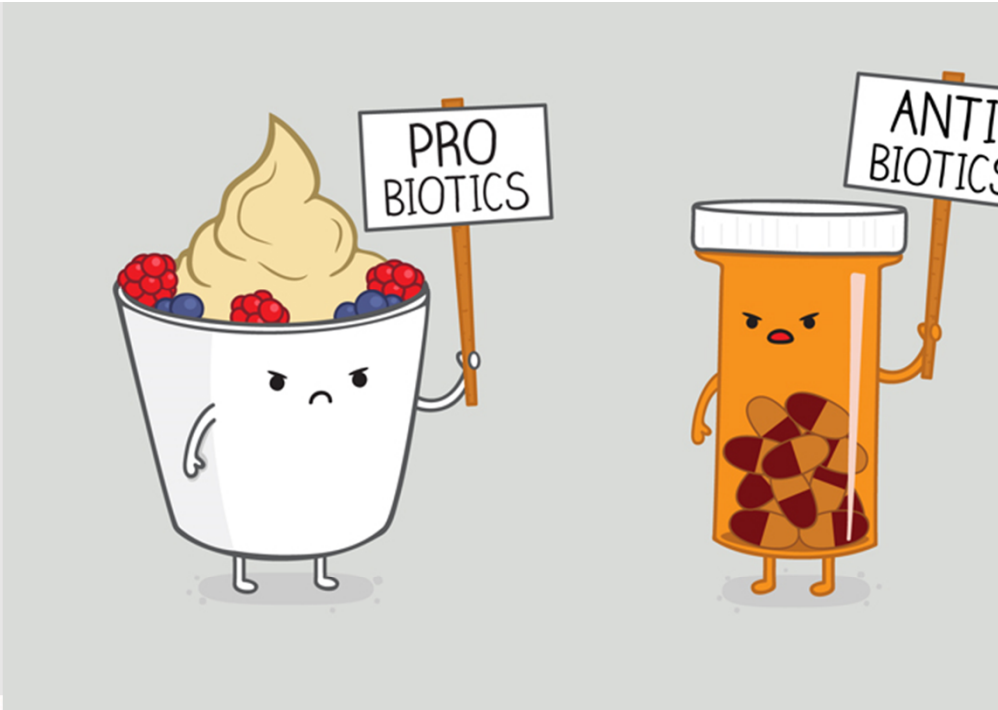
- The supplemented strains *L.reuteri* and *L.rhamnosus;* has colonized successfully and *L.reuteri* has shown specifically higher increase in prbiotic group demonstrating the benefit of supplementation

- L.gasseri and L.jensenii were always associated with normal vaginal flora and these two strains increased in the probiotic group suggesting that the probiotics may favorably affect the overall vaginal flora

- Therefore, it may be speculated that oral supplementation of probiotics in pregnant women can aid in restoring the normal flora of vagina and can reduce the relapse of BV infection

- The samples were also processed for Next Generation Sequencing and results awaited.

Thank You……


PRO
BIOTICS
ANTI
BIOTICS


ealthy
lactobacillus
yogurt
cultures
microflora
robiotics
tract
microorganisms
nutrients
kef
infection
dietary
health
absorb
live
living
active
intestinal
flora