The cervicovaginal microbiota, women's health and reproductive outcomes

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Short Narrative abstract

The human microbiome project has shown a remarkable diversity of microbial ecology within the human body. The vaginal microbiota is unique in that in many women it is most often dominated by *Lactobacillus* species. However, in some women it lacks *Lactobacillus* spp. and is comprised of a wide array of strict and facultative anaerobes, a state that broadly correlates with increased risk for infection, disease, and poor reproductive and obstetric outcomes. Interestingly, the level of protection against infection can also vary by species and strains of *Lactobacillus*, and some species although dominant are not always optimal. This factors into the risk of contracting sexually transmitted infections and possibly influences the occurrence of resultant adverse reproductive outcomes such as tubal factor infertility. The composition and function of the vaginal microbiota appear to play an important role in pregnancy and fertility

24 treatment outcomes and future research in this field will shed further translational mechanistic

25 understanding onto the interplay of the vaginal microbiota with women's health and

26 reproduction.

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29 Capsule of 30 words or less:

30 Insights into the composition and function of the vaginal microbiota will impact reproductive

31 health management.

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33 Running title: Vaginal microbiota and reproduction.

34 3-5 Keywords: sexually transmitted diseases, pelvic inflammatory disease, bacterial vaginosis,

35 *in vitro* fertilization, contraception.

36

Introduction

This review addresses recent advances into our understanding of the microbial ecosystem in the human vagina and its role on women's health and reproductive outcomes. We have summarised the most recent knowledge, in the context of prior understanding of how the vaginal microbiota is influenced by menstrual cycle and sex hormones, contraceptives and influences the risk of infections and disease, adverse pregnancy and fertility treatment outcomes.

Lactobacillus spp. often uniquely predominate the human vaginal microbiota

It is now well accepted that microbes present in or on the human body can impact immunity, nutrition, and physiology (1-3). The human vagina is unique in that, in healthy states, it is most often characterized by reduced bacterial diversity and the dominance of *Lactobacillus* spp. ($\sim 10^7$-10^9 per gram vaginal fluid in reproductive aged healthy women) compared to other microbiota (4). The presence of *Lactobacillus* spp., known to produce copious amount of lactic acid, is directly correlated with vaginal pH <4.5. Lactic acid driven acidity (low pH) has been strongly correlated with protection against cervico-vaginal infections, including HIV and other sexually transmitted infections (5-8).

Lactobacillus spp. dominated vaginal microbiota have been intrinsically linked to estrogen production and the accumulation of glycogen in the upper layers of the stratified vaginal epithelium (9, 10). Beyond lactic acid, *Lactobacillus* spp. beneficial properties are associated with the production of bacteriocins (antimicrobial compounds), adherence to the vaginal epithelia (competitive exclusion of other bacteria), and ability to competitively use available nutrients (11, 12). The physiology of the vaginal *stratum corneum* (*SC*), consisting of loosely

associated cells with glycogen stores, and innate defence mediators (13), is thought to contribute to this site being a niche for *Lactobacillus* spp.. However, the exact reason for *Lactobacillus* spp. dominance in the human vagina remains to be fully elucidated. Interestingly, other mammals do not harbor *Lactobacillus* spp. in their vaginal microbiota, and consequentially their vaginal pH is not acidic. However, while the composition of the vaginal microbiota is different, it is hypothesized that it could perform the same functions (14). Factors such as diet and unique environmental exposures have been proposed as potential reasons for these compositional differences (14).

The development of novel and high-throughput culture-independent methods to characterize the composition and structure of microbiota, supported by advances in next generation sequencing technologies and their reduced cost, have enabled a more in depth characterization of microbiota. In the vaginal microbiota (as discussed further in the next section) these advances have enabled the identification of strong correlations between different states of the vaginal microbiota and risk of infections (15). As a result, an improved understanding of the complexities of the microbial environment of the female reproductive tract is available. Approaches that do not rely on amplifying and sequencing specific taxonomically informative genes (i.e., 16S rRNA gene, *cpn60* (16)), such as metagenomics (17) (sequencing of all genes and genomes in a microbial community) or metatranscriptomics (18) (sequencing all gene transcripts expressed in a microbial community) are contributing to the functional characterization of the microbiota and its interaction with the human host.

Molecular, culture, and sequencing contributions to understanding the ecology of the human vagina

85   High-throughput 16S rRNA gene sequencing studies examining vaginal bacterial species

86   composition and abundance in reproductive-aged women have shown that there are at least

87   five major types of vaginal microbiota, termed community state types (CST) (19, 20). Four of

88   these CSTs are dominated by either *Lactobacillus crispatus* (CST I)*, L. gasseri* (CST II), *L.*

89   *iners* (CST III)*,* or *L. jensenii* (CST V)*.* Additionally, CST IV does not contain a significant

90   species or quantity of *Lactobacillus* but instead comprised of a polymicrobial mixture of

91   strict and facultative anaerobes including species of the genera *Gardnerella*, *Atopobium*,

92   *Mobiluncus*, *Prevotella* and other taxa in the order *Clostridiales* (19-21). Further examination

93   of CST IV has revealed distinct clusters within this polymicrobial community type, which

94   have since been denoted subgroups CST IV-A and CST IV-B (20). Subgroup IV-A can

95   contain moderate amounts of *Lactobacillus* spp. (typically *L. iners*) as well as strict anaerobes

96   including *Corynebacterium*, while conversely CST IV-B contains a higher proportion of

97   species associated with bacterial vaginosis (BV). The frequency of these CSTs has been

98   shown to differ in different ethnic backgrounds (19, 22), with CST I more common in

99   Caucasian women and CST IV more common (~40%) in African-American and Hispanic

100   women. The frequency of these CSTs differs not only by ethnicity but also by geographical

101   origins (22-24).

102   Daily (or frequent) fluctuations in the composition of the vaginal microbiota have been

103   documented by microscopy and cultivation studies (25-27). These findings were confirmed

104   and extended in longitudinal culture-independent analyses performed on vaginal swabs

105   collected twice weekly for 16 weeks (20, 28), or daily for 10 weeks (29) or 4 weeks (4). It was

106   observed that some vaginal microbial communities transitioned in and out of CST IV. The

107   amount of time spent in a particular CST could vary individually as some women experienced

108   consistent and stable CST longitudinal patterns, while others frequently transitioned between

109   CSTs, most frequently to CST IV (20, 29). In some cases, CST transitions were triggered by

110 menstruation or sexual behaviors, but in other cases they seem to be driven by uncharacterized

111 factors (20). In another longitudinal study, presence of *Gardnerella* was found to be predictive

112 of an impending CST change (30). Phase in the menstrual cycle greatly affects community

113 stability. During ovulation, when estradiol production peaks, stability is highest, while during

114 menstruation, *Lactobacillus* spp. tend to decrease in relative abundance (31), with the exception

115 of *L. iners* (20). In general, molecular and culture-based methods are somewhat in agreement

116 that menses significantly alters the composition of the vaginal microbiota (27, 32-34), but

117 change appears to depend on the initial CST present, as well as other factors (20) such as the

118 use of menstrual pads or tampons (20, 35). See Figure 1 that shows the interplay of microbiome

119 status throughout the menstrual cycle, (reproduced from (20)). These longitudinal studies

120 highlight the highly dynamic nature of vaginal microbial communities during the menstrual

121 cycle and emphasize the need to better understand the underlying biological factors modulating

122 fluctuations in composition and functions that affect host physiology. Bayesian network

123 analysis was used to further understanding of the complex interplay between behaviours in

124 menstrual hygiene and microbiota (36). The study highlighted that despite the relatively

125 reduced complexity of the vaginal microbiota, novel approaches integrating more elements of

126 the complex biological system will ultimately improve our understanding of the interactions

127 that drive the vaginal ecosystem and ultimately women's health.

128

129 **Impact of hormonal contraception on vaginal microbiota**

130 Because estrogen cycling appears to be linked to vaginal microbiota stability and to some

131 extend composition, several studies have evaluated the effect of contraception (oral, injected,

132 and implanted) methods on the composition of the microbiota. A large cohort study of 266

133 healthy women initiating contraception and aged 18-35 years in Harare, Zimbabwe, used

134   quantitative PCR (polymerase chain reaction) measurement of vaginal bacteria. No significant

135   impacts of most hormonal contraceptives were found on vaginal microbiota composition,

136   including on the abundance of *Lactobacillus* spp. Interestingly, copper intrauterine devices

137   were associated with a significant increase of BV-associated bacteria (assessed by species

138   specific quantitative PCR) over the 180-day study (p=0.005) (37). This finding contradicts a

139   study using Nugent and microscopic analysis of vaginal microbiota in Thai HIV-positive

140   women, which found no association with these BV microbial indicators and intrauterine

141   devices (IUDs) (38). In another study of 682 women using contraceptive measures in the

142   United States, combined oral contraceptives (progestin and estrogen) (COC) (39) users were

143   more likely to be colonized by *Lactobacillus* spp. and less likely to harbor BV-associated

144   bacteria than when using other forms of barrier (condoms) or hormonal contraceptives (depot

145   medroxyprogesterone acetate (DMPA), or the levonorgestrel-releasing intrauterine system

146   (LNG-IUS)) (adjusted Odds Ratio 1.94, 95% CI 1.25-3.02). A systematic review of HIV

147   acquisition studies that include microbiota and contraceptive usage in women identified that

148   there is some (limited evidence) that the combined oral contraceptive may pre-dispose to

149   candidiasis, which may in turn be a risk factor for HIV acquisition (40). Other studies have

150   reported the LNG-IUS can increase *Candida* colonization and temporally decrease

151   *Lactobacillus* dominance (41), enhance susceptibility to herpes simplex virus (HSV) infection

152   (42) or delay clearing *Chlamydia trachomatis* infection (43). Mitchell et al., in a small study

153   of 32 women have reported that after 12 months of use, DMPA was associated with a decreased

154   in vaginal *Lactobacillus* phenotyped by culture as producing H_2O_2, a surrogate for non-

155   *Lactobacillus iners* species (44). In that study, DMPA did not increase vaginal mucosal CCR5+

156   HIV target cells but did decrease CD3+ T lymphocytes. Borgdorff et al., found that

157   contraceptive use was not associated with vaginal microbiota composition, but they did find

158   sexual behaviour (inconsistent sexual partner OR 3.2 CI 1.0-9.9) and ethnicity correlate with a

159 polymicrobial BV-like microbiota when compared to a *Lactobacillus*-dominated microbiota

160 (22). Bassis et al., analysed the vaginal microbiota before, at 6 and 12 months following

161 insertion of copper (n=36) and progesterone (n=40) intrauterine devices, and found no

162 correlation with the device and microbiota changes over this relatively large time frame (45).

163 Interestingly, the literature is not always in agreement on the effect of contraception on the

164 composition of the vaginal microbiota or susceptibility to sexually transmitted diseases. A

165 major factor often not considered in several of these studies is ethnicity. Further studies are

166 needed to evaluate the effect of contraceptive methods on disease susceptibility and the

167 composition of the vaginal microbiota, while considering the previously reported association

168 between ethnicity and vaginal microbiota (19).

169

170

171 **Vaginal infections, disease and the microbiota**

172

173 We have chosen to include a section on how the vaginal microbiota interplays with infections

174 and disease, because these can result in infertility or adverse pregnancy outcomes and hence

175 are important in the context of reproduction. Using microscopic observation, the composition

176 of the vaginal microbiota has long been linked to disease risk, with the presence of

177 *Lactobacillus* spp. providing protection while a paucity in *Lactobacillus* spp. and the presence

178 of a diverse set of Gram-negative anaerobic species associated with increased risk to disease.

179 The latter is often defined as bacterial vaginosis, a conditions present in 29% women aged 14–

180 49 years in the general USA population, in over 50% of African-American women (46), and in

181 over 70% of women attending sexually transmitted infection clinics (47). High-throughput

182 molecular analyses afford a more in-depth and precise characterization of the vaginal

183   microbiota and insight into the role of specific species or clades in disease risk. In this section,

184   we address how these high-resolution analyses have advanced our understanding of disease

185   risks for BV, Pelvic Inflammatory Diseases (PID), and sexually transmitted infections (STIs).

186

187   **Bacterial vaginosis**

188   Diagnosis of BV in a clinical setting relies on the Amsel criteria (48) and in research settings

189   on the Nugent scoring system (49). Interestingly, despite the use of molecular analysis to define

190   BV states (50, 51), no one taxa has been confirmed as the etiological agent of the condition,

191   and BV remains ill-defined microbiologically as a polymicrobial state, basically characterized

192   by the lack of predominant *Lactobacillus* spp. That said, several bacteria, such as *Gardnerella*

193   (*G.*) *vaginalis*, have been shown to be associated with the condition in some studies but not

194   others (52). Early studies failed to reproduce the disease after direct vaginal inoculation of *G.*

195   *vaginalis* isolated from women with BV, while inoculation with whole vaginal secretions did

196   (53, 54), supporting that the condition is either polymicrobial or other factors contribute. It is

197   highly likely that the "pathogenic" potential of *G. vaginalis* might differ depending on the

198   specific strain of *G. vaginalis* colonizing and possibly the vaginal immune state or ethnicity

199   (55). Interestingly, it appears that *G. vaginalis* can be transferred sexually. A longitudinal study

200   of young women in Australia found that *Gardnerella* was more likely to be found in those

201   having penile sex (OR 11.82 95% CI:1.87-74.82; p= 0.009) (24). *Gardnerella* was also found

202   in approximately a third of girls (aged 10-12) in a pre-menarche vaginal microbiota study,

203   indicating that this organism is not only acquired/facilitated by sexual activity, potentially

204   could be transfer at birth from mother to daughter (56). Yet, *G. vaginalis* colonisation increased

205   after sexual activity in a cohort of young women who were monitored pre- and post-sexual

206   debut, (p=0.02) indicating sexual activity is a factor in the transmission of this bacteria (57). In

207   support of the polymicrobial nature of BV, in a longitudinal study of women who have sex

with women, BV incidence was associated with sexual behaviours, and most strongly

correlated with a new sexual partner with BV-associated symptoms (adjusted hazard ratio 2.8

CI;1.30-4.82) (58). Furthermore, a large cohort study of 1,093 women in general practice care

in Australia found that either a recent new female sexual partner or multiple male partners were

significantly associated with prevalent or incident BV cases (59). Interestingly, in this study

estrogen contraceptives were protective (AOR 0.6 CI 0.4-0.9) (59). A similar study in the USA

found that new sexual partners and oral vulvovaginal sex were both significant risk factors for

BV, while a *L. crispatus*-dominated (CST I) microbiota was protective (HR 0.18, CI:0.08-0.4)

(60). The lack of a clear definition for BV makes studying its etiology challenging. One study

attempted to improve the definition of BV using molecular methods, combining bacterial

composition (16S rRNA gene amplicon sequencing), eukaryotic composition (ITS

sequencing), and *Trichomonas* characterization (sequencing of the *tvk* loci) (61), but it failed

because of limitations associated with the study such as the lack of speciation of *Lactobacillus*

spp., highlighting the need for further development. An improved definition of BV would have

major implications in women's clinical management and women's health as a whole.

While new antibiotics are being developed or tested to treat BV (62-64), leveraging the vaginal

microbiota for the development of live biotherapeutic formulations to modulate the microbiota

is also considered (65) to restore a *Lactobacillus*-dominated protective vaginal microbiota. As

drug-based treatment failure can be high, with antibiotic resistance appearing (66), and

recurrence very common (67, 68), alternative approaches are needed. Vaginally delivered live

biotherapeutics are safe and can be used in combination therapy after antibiotic treatment (69-

71), however success has been limited, certainly because formulations do not take into account

the ecology of the vaginal microbiota and often rely on one strain of *Lactobacillus*, mostly *L.

crispatus* or non-vaginal *Lactobacillus* strains (70-73). Nonetheless, further work is needed to

develop and optimize an efficacious formulation.

233   The majority of trials in this space have been in the context of BV. An analysis of several trials

234   of probiotics, orally administered with presumed rectal transfer, or vaginally distributed

235   supported that greater than 10^8 cfu of leading probiotic strains for more than 2 months helped

236   some participants resolve BV (74). However, a trial comparing metronidazole treatment with

237   combined metronidazole and a vaginal probiotic of *Lactobacillus acidophilus* with estrogen

238   did not find a significant impact on BV recurrence (69). One interesting approach used an *ex*

239   *vivo* model to provide further evidence of *Lactobacillus* defence against HIV (75), further

240   supporting the potential for developments in this field to live biotherapeutics.

241   Alternative strategies, such as metabolite or receptor competitive molecules, along with

242   precision medicine approaches are likely to emerge. One example that has been proposed for

243   family members with a genotypically driven dectin-1 deficiency linked to recurrent vulvo-

244   vaginal candidiasis was supplementation with dectin-1 (76, 77). Also, antimicrobial proteins

245   and peptides that are mimics of those already produced as innate defence and used as vaginal

246   supplements are possible future therapeutic option (reviewed (76)).

247

248   **Pelvic Inflammatory Disease**

249   The composition of the vaginal microbiota appears to play a role in the development of another

250   important disease, pelvic inflammatory disease (PID). PID is associated with inflammation in

251   the upper reproductive tract in women, characterized by sudden onset of pain along with

252   cervical, adnexal, or uterine tenderness. Risk factors for PID include those that also affect the

253   composition of the vaginal microbiota, such as history of multiple sexual partners, or early age

254   of commencement of sexual activity (78). Microbial risk factors for PID include sexually

255   transmitted infections and bacterial vaginosis (79, 80). In addition to the endometrial presence

256   of sexually transmitted pathogens such as *C. trachomatis*, *Mycoplasma genitalium* and

257   *Neisseria gonorrhoeae*, a polymerase chain reaction (PCR) testing for BV-associated bacteria

258   in endometrial samples identified bacteria such as *Sneathia sanguinegens*, *Sneathia amnionii*,

259   *Atopobium vaginae* and BV-associated bacteria 1 (BVAB1) in women with PID (81). It is

260   common to fail to identify known "pathogens" in women with PID, although frequently many

261   other organisms are detected in the upper reproductive tract (reviewed (82)). Hence, it is likely

262   that a *Lactobacillus*-dominated vaginal microbiota could be protective for PID. However, as

263   yet there are no reports of extensive molecular analyses of the vaginal microbiota in the context

264   of PID and the question remains open.

265

266   **Sexually transmitted infections**

267   Risk for contraction of sexually transmitted pathogens has been associated with the

268   composition of the vaginal microbiota. The most insights have come from studies into

269   incidence and prevalence of *C. trachomatis*. *Chlamydia* is one of the most common sexually

270   transmitted bacterial pathogens and has been found in three independent studies to be more

271   likely detected in association with *L. iners* (CST III) (e. g. OR of 2.6 - 4.4) and/or CST IV (OR

272   4.2) (83-85). This may relate to availability of metabolites produced by these types of

273   microbiota that benefit the pathogen, as found in one study (86). On the other hand, the vaginal

274   microbiota, in particular *L. crispatus* (CST I), may have specific anti-chlamydial, anti-

275   gonococcal, and immune enhancing properties, as evidenced *in vitro* and on a porcine epithelial

276   model (87-89). *N. gonorrhoeae* infections are less common (compared to *Chlamydia*) in

277   women. Whilst there is little information on the composition of the vaginal microbiota in the

278   context of gonococcal infections, it has been shown *in vitro* that *Lactobacillus* spp.(especially

279   *L. gasseri* (CST II), and *L. jensenii* (CST V)) can directly compete with *N. gonorrhoeae* for

280   epithelial binding (90, 91). A large nested case-control study in African women identified that

281   there are several taxa within the vaginal microbiota that are associated with increased risk of

282   HIV acquisition (92). Bacterial vaginosis, STIs such as *Chlamydia* and Herpes, and vaginal

283 washing have also been associated with increased risk of transmission and/or acquisition of

284 HIV (93, 94). Altogether, these data support mechanisms such as competition, low pH, specific

285 anti-bacterial molecules (bacteriocin) (95, 96), through which the vaginal microbiota is a major

286 driver of protection to infectious agents.

287

288 **Pregnancy outcomes and the cervicovaginal microbiota**

289 In pregnancy the lack of menses and the increase in circulating estrogen are associated with a

290 microbiota characterized by an increased dominance of *Lactobacillus* spp. as gestation

291 progresses (97). This is a feature of the vaginal microbiota in pregnancy that has been

292 established by several studies of varied power and sampling intensities, both in the US and in

293 Europe (97-101). Interestingly, this inherent stability of the microbiota in pregnancy was also

294 true at other body sites (97). Post-partum, and up to a year after delivery, the vaginal microbiota

295 was characterized by a paucity of *Lactobacillus* spp. (CST IV), even in pregnancies where

296 *Lactobacillus* spp. were dominant during gestation (97, 98). While these finding support the

297 hypothesis that adverse postpartum outcomes such as endometritis and sepsis might be

298 mediated by vaginal microbes, its biological and reproductive implications remain unknown.

299 Overall summary of this data is presented in Figure 2, showing that generally *Lactobacillus*

300 spp. abundance increases, and community diversity decreases during pregnancy, with a shift

301 post-partum to high diversity.

302 Several studies have documented the composition of the vaginal microbiota associated with

303 adverse pregnancy outcomes such as pre-term birth (97, 102-110). While certain studies

304 found association with a few bacteria (mostly anaerobes), others did not, and no consistent

305 signature has been identified. Deciphering cause from effect remains a challenge in these

306 studies and it is likely that most suffered from a low number and poor phenotype (not all

307 preterm births were spontaneous) of preterm birth cases.

308 Preterm premature rupture of membranes (PPROM) is one adverse pregnancy outcomes that

309 have strongly correlated with the cervicovaginal microbiome in distinct studies. In one study,

310 and consistent with several others, women who experienced PPROM were less likely to have

311 *Lactobacillus*, or lower abundance of *Lactobacillus* in the vaginal microbiome composition

312 and high diversity of the microbiota (108). Furthermore, the presence of *Mollicutes* such as

313 *Mycoplasma* or *Ureaplasma* (although these are also common in healthy vaginal

314 microbiomes) have been found to be more frequently present in the vaginal microbiome of

315 women who experienced PPROM (108). Early and late miscarriages have long been

316 associated with BV or the presence of specific flora in the vagina (111-114).

317 Chorioamnionitis or intra-amniotic infections have also been associated with the vaginal

318 microbial community state, including a recent history of BV (115-117). Further research is

319 desperately needed and would involve well-powered prospective study designs, include

320 ethnically diverse populations, and aim at identifying predictive signatures in the cervico-

321 vaginal microbiota or its products, thereby ultimately providing novel strategies to restore a

322 protective vaginal microbiota.

323

324 **The cervico-vaginal microbiota in infertility and fertility treatment**

325 The composition of the vaginal microbiota is thought to influence fertility and outcomes of

326 fertility treatment. Most published studies were performed in the context of *in vitro* fertilization

327 procedures, which could be well-controlled. In these studies, BV-associated bacteria in the

328 vagina have been shown to be associated with a reduced pregnancy rate (118-120). The study

329 by Haahr and colleagues focused on using Nugent score and PCR detection of BV-associated

330 bacteria in the vagina and comparing to IVF success in 130 women. They found that Nugent

331 and PCR correlated highly, and that women with PCR detected BV-associated bacteria were

332 significantly less likely to obtain a clinical pregnancy (9%) compared to the overall rate of 35%

333 (p=0.004) (118). Interestingly, women undergoing IVF with tubal factor infertility (a pathology

334 associated with infections) were found to be more likely to have a vaginal microbiota consistent

335 with BV by analysis of smears (120), supporting a connection between these etiologies.

336 Although the authors acknowledge the lack of cause and effect in an infertility context, this

337 finding supports that precision medicine approaches around fertility treatment and the vaginal

338 microbiota could well inform practise in the fertility clinic. Further to this finding, using next

339 generation sequencing of the vaginal microbiota, women with idiopathic infertility were found

340 to have a microbiota profile consistent with BV compared to healthy women (121). Another

341 study identified trends for distinct microbiota in women with a history of infertility compared

342 to women with a history of fertility, albeit a retrospective study on a small sample size (122).

343 A study of the composition of the vaginal microbiota on the day of embryo transfer in women

344 undergoing IVF found that a lower vaginal microbiota diversity index correlated with a

345 resultant live birth (123). However, this study did not profile the taxa present but rather

346 compared the diversity index, providing a relatively limited insight into the role of the vaginal

347 microbiota in reproductive outcomes. It is likely that analysis of the upper reproductive tract

348 microbiota will shed further insights into fertility and fertility treatment outcomes, and this

349 topic is reviewed in this issue (119, 120, 124-126). However, additional studies are needed to

350 better understand fertility outside the controlled context of IVF procedures. Studies involving

351 time to pregnancy and detailed monitoring of the composition and function of the vaginal

352 microbiota would be extremely informative and would provide the evidence necessary to

353 develop ways to improve natural conception outcomes.

354

Future perspectives

The vaginal microbiota critically interplays with women's health and reproduction. We have summarised the factors reviewed here which are thought to drive or be associated with vaginal microbiota dysbiosis or eubiosis in Figure 3. It is becoming critical to further our understanding of the cervicovaginal microbiota from a mechanistic and functional aspect, so that causal relationships can be established between the microbiome and adverse outcomes. These mechanistic understandings could be leveraged to develop improved protective and curative strategies, or to optimize the vaginal microbiome using rationally designed live biotherapeutic products or metabolites. To achieve these goals, improved study designs and sampling strategies are needed that would maximize power and frequency of sampling in prospective study design. The applications of advanced and high-resolution approaches such as metagenomics, metatranscriptomics, metabolomics, and/or proteomics, detailed immunological characterizations in combination with novel systems biology, modelling and statistical approaches will be critical to advancing the field and improving women's reproductive health.

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Figure Legends

Figure 1. Vaginal microbiota stability and sex hormone levels during the menstrual cycle.

Reproduced from 'Temporal Dynamics of the Human Vaginal Microbiota, Gajer P, Brotman

RM, Bia G, Sakamoto J, Schutte UME, Zhong X, Koenig SSK, Fu L, Ma Z, Zhou X, Abdo Z,

Forney LJ, Ravel J, Sci Transl Med, 2012, 4(132): 132ra52' (20). Reprinted with permission

from AAAS. The highest stability correlates with high estrogen or progesterone levels, but can

be affected by the community state type of the vaginal microbiota, behaviours, and other host

factors (20).

Figure 2. The vaginal microbiota decreases in diversity during pregnancy, often with an

increased relative abundance in *Lactobacillus* spp.. In the post-partum phase an immediate

increase in vaginal microbiome diversity and decrease in *Lactobacillus* spp. has been observed.

747 **Figure 3. Factors driving or associated with dysbiosis or eubiosis of the vaginal**

748 **microbiota in reproductive age women.**

749