The *Lactobacillus* flora in vagina and rectum of fertile and postmenopausal healthy Swedish women

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Abstract

**Background:** *Lactobacillus* species are the most often found inhabitants of vaginal ecosystem of fertile women. In postmenopausal women with low oestrogen levels, *Lactobacillus* flora is diminishing or absent. However, no studies have been performed to investigate the correlation between oestrogen levels and the lactobacilli in the gut. The aim of the present study was to investigate the relation in healthy women between vaginal and rectal microbial flora as well as possible variations with hormone levels.

**Methods:** Vaginal and rectal smears were taken from 20 healthy fertile women, average 40 years (range 28-49 years), in two different phases of the menstrual cycle, and from 20 postmenopausal women, average 60 years (range 52-85 years). Serum sex hormone levels were analyzed. Bacteria from the smears isolated on Rogosa Agar were grouped by Randomly Amplified Polymorphic DNA and identified by multiplex PCR and partial 16S rRNA gene sequencing.

**Results:** *Lactobacillus crispatus* was more often found in the vaginal flora of fertile women than in that of postmenopausal (p = 0.036). Fifteen of 20 fertile women had lactobacilli in their rectal smears compared to 10 postmenopausal women (p = 0.071). There was no correlation between the number of bacteria in vagina and rectum, or between the number of bacteria and hormonal levels. Neither could any association between the presence of rectal lactobacilli and hormonal levels be found.

**Conclusion:** *Lactobacillus crispatus* was more prevalent in the vaginal flora of fertile women, whereas the *Lactobacillus* flora of rectum did not correlate to the vaginal flora nor to hormonal levels.

Background

Lactobacilli are non-spore-forming, gram-positive rods that form an important part of the normal human bacterial flora commonly found in the mucosa of the mouth [1-3], gastrointestinal (GI) tract [1-4] and female genitourinary tract [1,2,5,6]. They are considered protective organisms required to maintain health by producing lactic acid and other metabolites inhibiting growth of pathogenic organisms [7].

The relationship between vaginal microbial flora, menstruation and levels of oestrogen is complex [8]. The most drastic changes in the vaginal flora occur at the onset of puberty, and are related to an increase of glycogen amount. The elevated glycogen level stimulates the growth of lactic acid-producing bacteria, especially *Lactobacillus* and *Streptococcus* [9]. Those conditions are usually maintained during the fertile years in a healthy vagina and start to change during the menopause. The oestrogen level in fertile women is believed to change during menstrual cycle, and the recovery of the *Lactobacillus* varies slightly [8]. The oestrogen level seems to be a determining factor for colonization of lactobacilli although there is still not any convincing data [10].

Postmenopausal women, who suffer from depletion in vaginal lactobacilli, are sometimes colonized by adverse microbial flora that may cause urinary tract infection

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[11] and bacterial vaginosis [12]. Nothing is known about the changes in rectal microbial flora in relation to hormonal changes, but some GI diseases tend to have their first onset during the years of the menopause [13,14]. Therefore, our hypothesis was that changes in rectal microbial flora may be an important etiological factor in these entities.

The aim of the present study was to investigate the relationship in healthy women between vaginal and rectal Lactobacillus flora, as well as possible variations with sex hormone levels with special references to changes in menstrual phases as well as in the menopause.

Methods
The study was approved by the Ethics Committee at Lund University. The women gave written informed consent before entering the study.

Study Design
Twenty healthy fertile women (28-49 years) average 40 years, in two different phases of the menstrual cycle (day 7 and day 21), and 20 healthy postmenopausal women (52-85 years) average 60 years, took part in the study. A basic clinical examination including routine blood samples was performed in the women to ensure a healthy status. Furthermore, a gynecological examination was carried out, including a PAP-smear. The bacterial flora in vagina was normal, excluding bacterial vaginosis or any other infection. All answered a written questionnaire regarding the intake of products containing lactobacilli. The fertile women were asked not to alter their use of hygienic products between the two occasions. All women were asked to report present or single use of drugs for example proton pump inhibitors (PPI), non-steroidal anti inflammatory drugs (NSAID) and antibiotics. The use of any hormonal contraceptive methods or oestrogen hormone replacement therapy was not allowed. Blood samples were collected, centrifuged and serum were stored at -20°C until analyze. Smears from vagina and from rectum were collected with a cotton-tipped swab that were placed in transport medium, on ice and, without delay, transported for cultivation of lactobacilli.

Cultivation and identification of lactobacilli
Vaginal and rectal smears were treated in an ultrasonic bath for two minutes and diluted before plating on Rogosa agar plates and incubated anaerobically at 37°C for 72 hours. Two to three colonies were randomly picked and characterized by Randomly Amplified Polymorphic DNA (RAPD) as described by Quednau et al. [15], and those having the same RAPD pattern within the same sample were regarded as belonging to the same species. Species identification was performed by multiplex PCR as described by Song et al. [16], slightly modified by Vasquez et al. [17], or if not applicable by partial 16S rDNA sequencing. The multiplex-PCR identified isolates of the following species: L. crispatus, L. acidophilus, L. gasseri, L. jensenii, L. reuteri, L. delbrueckii, L. plantarum, L. salivarius and L. paracasei. Isolates (altogether 13) of L. vaginalis, L. cohnii and L. ruminis were identified by sequencing of approximately 800 bp long fragments of the 16S rRNA gene.

Sex hormone analyses
Serum oestradiol, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were analyzed for the fertile women at day 7 and 21 and for the postmenopausal women at the Department of Clinical Chemistry, Skåne University Hospital, Malmö. Oestradiol and progesterone were analyzed by a one-step competitive immunoassay with alkalic phosphatase (ALP), enzyme marking and magnetic separation. FSH and LH were analyzed by a two-step immunometric assay with ALP, enzyme marking and magnetic separation.

Statistical analyses
Fisher’s exact test was used to compare fertile women between day 7 and 21, and fertile and postmenopausal women, in the presence of specific lactobacilli in vagina and rectum. Spearman’s test was used to examine correlations between the number of bacteria in vagina and rectum, and between the number of bacteria and hormonal levels. P < 0.05 was considered statistical significance.

Results
Vaginal smears
The colony forming units (CFU) bacteria per gram vaginal smear varied in fertile women from 8.3 × 10^2-1.8 × 10^9 at day 7, and from 4.0 × 10^2-4.0 × 10^7 at day 21 (Table 1 and 2). In the postmenopausal women it varied from 1.7 × 10^3-3.0 × 10^6 (Table 3).

Lactobacilli were found and isolated from 11 out of 20 fertile women as well as from 11 out of 20 menopausal women (Table 4 and 5). Altogether 39 isolates were further characterized. The most commonly identified species of the fertile women was L. crispatus (7 women), followed by L. acidophilus (2 women), L.gasseri (2 women), L. jensenii (2 women) and L. vaginalis (2 women) (Figure 1 and 2). Menopausal women were most often colonized by strains of L. gasseri (5 women) and L. crispatus (2 women) (Figure 3). Lactobacillus crispatus was significantly more often found in the vaginal flora of the fertile women than in that of postmenopausal women (p = 0.036).

Rectal smears
The CFU bacteria per gram rectal smear of the fertile group varied between 1.8 × 10^2-1.9 × 10^7 at day 7, and
### Table 1 Serum hormone levels and bacterial smear numbers in fertile women at day 7 of the menstrual cycle

| Subject No. | Oestradiol (E2) (pmol/L) | Progesterone (nmol/L) | FSH (IU/L) | LH (IU/L) | Vaginal smear (CFU/g) | Rectal smear (CFU/g) |
|-------------|-------------------------|-----------------------|------------|-----------|-----------------------|---------------------|
| 1           | 689                     | 3                     | 4.6        | 3.9       | –                     | 180                 |
| 2           | 737                     | < 3                   | 7.7        | 4.5       | 1600000               | 840000              |
| 3           | 653                     | 5                     | 18.2       | 24.7      | 980000                | 950000              |
| 4           | 138                     | < 3                   | 9.5        | 5.6       | –                     | 320000              |
| 5           | 310                     | 3                     | 8.5        | 4.4       | 18000000              | 230000              |
| 6           | 238                     | < 3                   | 7.2        | 3.5       | –                     | 850000              |
| 7           | 1053                    | 3                     | 6.7        | 4.3       | 42000000              | 350000              |
| 8           | 240                     | 3                     | 6.2        | 3.5       | –                     | 280000              |
| 9           | 166                     | < 3                   | 8.9        | 6.5       | 30000000              | 190000              |
| 10          | 282                     | < 3                   | 9.2        | 2.8       | 57000000              | 380000              |
| 11          | 520                     | < 3                   | 7.1        | 5.7       | 72000000              | 280000              |
| 12          | –                       |                       |            |           |                       | 130000              |
| 13          | 1111                    | 3                     | 5.7        | 8.5       | 36000000              | 16000000            |
| 14          | < 125                    | 3                     | 5.12       | 12.9      | 42000000              | 12000000            |
| 15          | 253                     | 3                     | 5.7        | 5.8       | –                     | –                   |
| 16          | 929                     | 4                     | 4.8        | 5.5       | 15000000              | 4300                |
| 17          | 598                     | < 3                   | 4.7        | 8.4       | 83000000              | 18000000            |
| 18          | 328                     | 6                     | 7.8        | 7.1       | –                     | –                   |
| 19          | 150                     | 3                     | 8.9        | 9.5       | –                     | 19000000            |
| 20          | 189                     | 3                     | 52.7       | 19.8      | –                     | –                   |

*= not detectable. One woman did not complete the hormone analyses. CFU = colony forming units, IU = international units, FSH = follicle-stimulating hormone, LH = luteinizing hormone.

### Table 2 Serum hormone levels and bacterial smear numbers in fertile women at day 21 of the menstrual cycle

| Subject No. | Oestradiol (E2) (pmol/L) | Progesterone (nmol/L) | FSH (IU/L) | LH (IU/L) | Vaginal smear (CFU/g) | Rectal smear (CFU/g) |
|-------------|-------------------------|-----------------------|------------|-----------|-----------------------|---------------------|
| 1           | 318                     | 52                    | 5.9        | 2.2       | –                     | 30000000            |
| 2           | 656                     | 46                    | 4.8        | 5.8       | 25000000              | 790000              |
| 3           | 272                     | 12                    | 2.9        | 1.4       | 830000               | 90000               |
| 4           | 248                     | 35                    | 4.9        | 4.6       | –                     | 40000000            |
| 5           | 499                     | 52                    | 2.9        | 2.2       | 26000000              | 18000000            |
| 6           | 253                     | 18                    | 3.1        | < 1.0     | 400                   | 31000               |
| 7           | 374                     | 3                     | 8.0        | 2.8       | 40000000              | 1200000             |
| 8           | 274                     | 14                    | 2.6        | 2.4       | –                     | 4800000             |
| 9           | 158                     | < 3                   | 11.9       | 20.2      | 22000000              | 6200000             |
| 10          | 389                     | 21                    | 3.1        | 1.4       | 87000000              | 3500000             |
| 11          | 635                     | 31                    | 3.8        | 4.9       | 30000000              | 55000               |
| 12          | –                       |                       |            |           |                       | –                   |
| 13          | 221                     | 18                    | 8.3        | 3.1       | 550000               | 18000               |
| 14          | 705                     | 8                     | 25.2       | 19.8      | 7100000              | 17000000            |
| 15          | 326                     | 35                    | 6.0        | 7.2       | 170000               | 5100000             |
| 16          | 4.1                     | 3.1                   | 150000     | 2200      | –                     | –                   |
| 17          | 164                     | 15                    | 4.8        | 2.5       | 1200                  | 3800000             |
| 18          | 607                     | 48                    | 5.0        | 8.4       | –                     | 210000              |
| 19          | 568                     | 70                    | 3.1        | 3.0       | –                     | –                   |
| 20          | 1248                    | 5                     | 2.9        | 3.5       | –                     | 31000               |

*= not detectable. One woman did not complete the hormone analyses and one women only completed FSH and LH analyses. CFU = colony forming units, IU = international units, FSH = follicle-stimulating hormone, LH = luteinizing hormone.
between $2.2 \times 10^3 - 4.8 \times 10^6$ at day 21 (Table 1 and 2). In postmenopausal women, the CFU/g varied between $1.0 \times 10^3 - 4.9 \times 10^6$ (Table 3).

Lactobacilli were found and isolated from 15 out of 20 fertile women as compared to 10 out of 20 menopausal women ($p = 0.071$) (Table 4 and 5). Altogether 67 isolates were further characterized. The most often occurring Lactobacillus species in the rectal smear of fertile women was L. plantarum (8 women), followed by L. vaginalis (5 women), L. crispatus (3 women), L. delbrueckii (2 women) and L. salivarius (2 women) (Figure 1 and 2). Also in postmenopausal women, the most commonly found lactobacilli was the species L. plantarum (4 women), followed by L. gasseri (2 women) and L. ruminis (2 women) (Figure 3).

**Correlation between vaginal and rectal smears**

Eight women had the same Lactobacillus species in their vaginal and rectal smears, six in the fertile group and two in the menopausal group (Table 4 and 5). This difference was not statistically significant. The number of bacteria in vaginal and rectal smears within a woman was not correlated (data not shown).

Seven fertile women were in the vagina colonized by the same Lactobacillus species on day 7 and 21 of the menstrual cycle. Three of those were also colonized by that species in the rectum. The remaining were, both in vagina and rectum, colonized by different species during those days (Table 4).

In two menopausal women, the same species of lactobacilli (L. gasseri and L. ruminis) was found to dominate in both vaginal and rectal smears (Table 5).

There was no statistically significant fluctuation of the number of bacteria in the rectal flora between day 7 and day 21 of the menstrual cycle, nor between fertile and postmenopausal women (data not shown).

**Sex hormone levels in serum**

Oestriol levels were lower than 125 pmol/L for most of the postmenopausal women (Table 3). In the fertile women it varied from < 125-1111 pmol/L at day 7, and from 158-1248 pmol/L at day 21 (Table 1 and 2). For the fertile women at day 7 and for all the postmenopausal women, progesterone levels were equal or lower than 6 nmol/L, and for the fertile women at day 21 the levels varied from < 3-70 nmol/L. FSH levels were 4.6-52.7 IU/L in the fertile women at day 7, and 2.6-25.2 IU/L at day 21. FSH was much higher in the postmenopausal women, 33.7-165.0 IU/L. The same was seen for LH which had lower levels in the fertile women, 2.8-24.7 IU/L at day 7 and < 1.0-20.2 IU/L at day 21, than the postmenopausal women, 15.7-

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**Table 3 Serum hormone levels and bacterial smear numbers in postmenopausal women**

| Subject No. | Oestradiol (E2) (pmol/L) | Progesterone (nmol/L) | FSH (IU/L) | LH (IU/L) | Vaginal smear (CFU/g) | Rectal smear (CFU/g) |
|-------------|-------------------------|----------------------|-----------|-----------|----------------------|--------------------|
| 21          | > 125                   | > 3                  | 66.3      | 36.5      | –                    | 1100000            |
| 22          | 126                     | > 3                  | 33.7      | 16.3      | 170                  | 5500               |
| 23          | > 125                   | > 3                  | 65.4      | 24.6      | 10000000             | 3100000            |
| 24          | 133                     | > 3                  | 84.2      | 31.4      | –                    | 300000             |
| 25          | 193                     | > 3                  | 58.6      | 21.0      | –                    | 3800000            |
| 26          | > 125                   | > 3                  | 58.8      | 26.7      | 360000               | 1400000            |
| 27          | > 125                   | > 3                  | 73.5      | 28.9      | 13000               | 5900000            |
| 28          | > 125                   | > 3                  | 145.0     | 50.9      | –                    | 1700000            |
| 29          | > 125                   | > 3                  | 80.9      | 16.2      | 8300000              | 470000             |
| 30          | > 125                   | > 3                  | 48.7      | 15.7      | 180000               | 4900000            |
| 31          | > 125                   | > 3                  | 65.1      | 33.4      | –                    | 10000              |
| 32          | –                       | > 3                  | 50.8      | 37.8      | 8900000              | –                  |
| 33          | > 125                   | > 3                  | 89.6      | 39.9      | 510000               | 500000             |
| 34          | > 125                   | 3                    | 126.0     | 53.7      | 30000000             | 9200000            |
| 35          | > 125                   | 3                    | 88.1      | 43.8      | 22000               | 2100000            |
| 36          | > 125                   | > 3                  | 53.3      | 19.0      | –                    | 910000             |
| 37          | > 125                   | > 3                  | 89.7      | 23.1      | –                    | 550000             |
| 38          | > 125                   | > 3                  | 110.0     | 38.2      | 1900000              | 310000             |
| 39          | > 125                   | > 3                  | 165.0     | 53.8      | –                    | 2000000            |
| 40          | > 125                   | > 3                  | 90.9      | 22.3      | –                    | –                  |

= not detectable. One woman did not complete the hormone analyses. CFU = colony forming units, IU = international units, FSH = follicle-stimulating hormone, LH = luteinizing hormone.
53.8 IU/L (Table 1, 2 and 3). These values were within the normal ranges.

Correlations between Lactobacillus and sex hormone levels

Lactobacillus crispatus was present in the vaginal ecosystem in fertile women with high (1053 pmol/L) and medium (310-737 pmol/L) oestradiol levels. This reached statistical significance compared to postmenopausal women (p = 0.036). However, one woman with the lowest oestradiol level (< 125 pmol/L) was colonized by this species as well. Lactobacillus jensenii, L.

reuteri and L. vaginalis were present in the vaginal ecosystems in women with high and medium oestradiol values. There was no correlation between the number of vaginal bacteria and hormone levels. No associations were found between rectal microbial flora and sex hormone levels (data not shown).

Discussion

The purpose of this pilot study was to investigate the relationship between vaginal and rectal Lactobacillus flora in healthy women, and possible variations with sex hormonal levels. Several studies have shown that the vaginal microbial flora varies in relationship to hormonal levels. However, no studies have been performed to investigate the correlation between hormonal levels and lactobacilli in the gut. Therefore, we analyzed vaginal and rectal smears from fertile and postmenopausal women as well as serum levels of oestradiol, progesterone, FSH and LH. In the fertile women, smears were collected both in day 7 and day 21 of the menstrual cycle.

The main finding in our present study was that L. crispatus was more often found in the vaginal flora of fertile women than in postmenopausal women [5,17,18].

No relation was found between numbers or occurrence of species of lactobacilli found in the vagina and rectum in a specific woman. However, this was a small study. In a recent large study of 531 fertile women in the age of 14-35 years, 43% of those having L. crispatus in vagina also had this species in rectum [19]. These authors found L.
crispatus, together with L. jensenii, to be the most common species in vagina and in rectum. Interestingly, L. vaginalis and L. plantarum that was found as dominating lactobacilli from rectal smears in our study was not at all identified as part of rectal flora in the study of Antonio et al. [19]. However, the age of our study group was considerably higher (28-49 years) and the methodology of identification of lactobacilli was different.

Lactobacillus vaginalis was first described by Embley et al. 1989 [20]. It was isolated from the vagina of patients suffering from trichomioniasis. Lactobacillus vaginalis is a hydrogen peroxide (H$_2$O$_2$)-producing lactobacilli, and is together with L. crispatus, L. jensenii and L. gasseri the most commonly recovered species of H$_2$O$_2$-producing lactobacilli in the vagina [5,21-23]. There are so far not many studies of L. vaginalis, but it has been shown that the absence of H$_2$O$_2$-producing lactobacilli in the vagina is associated with an increase of bacterial vaginosis [24,25]. In a previous study, vaginal and rectal colonization by H$_2$O$_2$-producing lactobacilli was associated with a lower prevalence of bacterial vaginosis, compared with vaginal colonization alone. This may suggest that rectal colonization by H$_2$O$_2$-producing lactobacilli may contribute to maintain the vaginal ecosystem [19]. In the present study no women with bacterial vaginosis were included, why comparisons according to presence or absence of different lactobacilli in rectum in this entity could not be performed.

![Figure 1 Presence of Lactobacillus species in fertile women at day 7 in the vagina and rectum](image1)

![Figure 2 Presence of Lactobacillus species in fertile women at day 21 in the vagina and rectum](image2)
Menopausal and postmenopausal women are at risk for autoimmune as well as metabolic and cardiovascular diseases [26-28]. Different hormonal, immunological and vascular changes might contribute to the increased risk for GI dysfunction in ageing women [13,14]. Our hypothesis that changes in rectal microbial flora may be an important etiological factor in these entities could not be confirmed in the present study.

One of the limitations of our study is the low number of examined women. However, this study was performed as a pilot study, aimed to generate a larger study if any positive results were found. Furthermore, established bacterial growth was found in only half of the smears. The explanation to this may partly be that we did not search for L. iners, which differs from other Lactobacillus species due to its peculiar culture requirements [29]. It has been shown that L. iners is part of the normal vaginal microbial flora [17], but further studies show that L. iners is a dominant part of the vaginal microbial flora when it is in a transitional stage between abnormal and normal due to treatment, physiological changes or oestrogen levels [30-32]. The fact that the smears were taken from the distal colon should not influence our results as there is little regional difference in human colonic microbial flora [33].

**Conclusions**

In this pilot study, we did not find any correlation between the overall levels of Lactobacillus species in vagina and rectum, and variations in sex hormone levels. However, L. crispatus was more often found in the vaginal flora of fertile women than in that of postmenopausal women.
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