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Changes in faecal bacteria and metabolic parametersin foals during the first six weeks of life

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Abstract

Many foals develop diarrhoea within the first two weeks of life which has been suggested to coincide with postpartum oestrus in their dams. To analyse the pathogenesis of this diarrhoea we have determined faecal bacteria in foals and their dams (n=30 each), and serum IGF-1 and γ-globulins for 6 weeks after birth. In addition, effects of ß-carotene supplementation to mares (group 1: 1000 mg/day, n=15, group 2: control, n=15) on diarrhoea in foals were studied. Diarrhoea occurred in 92 and 79% of foals in groups 1 and 2, respectively, but was not correlated with oestrus in mares. Beta-carotene supplementation was without effect on foal diarrhoea. In mares, bacterial flora remained stable. The percentage of foals with cultures positive for *E. coli* was low at birth but increased within one day, the percentage positive for *Enterococcus sp.* was low for 10 days and for *Streptococcus* sp. and *Staphylococcus* sp. was low for 2 to 4 weeks. By 4 weeks of age, bacterial flora in foals resembled an adult pattern. Concentration of serum IGF-1 was low at birth (group 1: 149±11, group 2: 166±17 ng/ml), increased after day 1 (day 7 group 1: 384±30, group 2: 372±36) but at no time differed between groups. Serum γ-globulin concentration in foals was low before colostrum intake and highest on day 1 ($p<0.001$ over time). In conclusion, neonatal diarrhoea in foals does not coincide with postpartum oestrus in their dams but with changes in intestinal bacteria and is not influenced by β-carotene supplementation given to mares.

Keywords: foal; diarrhoea; faecal bacteria; IGF-I; carotene supplementation

1. Introduction

Many foals develop transient diarrhoea in the first two weeks of life. This condition has been suggested to coincide with first oestrus after parturition in their dams and is often named foal heat diarrhoea. This diarrhoea is non-infectious and self-limiting but little is known about the causative factors. Changes in milk composition (Johnston et al. 1970), postnatal maturation of the gastrointestinal tract and establishment of intestinal bacterial flora have been suggested (Masri et al. 1986). Foals are born with virtually no immunoglobulins and passive transfer of maternal immunoglobulins via colostrum is required to provide protection against infections in the neonatal period (McGuire et al. 1977, LeBlanc et al. 1992). In addition to immunoglobulins, colostrum contains a variety of growth factors (Hess-Dudan et al. 1994, Berg et al. 2007, Stelwagen et al. 2009). In cattle and pigs, positive effects of colostrum on development of the intestinal mucosa have been demonstrated (Xu 1996, Bühler et al. 1998).

Beta-carotene is present in equine colostrum at high concentrations (Schweigert and Gottwald 1999) and transferred to the foal via colostrum (Kuhl et al., 2010). In calves, β-carotene status may affect the occurrence of diarrhoea (Kume and Toharmat 2001). Clinical reports but no controlled studies indicate positive effects of β-carotene supplementation to mares on diarrhoea in their foals (Enbergs and Klemt 1987).

In the current study, changes in faecal bacteria and parasites of newborn foals and their dams were analysed from foaling until 6 weeks after birth. We hypothesized that foal heat diarrhoea is not linked to postpartum oestrus but is associated with changes in intestinal bacterial flora. Factors potentially influencing intestinal maturation, function and immunity such as IGF-1 concentration and serum γ-globulins were analysed as well. In addition, effects of ß-carotene supplementation to mares on faecal bacterial flora and occurrence of neonatal diarrhoea in their foals were studied.

2. Material and Methods

2.1. Animals and sampling procedures

A total of 30 mares and their foals of the Brandenburg State Stud (Neustadt/Dosse, Germany) were included into the study. Foals were born between March and May. Details on the animals are given in table 1.

Mares were kept in group stables and had access to an outdoor paddock for several hours every day. Approximately two weeks before the expected date of foaling, mares were transferred to individual boxes and were returned to their group together with the foal 5 days after parturition. Mares were fed concentrates and hay twice daily and had free access to fresh water at all times. Foals were fed a pelleted foal starter in an area of the group stable not accessible to the mares and had also access to the mares´ hay. When the foals had reached an age of 3 to 4 weeks, the group was turned out on pasture. Mares were dewormed with ivermectin (Eraquell, Virbac, Bad Oldesloe, Germany) on the day of foaling and on day 28 thereafter if not yet pregnant again. All foals received ivermectin on day 28 of life. Three foals were excluded from the study for reasons not related to the experiment (one foal treated for meconium impaction in a veterinary clinic from days 2 to 7 of life, one foal receiving systemic antibiotic treatment because of a superficial wound with local swelling on the first day of life, one foal receiving antibiotic treatment because of profound diarrhoea with signs of clinical illness between days 22 and 32 of life).

2.2. Experimental design

Mares were tested daily for oestrous behaviour with a teaser stallion, beginning on day 6 postpartum. In addition, signs of oestrus were checked by rectal palpation and

ultrasonography of the ovaries and uterus at one to two-day intervals. Oestrus was defined using standard criteria (Behrens et al. 1993). Foals were checked daily for signs of diarrhoea and/or illness. The presence of watery faeces, smearing of faeces and loss of hair in the perianal area were used as indicators for diarrhoea. To characterize diarrhoea, a score from 0=absent, 1=mild, 2=moderate to 3=high grade diarrhoea was used.

For bacteriological analysis, faecal samples were taken from the rectum of the horses using a sterile swab (WDT, Garbsen, Germany) on day -14, 0 (=day of foaling), 14, 28, 42 in mares and on day 0 (=day of birth), 4, 6, 8, 10, 12, 14, 28, 30, 32, 34, 36 in foals, respectively. The sampling regime takes into account the expected time of first and second postpartum oestrus in the mares. To observe infestation with intestinal parasites, faecal samples from the mares were taken on days -14, 0, 14, 28 and 42. At the time of collection of faecal swabs from foals, it was always attempted to take also faecal material for parasitological analysis but sufficient amounts of faeces for parasitology (50 g) could not be obtained at all times. The number of samples evaluated is indicated in the results section. Bacteriologic samples were placed in transport medium (Amies medium without charcoal, WDT), chilled at 5°C and transported to the laboratory within 24 hours. Samples for parasitological analysis were shipped without transport medium.

Blood samples for plasma and serum analysis were taken via jugular venipuncture on day -14, -7, 0, 7, 14, 21, 28, 34, 42 in mares and on day 0 (= within 1 hour after birth and before first nursing), 1 (=18-36 hours after birth), 3, 7, 21, 28, 34, 42 in foals. Plasma was centrifuged at 1600 g for 10 min, serum was centrifuged at 2200 g for 15 min after a waiting period of 2 hours at room temperature. Aliquots of all samples were frozen at -20°C until further processing.

The mares were randomly allocated to two groups. Mares of group 1 (n=15) received β-carotene orally (1000 mg per day; Blattiviko beta 8000, Blattin Mineralfutterwerk Seitschen, Göda, Germany) from 14 days before the expected date of foaling to 42 days after parturition. Mares of group 2 (n=15) remained untreated as controls. The study was approved by the Committee on Animal Experimentation of the Brandenburg State Office for Consumer Protection, Agriculture and Rural Development.

2.3. Experimental procedures

Detection and identification of bacteria in faecal swabs

Faecal swabs were plated onto a set of standard media including Columbia agar supplemented with 5% sheep blood (CA), Gassner agar (GA), Yersinia selective agar (YA; all Oxoid, Wesel, Germany), and anaerobic agar supplemented with 5% sheep blood and 0.01% neomycin (AA) (Heipha, Eppelheim, Germany). Incubation was performed as follows: CA and GA aerobically at 36°C overnight, YA aerobically at 29°C for 48 hours, and AA anaerobically at 36°C overnight. For the detection of salmonellae, the swab was inoculated into 10 ml of Preuss's potassium tetrathionate enrichment broth (Merck, Darmstadt, Germany). After overnight incubation at 36°C, aliquots of 10 µl were plated onto Xylose Lysine Desoxycholate agar and Brilliance Salmonella agar (Oxoid). Inoculated agar plates were incubated again at 36°C overnight. Identification of bacterial isolates was performed by standard bacteriological procedures described elsewhere (Songer and Post 2005).

Bacterial quantities on solid media (except post-enrichment) were classified as no growth (score 0), sparse growth (<30 colony forming units, cfu; score 1), moderate growth (30-100 cfu; score 2) and pronounced growth (>100 cfu; score 3).

Parasitology

Flotation using a saturated sodium chloride solution (specific gravity of 1.18-1.20), sedimentation and the Baermann technique were carried out for each faecal sample. Parasitic development stages were identified by morphologic characteristics using a reticule equipped light microscope (magnification 100-400x).

Insulin-like growth factor 1

Serum IGF-1 in foals was measured by RIA using a non-extraction method (Daxenberger et al. 1998). The antiserum was raised in rabbits against bovine IGF-1 and showed a cross-reactivity with IGF-2 <0.002%. Intra and interassay coefficients of variation were 5.1 and 13.4%, respectively.

Gamma-globulins and total protein

Serum electrophoresis was performed by an automated electrophoresis system and densitometric scanning (Menarini Diagnostics, Vienna, Austria). A proportion of the 20 µl serum sample was blotted on a cellulose acetate strip with a plastic support (Mylor). The strip was transferred into the migration chamber filled with buffer solution (1.09% Tris-hydroxymethyl-aminomethan and 0.5% 55-diaethylbarbiturate-sodium), the migration time was 15 min at 140 V. After migration, the strip was blotted and transferred into a staining solution (Ponceau-red-5, 1.5% trichloracetic acid) for 180 sec and then transferred into a destaining solution (5% citric acid) for 120 sec and into a clearing solution (32% N-methyl-pyrrolidon, acetic acid, methanol) for 120 sec. Drying took 620 sec. Densitometric scanning was performed in green light and the readings were performed with Elfolab software (Menarini Diagnostics).

Statistical analysis

Statistical comparisons were made with the SPSS statistics package (version 17.0 SPSS, Chicago, Illinois, USA). Because part of the data are based on scoring, non-parametric tests were used throughout. For comparisons between times within

groups test for related samples were used, taking into account the repeated measures in the same animals (Friedman-test: > two time points, Wilcoxon-test:two time points). Comparisons between groups for individual time points were made by Mann-Whitney-U-test. A p-value <0.05 was considered significant. All data given are means ± standard error of mean (S.E.M.).

3. Results

Clinical findings

All but one mare showed signs of oestrus within two weeks after foaling with a mean interval of 9.2±0.4 days until first observation of oestrus in group 1 and 8.6±0.5 days in group 2. Occurrence of first postpartum oestrus did not differ between groups (figure 1). All foals were viable at birth and no problems with regard to first standing and suckling and colostrum intake were observed. Diarrhoea occurred in 92 and 79 % of foals in groups 1 (foals born to ß-carotene supplemented mares) and 2 (foals born to control mares), respectively, at least once during the study period (n.s.). Diarrhoea was seen for 4.1±1.0 and 2.6±0.8 days in groups 1 and 2, respectively, and was detected for the first time on day 11.0±1.8 and 17.9±3.0 in foals of group 1 and 2 (n.s.). Within the first two weeks of life 62% and 43% of foals in groups 1 and 2, respectively, showed diarrhoea at least on one day (n.s.). With the exception of the first 5 days and days 24 to 42 of life, the occurrence of diarrhoea was spread over the whole observation period and not correlated to signs of first postpartum oestrus in the mares (figure 1).

Bacteriology

A variety of bacterial species considered as markers of gastrointestinal flora was isolated from faecal swabs. Bacteria species isolated at least in two individuals per group and at least on two days were *E. coli, Enterococcus* sp., β-haemolytic *Streptococci* and coagulase-negative *Staphylococci* in both mares and foals,

Acinetobacter sp. in mares only and *Proteus* sp. and *Klebsiella* sp. in foals only. In foals *Pantoea* sp., *Acinetobacter* sp., *Clostridium perfringens* and *Citrobacter* sp. were seen less frequently, i.e. in less than two individuals per group or not on two days. Only occasionally *Pseudomonas* sp., *Staphylococcus intermedius*, *Bacillus* sp., *Acinetobacter* sp., *Corynebacterium* sp. and *Enterobacter* sp. were detected. Coagulase-positive *Staphylococci*, *Kluyvera* sp., *Arthrobacter* sp. and apathogenic *Yersina entercolitica* occurred in one sample each. In mares, single isolates of *Proteus* sp., *Pseudomonas* sp., *Pantoea* sp. and *Bacillus* sp. could be detected. *Klebsiella* sp. and *Citrobacter* sp. were found in one sample each.

In mares, bacterial flora remained largely stable throughout the period studied. *E. coli* was present in all swabs of group 1 and 2 mares throughout the study except for two swabs of group 2 on the day of foaling. *Enterococcus* sp. was isolated over the whole observation period from between 57 to 93% of samples with no significant differences between groups. The occurrence of β-haemolytic *Streptococci* decreased at the time of parturition in both groups compared to all later time points ($p=0.05$). Coagulase-negative *Staphylococci* did not change significantly over time in group 1. In group 2, they were detected in fewer samples on day 0 and 42 compared to day -14 ($p<0.05$) but did not differ when day -14 was compared to days 14 and 28 and on day 0 compared to days 14 and 42 (figure 2a and c). Average intensity of bacterial growth is summarized in table 2.

In foals, occurrence of bacterial species and intensity of growth changed over time. *E. coli* occurred in 2 out of 13 and 5 out of 14 samples in groups 1 and 2, respectively, directly after birth (n.s.). On day four 12/13 and 13/14 faecal swabs in groups 1 and 2, respectively, (n.s.) contained *E. coli* which could be isolated in all samples from the age of 10 days onwards (Figure 2b). *Enterococcus* sp. were present in 4/13 and 6/14 samples of group 1 and 2, respectively, on the day of birth and in 5/13 and

2/14 swabs of group 1 and 2 respectively on day 8. After the first week of life, the percentage of samples positive for *Enterococcus* sp. increased significantly ($p<0.05$) in group 2 but only tended to increase in group 1 ($p=0.08$). On day 32, *Enterococcus* sp. occurred in more faecal swabs from foals of mares treated with β-carotene than from foals of non-treated mares ($p<0.05$; figure 2b). Beta-haemolytic *Streptococci* were first isolated on day 4 of life and until day 10 detected in only 1 to 2 samples in both groups. On day 30, β-haemolytic *Streptococci* were detected in more samples, i.e. 9/12 in group 1 and 8/10 in group 2, compared to day 8 ($p<0.05$). No samples could be obtained from one foal in group 1 and 4 foals in group 2. Coagulase-negative *Staphylococci* were isolated in 10/13 and 12/14 swabs in groups 1 and 2, respectively, at the day of birth and in none (group 2) or only one (group 1) of the samples until day 10 where 3/11 and 2/14 samples in group 1 and 2, respectively, were positive for coagulase-negative *Staphylococci*. Thereafter, they were present in up to 50% and 36 % of samples in groups 1 and 2, respectively. *Clostridium perfringens* was present in one sample of 3 foals (n=2 group 1, n=1 group 2), in two consecutive samples of one foal (group 1) and in 4 consecutive samples of two foals (group 2). *Clostridium perfringens* could be detected during the first two weeks of life only and not at later times. Its occurrence was not associated with diarrhoea in these foals. Intensity of bacterial growth in faecal swabs of foals is summarized in table 3.

Parasitology

Eggs of small strongyles occurred in 12 of 15 faecal samples in mares of both groups on day -14 and in 13 (group 1) and 12 (group 2) of 15 samples on the day of parturition. Eggs of large strongyles occurred in 7 (group 1) and 2 (group 2) of 15 faecal samples on day -14 (n.s.) and in 8 (group 1) and 5 (group 2) of 15 samples on the day of parturition (n.s.). No eggs of endoparasites could be detected thereafter. In the foals, one faecal sample was positive for eggs of small strongyles on day 8. In

the remaining samples (n=32) parasitic stages were non-detectable throughout the study period.

IGF-1

Concentrations of IGF-1 in serum of foals were low on the day of birth (149±11 and 166±17 ng/ml in groups 1 and 2, respectively) and decreased slightly but significantly ($p<0.05$) until day 1 (133±13 and 154±12 ng/ml in groups 1 and 2, respectively). Thereafter, IGF-1 concentrations increased continuously during the first week of life (day 7: 384±30 and 372±36 ng/ml in groups 1 and 2, respectively, $p<0.001$ versus day 0). No further changes were seen between days 7 and 42. At no time serum IGF-1 concentrations differed between foals born to ß-carotene-treated mares and control mares (figure 3a).

Gamma-globulins

Concentration of γ-globulins in serum was lowest on the day of birth before colostrum intake (group 1: 315±56, group 2: 317±51 mg/dl), and highest on day 1 (group 1: 958±140, group 2: 943±161 mg/dl, $p<0.01$ versus day 0). Thereafter, γ-globulin concentration decreased continuously during the first 4 weeks of life (day 28: 492±46 and 509±62 mg/dl in groups 1 and 2, respectively, $p<0.05$ versus day 1). Serum γ-globulin concentrations at no time differed between foals born to ß-carotene-treated and control mares (figure 3b).

4. Discussion

The majority of foals showed signs of diarrhoea for some time during the first 6 weeks of life. Diarrhoea was transient and none of the foals received antibiotic or other treatment. Diarrhoea in the foals occurred throughout the experimental period but was most pronounced during the second and third week of life. There was no

292 temporary relation between diarrhoea in foals and first or second postpartum oestrus
293 in their dams. During the first two weeks of life, diarrhoea occurred in approximately
294 50% of foals only. While older studies have reported a prevalence of foal heat
295 diarrhoea between 75 and 80% (Rumbaugh 1983, Masri et al. 1986), the total
296 prevalence of diarrhoea in newborn foals between days 5 and 15 of life in our study
297 is in the same range as reported recently by Sgorbini et al. (2008). Onset and
298 duration of diarrhoea in foals of our study was in accordance with occurrence of
299 diarrhoea in orphan foals raised on a milk replacer diet (Cymbaluk et al. 1993). Foal
300 diarrhoea is thus neither an effect of first postpartum oestrus in their dams nor does it
301 occur in all foals. Because no close temporal relation between foal heat and
302 diarrhoea in foals exists, the term foal heat diarrhoea is confusing and its adequacy
303 should be questioned.
304
305 Beta-carotene supplementation to periparturient mares was without effect on foal
306 diarrhoea. This contradicts the report by Enbergs and Klemt (1987), however in that
307 study, data were not submitted to critical statistical evaluation. In calves, ß-carotene
308 status at 6 days of age affects the occurrence of diarrhoea (Kume and Torhamat
309 2001), however, the aetiology of diarrhoea in calves and foals is not comparable.
310 Diarrhoea in neonatal dairy calves is caused either by inadequate feeding manage-
311 ment or by infectious agents (reviewed by Bartels et al. 2010).
312
313 Frequency and intensity of bacterial growth in faeces of foals underwent changes
314 especially within the first two weeks of life, i.e. at a time when neonatal diarrhoea
315 occurred in most foals. Faecal bacterial flora represents a combination of shed
316 mucosal bacteria and non-adherent luminal bacteria. It can thus be assumed that the
317 changes in faecal bacterial flora observed in our study are due to changes in the
318 intestinal bacterial flora. Changes in intestinal flora occur in all species and have
319 been analysed in depth in human infants (Palmer et al., 2007). In infants, a first

rearrangement of the faecal bacterial population occurred around 5 days of age while the transition to an adult-like pattern is found later and usually after the introduction of solid foods. In contrast to humans, foals start to consume small amounts of concentrates already around 10 days of age. To our knowledge, the horse is the only domestic animal species where a non-infectious diarrhoea occurs regularly in most neonates. The temporal coincidence with changes in bacterial flora is no proof yet of a causal relationship and our data do not allow excluding postnatal maturational changes of the intestinal epithelium as a cause of foal diarrhoea.

The percentage of foals with faecal cultures positive for *E. coli* was low on the day of birth but had increased to near 100% within one day. In contrast, the percentage of foals with faecal cultures positive for *Enterococcus sp.* remained low for approximately 10 days before increasing to the same range as in mares. The percentage of faecal cultures positive for *Streptococcus* sp. and *Staphylococcus* sp. increased even later, i.e. between two and 4 weeks of life. Consistent with other reports which consider *Clostridium perfringens* part of the normal intestinal flora in neonatal foals (Yuyama et al. 2004, Tillotson et al. 2002), its presence was not associated with diarrhoea in the foals of our study. By 4 weeks of age, the bacterial flora in foals largely resembled the spectrum isolated from faeces of their dams, indicating that the intestinal bacterial flora had changed from a neonatal to a postnatal pattern. The time window of these changes was closely correlated with the time range for foal diarrhoea. During the first 10 days of life, in foals also coprophagy can be seen (Kazunori et al.1985, Thompson et al. 1988, Cymbaluk et al. 1993). Microorganisms from the faeces of the mares present on the floor of the stable might therefore colonise the intestinal tract of the foals at this time. Interestingly, diarrhoea occurred in approximately 50% of foals only during the first two weeks of life, although changes in bacterial flora did not differ between foals with and without signs of diarrhoea. Some foals are thus able to develop a postnatal intestinal flora without

348 diarrhoea. This might be an advantage with regard to growth and weight gain in
349 these foals.
350
351 Results of a study using the same deworming regimen of the dams as in our
352 experiment indicated that the regimen was efficient to reduce shedding of
353 *Strongyloides* eggs and that foal diarrhoea was not caused by infestation with
354 *Strongyloides westeri* (Ludwig et al. 1982). As no mare was shedding eggs of
355 endoparasites postpartum, worm burden can largely be excluded as a cause of
356 diarrhoea in our foals.
357
358 Colostrum uptake does not only provide the neonate with immunoglobulins but, at
359 least in piglets, enhances also postnatal maturation of the intestinal epithelium (Xu
360 1996, Jensen et al. 2001, Blum and Baumrucker 2008). In pigs, intestinal maturation
361 is not fully complete before 3 weeks of age (Jarvis et al. 1977). It can be assumed
362 that also in foals, maturation of the intestinal tract at large occurs during a
363 comparable time period and may in part be linked to foal heat diarrhoea.
364
365 In our study, serum IGF-1 reached highest levels within the first week and remained
366 constant thereafter whereas Hess-Dudan et al. (1994) reported an increase over a
367 period of 3 weeks until a plateau phase was reached. Concentrations of IGF-1 in
368 colostrum of mares are high but decrease dramatically within less than 24 hours
369 (Hess-Dudan et al. 1994, Berg et al. 2007). The postnatal increase in IGF-1
370 concentrations in foals therefore is not due to absorption of colostral IGF-1 but to
371 IGF-1 synthesis by the foal. Although, as in calves (Baumrucker et al. 1994) and
372 piglets (Xu 1996), colostral IGF-1 might have local effects on postnatal development
373 of the intestinal epithelium in foals, these effects will take place before the occurrence
374 of neonatal diarrhoea.
375

Gamma-globulin concentrations in foals were low before first suckling and, as expected, increased rapidly after ingestion of colostrum (Jeffcott 1974, Hess-Dudan et al. 1994). The concentration of γ-globulins was highest one day after foaling and decreased thereafter. Another increase was seen after week 4 of life and is apparently due to immunoglobulin synthesis by the foals (Holznagel et al. 2003). There was no period of markedly reduced γ-globulin concentrations in foals and thus no gap in immunological protection. Neither the occurrence nor the characteristics of neonatal diarrhoea differed between foals with high and with lower plasma γ-globulin concentration, indicating that neonatal diarrhoea in foals is not affected by passive transfer of immunity via colostrum and not caused by reduced γ-globulin transfer in our study. Gamma-globulins were already detectable before first intake of colostrum. This is in agreement with a recent study in cattle, indicating that more than 50% of calves had detectable serum IgG concentrations before first colostrum uptake (Chigerwe et al. 2008). However, further investigations are needed to determine causes for the comparably high concentration of γ-globulins prior to first nursing in our foals. Most probably this is caused by the fact that in our study, total γ-globulins in serum were measured which consist not only of immunoglobulins but also of additional proteins with no known immune function.

In conclusion, neonatal diarrhoea in foals is not linked to postpartum oestrus in their mares but is observed at a time when changes in faecal bacterial flora from a neonatal to a postnatal pattern occur. These changes in bacterial flora in many foals are accomplished without clinical signs of diarrhoea. Beta-carotene supplementation to mares was without effect on neonatal diarrhoea in foals.

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495

496 Table 1: Age of the mares and parity (number of foals produced so far)

	Age (years) Range (means ±SEM)	Parity (number of foals) Range (means ±SEM)	Number of mares rebred within the study period
Group 1 (n=15)	5 – 17 (8.0 ± 1.0)	1 – 13 (4.0 ± 0.9)	10/15
Group 2 (n=15)	4 – 19 (8.5 ± 1.1)	1 – 13 (4.7 ± 1.0)	14/15
Total (n=30)	4 – 19 (8.3 ± 0.7)	1 – 13 (4.4 ± 0.6)	24/30

497

498

499 Table 2: Intensity of bacterial growth in mares (n=15 group 1, n=15 group 2; score

500 from 0 to 3;data are means ± SEM)

Isolated bacteria	Group	Day -14	Day 0	Day 14	Day 28	Day 42
E.coli	1	2.4±0.1*	2.3±0.2	2.6±0.2	2.5±0.1	2.8±0.1
	2	2.9±0.1*	2.3±0.3	2.4±0.2	2.7±0.1	2.9±0.1
Enterococcus sp.	1	2.1±0.2*	2.1±0.3	2.3±0.3	2.3±0.3	1.6±0.4
	2	2.5±0.2*	2.1±0.3	1.5±0.3	1.9±0.3	1.9±0.3
β-haemolytic	1	1.5±0.3 a,b	0.6±0.3 a	1.8±0.2 b	2.1±0.2 b	2.5±0.2b
Streptococci	2	1.8±0.3 a,b	0.9±0.3 a	1.5±0.3 b	1.5±0.3 b	1.9±0.3 b
Coagulase-neg.	1	0.4±0.3 a	1.,5±0.3 b	1.1±0.3 a,b	1.1±0.4 a,b	0.9±0.4 a,b
Staphylococci	2	0.1±0.1 a	1.3±0.3 b	0.5±0.2 a,b	0.6±0.3 a,b	1.7±0.4 b
Proteus sp.	1	0	0.1±0.1	0	0	0
	2	0.1±0.1	0.1±0.1	0	0	0
Pantoea sp.	1	0	0.1±0.1	0.4±0.3	0.1±0.1	0
	2	0	0	0	0.1±0.1	0
Acinetobacter	1	0.2±0.2	0.8±0.4	0.6±0.3	0.4±0.3	0.4±0.3
sp.	2	0	1.2±0.4	0.4±0.3	0.2±0.2	0.5±0.3

501 * significant differences between groups ($p<0.05$); a,b: values with different

502 superscript letters in the same line differ significantly ($p<0.05$)

Table 3: Intensity of bacterial growth in foals from the day of birth to day 36 of life (n=13 group 1, n=14 group 2; score from 0 to 3; data are means ± SEM)

Isolated bacteria	Group	Day after birth											
		0	4	6	8	10	12	14	28	30	32	34	36
E.coli	1 a	0.2±0.2	2.5±0.2	2.5±0.2	2.7±0.1*	2.6±0.2	2.8±0.1	2.7±0.1	2.9±0.1	2.9±0.1	2.8±0.1	2.8±0.1	2.9±0.1
	2 a	0.8±0.3	2.2±0.2	2.4±0.1	2.2±0.1*	2.4±0.1	2.4±0.2	2.6±0.1	2.9±0.1	2.6±0.2	2.7±0.1	2.6±0.1	2.7±0.1
Enterococcus	1 a	0.6±0.3	1.4±0.4	0.7±0.4	1.0±0.4	2.3±0.4	2.1±0.4	1.9±0.4	2.3±0.3	1.9±0.4	2.8±0.1*	2.0±0.4	2.4±0.4
	2 a	0.8±0.3	0.4±0.2	0.4±0.2	0.3±0.2	1.4±0.3	2.1±0.3	1.3±0.3	2.3±0.4	1.9±0.4	1.5±0.4*	2.3±0.3	2.2±0.3
β-haem.	1 a	0	0.2±0.2	0.1±0.1	0.2±0.2	0.7±0.4	0.4±0.2	0.2±0.2	1.2±0.3	1.9±0.4	1.6±0.4	1.6±0.3	1.6±0.4
Streptococci	2 a	0	0.1±0.1	0.3±0.2	0.4±0.2	0.4±0.2	0.4±0.2	0.7±0.3	1.3±0.4	2.1±0.4	1.5±0.3	1.6±0.3	1.7±0.4
Coagulase-	1 a	1.4±0.3	0	0.2±0.2	0.2±0.2	0.8±0.4	0.5±0.3	0.9±0.4	1.5±0.5	1.4±0.4	0.7±0.4	0.7±0.4	0.3±0.3
neg. Staph.	2 a	1.9±0.3	0	0	0	0.4±0.3	0.8±0.4	0.9±0.4	0.7±0.4	0.6±0.4	0.9±0.4	0.8±0.4	1.0±0.4
Proteus	1 a	0.1±0.1	0.9±0.1	1.1±0.2	0.8±0.2	0.4±0.2	0.3±0.1	0.5±0.2	0	0	0	0	0
	2 a	0.1±0.1	1.0±0.0	0.9±0.1	0.6±0.1	0.4±0.1	0	0.1±0.1	0	0	0.1±0.1	0	0
Clostridium	1	0	0.2±0.1	0.4±0.3	0.4±0.3	0.5±0.3	0.3±0.3	0	0	0	0	0	0
perfringens	2	0	0	0.2±0.2	0.1±0.1	0.1±0.1	0	0	0	0	0	0	0
Klebsiella	1 a	0	0.7±0.3	0.5±0.2	0	0	0	0	0	0	0	0	0
	2 a	0	0.3±0.2	1.1±0.3	0.4±0.3	0	0	0.4±0.3	0	0	0	0	0
Pseudomonas	1	0	0	0	0	0	0.3±0.3	0	0.2±0.2	0	0.5±0.3	0.2±0.2	0
	2	0.1±0.1	0	0	0	0	0	0	0.3±0.3	0.3±0.3	0	0	0
Pantoea	1 a	0.7±0.3	0.2±0.2	0	0	0	0	0	0	0	0	0	0
	2 a	0.9±0.3	0	0	0	0	0	0	0.5±0.3	0	0	0	0
Acinetobacter	1	0.4±0.3	0.2±0.2	0	0	0.3±0.3	0	0.2±0.2	0.3±0.3	0	0.3±0.3	0	0.2±0.2
	2	0.5±0.3	0	0	0	0.1±0.1	0	0.4±0.3	0.6±0.4	0.3±0.3	0.2±0.2	0	0
Citrobacter	1	0	0.5±0.3	0.5±0.3	0.2±0.2	0.3±0.3	0	0	0	0	0	0.2±0.2	0
	2	0	0.3±0.2	0.1±0.1	0	0.2±0.2	0	0	0	0	0	0	0

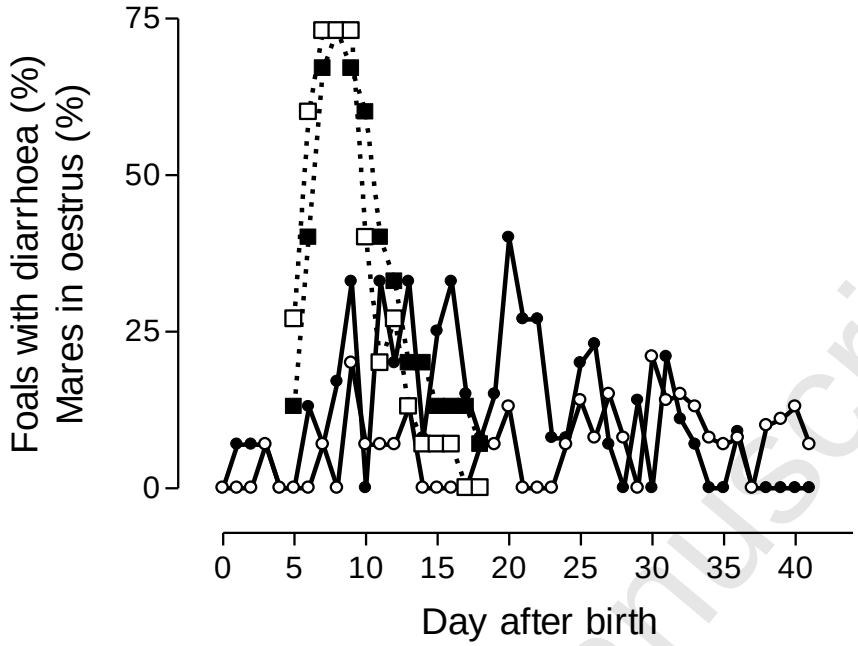
*significant differences between groups ($p<0.05$); a: significant changes within group over time ($p<0.01$; Friedman-test)

Figure legends

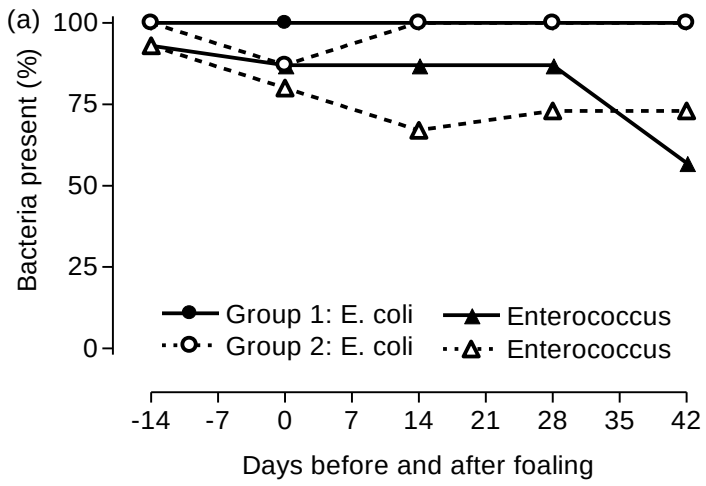
Figure 1: Percentage of mares in first postpartum oestrous (■ β-carotene treated, n=15; □ control, n=15) and foals showing signs of diarrhoea during 42 days after foaling and birth, respectively (● foals born to β-carotene-treated mares, n=13; ○ foals born to control mares, n=14)

Figure 2: Percentage of (a, c) mares (n=15 per group) and (b, d) foals (n=13 group1, n=14 group 2) with *E. coli, Enterococcus* spp., β-haemolytic *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. in faecal swabs throughout the experimental period. Significant changes over time in mares of both groups for β-haemolytic *Streptococcus* spp. ($p<0.05$) and group 2 mares for coagulase-negative *Staphylococcus* spp. ($p<0.01$); in foals for *E. coli, Enterococcus* spp., β-haemolytic *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. ($p<0.001$); *$p<0.05$ between groups.

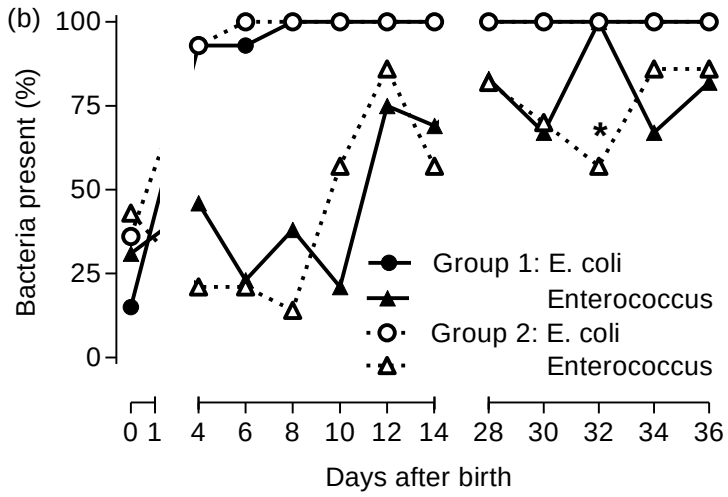
Figure 3: (a) Serum IGF-1 and (b) γ-globulin concentrations in foals born to ß-carotene-treated (●; n=13) and control mares (○; n=14). Significant changes over time for both IGF-1 ($p<0.001$) and γ-globulins ($p<0.001$).



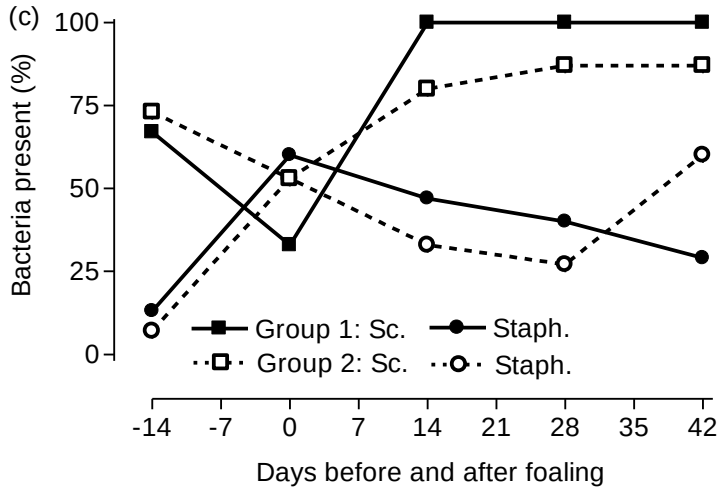
Kuhl et al., Figure 1



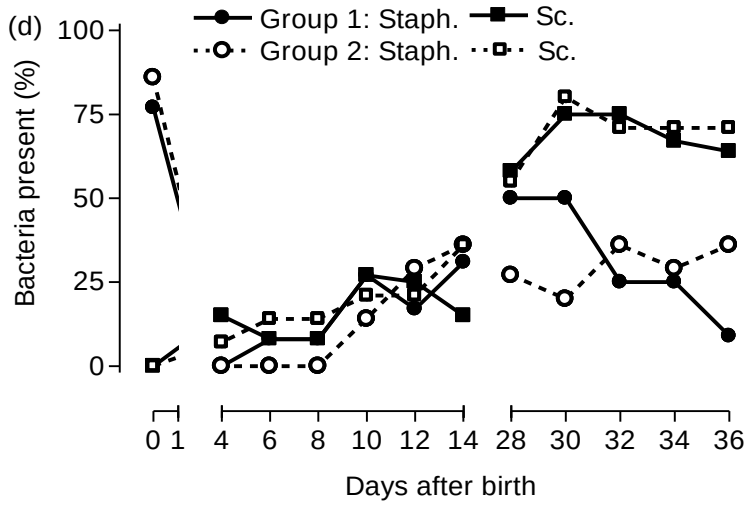
Kuhl et al., Figure 2a



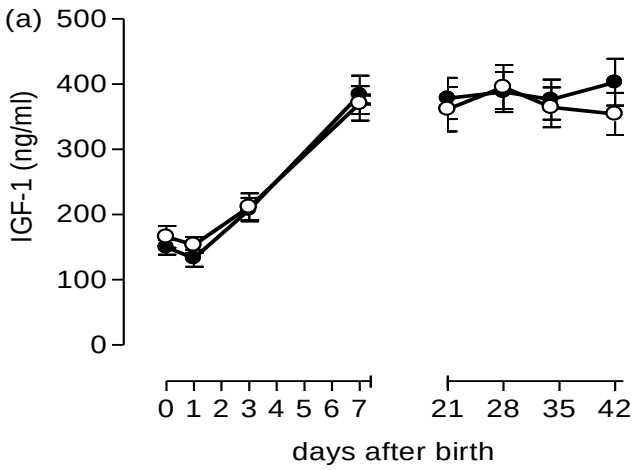
Kuhl et al., Figure 2b



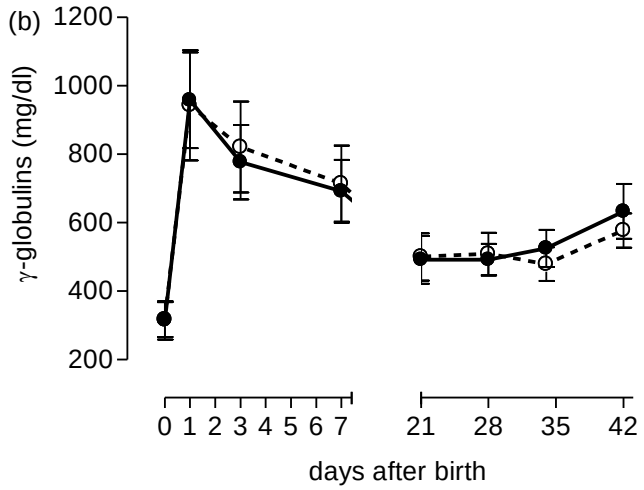
Kuhl et al., Figure 2c



Kuhl et al., Figure 2d



Kuhl et al., Figure 3a



Kuhl et al., Figure 3b