Journal of Equine Veterinary Science 35 (2015) 584–590

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Colostrum Withdrawal is Without Effect on Duodenal Development in Newborn Foals

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ARTICLE INFO

Article history:
Received 15 November 2014
Received in revised form 27 February 2015
Accepted 25 March 2015
Available online 31 March 2015

Keywords:
Foal
Intestinal development
Colostrum

ABSTRACT

A lack of colostrum may delay postnatal intestinal development in foals. Therefore, in this study, the effect of colostrum withdrawal on duodenal development of newborn foals was investigated. Shetland foals were fed either colostrum (group COL, n = 5) or milk replacer (group MR, n = 8) by nasogastric tube over the first 24 hours of life, and all foals received one liter of hyperimmune plasma. On day 3 of life, duodenal biopsies were taken via gastroduodenoscopy. Expression of mRNA for receptors for insulin-like growth factor 1 (IGF1), IGF2, and transforming growth factor β (TGFβ) was analyzed by real-time PCR. Biopsies were analyzed for IGF receptors 1 and 2, TGFβ receptor 1, immunostaining for Ki-67 proliferation marker, lectin binding, and histomorphology. The mRNA expression of receptors for IGF1, IGF2, and TGFß in the duodenum of newborn foals was not altered by withholding colostrum. The number of goblet cells in the epithelium of the duodenum was greater in MR compared with COL foals (1.95% ± 1.15 vs. 1.25% ± 0.75), whereas villus length did not differ between groups. Immunohistochemistry of Ki-67 and lectin-binding patterns was similar in foals of both groups. Results indicate that a lack of colostrum is without detrimental effects on postnatal duodenal development in foals.

1. Introduction

Foals may have to be raised without receiving colostrum due to death of the mare at foaling, inadequate development of the udder, or aggression of the mare toward its newborn. A failure of passive transfer of immunity in these foals can be overcome by treatment with hyperimmune plasma [1,2], but in addition to immunoglobulins, colostrum contains different bioactive substances, which in piglets and calves have been suggested to stimulate intestinal maturation [3–6].

Colostral bioactive substances that potentially stimulate development of the intestinal epithelium in cattle and pigs include insulin-like growth factor 1 (IGF1) and transforming growth factor β (TGFβ) [4,7,8]. Differences at the histomorpholgical level between piglets fed colostrum or milk replacer included enhanced mitotic activity in the intestinal crypts, less pronounced apoptosis, and faster replacement of fetal-type vacuolated enterocytes by adult-type enterocytes in animals that received colostrum [6,9,10].

In neonates, different IGFs are produced or absorbed from colostrum and IGF receptors are present in the intestinal mucosa of neonatal calves [11–14]. Abundance of the insulin-like growth factor 2 receptor (IGF2R) was positively correlated with villus size and crypt depth.

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0737-0806/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2015.03.195

Transforming growth factor β is present in milk and colostrum and participates in enterocyte proliferation and differentiation as demonstrated in different species [15–17]. Maturational changes potentially induced by TGFβ in piglets include an increase in villus height, deepening of crypts and a transient decrease in TGFβ receptor density on the villus epithelium [18,19].

We hypothesized that in neonatal foals, intestinal histomorphology and expression of receptors for growth factors are influenced by colostrum and that colostrum-derived growth factors enhance development of the duodenal epithelium. To investigate effects of colostrum on intestinal development in the equine neonate, duodenal biopsies were collected from 3-day-old foals and analyzed for histomorphology, IGF1R, IGF2R, and TGFβR1 mRNA expression, Ki-67 proliferation marker, and lectin-binding patterns.

2. Material and Methods

2.1. Animals

Horses included in this study were 13 newborn Shetland foals. Duration of pregnancy was 327 ± 3 days. All mares foaled spontaneously and without veterinary intervention, and foals were viable at birth. Birth weight was 20.1 ± 3.9 kg with no significant difference between groups. Clinical and hematological data from these foals have in part been published elsewhere [20].

The study was approved by the competent authority for animal experimentation in Austria (Federal Ministry for Science and Research, license number, 68.205/0130-11/10 b/2009).

2.2. Experimental Procedures

Before first suckling, foals were fitted with an oral muzzle to prevent them from suckling their dam's udder and received a nasogastric tube (Stallion urinary catheter, 6.6 mm × 140 cm; Andreas Bones, Straelen-Dam, Germany). An IV catheter (Milacath; Mila, Florence, KY, USA) was placed into one jugular vein. The foals were fed either colostrum from their dams by nasogastric tube over 24 hours (group COL; n = 5) or received milk replacer (Pavo foal milk; Boxmeer, Netherlands) instead (group MR, n = 8). Colostrum or milk replacer was administered hourly beginning at 1.5 hours after birth. The total amount of colostrum or milk replacer fed within 24 hours corresponded to 15% of body weight measured directly after birth. Mares were milked every hour and to ensure that feeding of group COL foals was representative of normal equine colostrum, volume was recorded, and immunoglobulin G (IgG) content determined by sugar refractometer [21] in each milking. Group COL foals received 61.7 ± 3.7 g of IgG over 24 hours. For adequate immunoglobulin supply, all foals received 1 L of equine hyperimmune plasma (Hypermune; Veterinary Immunogenics, Penrith, UK). After 24 hours of life, foals were allowed to suckle their dams without restriction.

2.3. Gastroduodenoscopy

On day 3 of life, duodenal biopsies were taken from the foals by gastroduodenoscopy under standard general anesthesia with isoflurane (Vetflurane; Virbac, Vienna, Austria). Biopsies of the duodenal mucosa were collected by use of a flexible human gastroscope (Olympus GIF-Q165; Olympus, Vienna, Austria) with a working length of 1,030 mm, an insertion tube diameter of 9.2 mm, and a 2.8-mm working channel. The anesthetized foal was positioned in the left lateral recumbency, and the endoscope was carefully inserted through the protected oral cavity, esophagus, stomach, and pylorus into the duodenum. Three biopsies for real-time PCR were taken with endoscopic fenestrated alligator tooth biopsy forceps (Olympus FB-36k-1; Olympus, Vienna, Austria) and snap frozen in liquid nitrogen and stored at −80°C until use, whereas another four biopsies were fixed in 4% formaldehyde for histological examination. After recovery from anesthesia, the foals were returned to their dams. One foal from group COL died 24 hours after anesthesia because of respiratory problems.

2.4. Qualitative Reverse Transcriptase PCR

Total RNA was extracted using the RNeasy Micro Kit (Qiagen, Hilden, Germany) after the manufacturer's instructions. The RNA obtained free of genomic contamination was used for reverse transcription followed by amplification using specific primers designed from equine sequences by use of Basic Local Alignment Search Tool (BLAST) primer design tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast) (Table 1). Qualitative reverse transcriptase PCR, separation by electrophoresis and visualization, was performed as described [22]. The bands were cut out from the gel, and cleaned (GeneJET Gel Extraction Kit; Thermo Scientific, Vienna, Austria) and sequenced using the amplification primers (Microsynth; Balgach, Switzerland). Finally, the sequences were compared with the database by BLAST (http://www.nbci.nlm.nih.gov/blast/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) algorithm.

Table 1
Primer sequences for the receptors type 1 of transforming growth factor β (TGFβR1) and receptors of insulin-like growth factor 1 (IGF1R) and 2 (IGF2R) used for qualitative RT-PCR.

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Amplicon Length (bp)	Accession Number
IGF1R	GCAGCTGGAGGGGAATTACT	GGGTGGGTTCCACTTCACAA	667	XM_001489765
IGF2R	CTTCCTCCGGGAGTGTTGAT	GGTGTATTCACCGGCACTCA	686	XM_005608119
TGFβR1	AACCGCACTGTCATTCACCA	CTTCAGGGGCCATGTACCTTT	700	XM_005606000