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Effects of periovulatory gonadotrophin treatment on luteal function and endometrial expression of selected genes in cyclic pony mares

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Progestin concentration in plasma during the early luteal phase is crucial for endometrial function and conceptus development. We hypothesized that periovulatory gonadotrophin treatment via support of luteal function affects endometrial gene expression in horses. Effect of age was analyzed as well. Shetland mares ($n = 8$, age 4–25 years) were assigned to the following treatments during five consecutive cycles in alternating order following a cross-over design: treatment hCG/-: preovulatory injection of hCG, but no gonadotrophin injection at detection of ovulation, treatment -/hCG: no preovulatory gonadodrophin injection, but injection of hCG at detection of ovulation, treatment eCG/-: preovulatory injection of eCG, but no gonadotrophin injection at detection of ovulation, treatment -/eCG: no preovulatory gonadotrophin injection, but injection of eCG at detection of ovulation, treatment control: no treatment. Concentration of progestin was analyzed by ELISA from the day of ovulation until Day 10. On Day 10, endometrial cells were collected transvaginally by cytobrush technique. Expression of mRNA of cyclooxygenase-2 (COX-2), prostaglandin F2α-synthase, prostaglandin E-synthase, progesterone receptor (PR), estradiol receptor (E2R), acyl-CoA-dehydrogenase (ACAD), uteroglobin (UGB), uteroferrin, and uterocalin was analyzed by RT qPCR. Immunohistological staining of endometrial tissue, obtained *via* biopsy, was performed for COX-2, PR and UGB. The P4 concentration was influenced by day of cycle ($P < 0.01$), but not by treatment. No effects of age on gene expression were determined. Neither of the periovulatory gonadotrophin treatments nor age influenced mRNA expression of the genes of interest. Treatment did also not affect immunohistological staining of the endometrium. In contrast, age affected the percentage of PR positive stromal cells (e.g. mare 1 (4 years): 65.5 ± 2.6, mare 2 (24 years): 82.7 ± 2.2%, $P < 0.05$) and COX-2 positive stained ciliated cells (e.g. mare 1: 15.8 ± 2.9, mare 2: 33.4 ± 6.0%, $P < 0.05$). In conclusion, no effects of periovulatory gonadotrophin treatment and age on endometrial gene expression in luteal phase pony mares were reported. A lack of treatment effects on luteal function and expression of PRs in the endometrium can at least in part be explained by differences in the reproductive physiology between horses and ponies.

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1. Introduction

In all mammals, establishment and maintenance of pregnancy depends on the presence of progesterone. Its main task is the preparation of the endometrium for pregnancy. Paradoxically, this requires downregulation of progesterone receptors (PRs) in endometrial epithelia as a prerequisite for the expression of pregnancy-associated proteins [1]. Altogether, the importance of a progesterone-primed endometrium for conceptus survival during the preimplantation period so far has been much better investigated in ruminants and pigs [2] than in the horse [3]. In agreement with findings from these species, supplementation of luteal phase mares with the progestin altrenogest resulted in earlier downregulation of endometrial PRs [4]. A direct influence of this treatment on equine endometrial receptivity has not been proven so far, nevertheless, it enhanced conceptus development in aged mares at the onset of placentation [5]. In cattle, controversial results of direct progesterone supplementation on pregnancy rates have been reported [6]. Therefore, stimulation of luteal function for increasing endogenous progesterone production has been suggested to be a better approach [7]. Improvement of luteal function may be achieved by treatment with gonadotrophins. Positive effects of hCG (human chorionic gonadotrophin) and eCG (equine chorionic gonadotrophin) treatment on luteal function and fertility in cattle have been reported (hCG: [8]; eCG: [9]). We recently reported beneficial effects of preovulatory hCG treatment on luteal function and conceptus size during the late preimplantation period in horses [10]. In this species, also a luteoprotective effect of eCG treatment after implantation has been shown before [11,12]. To the best of our knowledge, luteotrophic effects of eCG during the periovulatory phase of the estrous cycle have not been investigated in the horse.

In contrast to dairy and beef cattle, where an age-related decline in fertility is not a major issue, equine stud farm veterinarians are often confronted with breeding of aged mares. With increasing age, rates of early pregnancy loss in horses increase [13] which is mainly a result of histologic degeneration of the endometrium [14,15]. Stimulation of endogenous luteal function during the early luteal phase is suggested to improve endometrial function also in horses [4,5]. In the present study, we investigated possible effects of different periovulatory gonadotrophin treatments on luteal and endometrial function in pony mares. We also analyzed the endometrial expression of selected candidate genes that are suggested to be involved in either maternal recognition of pregnancy (cyclooxygenase 2, COX-2; prostaglandin E synthase, PGES; prostaglandin F synthase, PGFS) or to contribute to composition of histotrophe in the mare (PR; uteroferrin, uteroglobin (UGB), uterocalin, acyl-coenzyme-A-dehydrogenase, ACAD) [3]. Because we hypothesized that effects of treatment might be more apparent in mares which suffer from degenerative alterations of the endometrium [15] we also included aged mares into the experiment.

2. Material and methods

2.1. Animals

The experiment was performed according to Austrian animal welfare legislation and was approved by the Austrian Ministry for Science, Research and Commerce (BMWF-68.205/0047-WF/II/3b/2014). Shetland pony mares ($n = 8$; mean weight 208.7 ± 18.3 kg, mean age 13.7 ± 2.9 years) belonging to the Centre for Artificial Insemination and Embryo Transfer, Vienna, were included into the study. The mares were kept in their established group and housed in spacious outdoor paddocks. They were fed hay twice daily and always had access to water.

2.2. Experimental design and management of mares

With the beginning of April, mares were checked for signs of estrus thrice a week by ultrasound examination with a7.0-MHz linear ultrasound scanner (DP-6600Vet, Mindray, Shenzhen, China). If found to be in estrus (presence of a follicle $\geq$3.0 cm in diameter and pronounced uterine edema), mares were included into the experiment. Each mare was assigned to four different periovulatory gonadotrophin treatment protocols and one untreated control cycle in a crossover design. Therefore each mare underwent each treatment in succeeding estrous cycles but alternating order to exclude any individual differences. Transrectal ultrasound of the genital tract was performed daily until ovulation occurred. Ovulation was characterized by disappearance of the formerly detected preovulatory follicle. In case of multiple ovulations ($n = 1$), the data from the respective estrous cycle were excluded and the mare underwent the same treatment in the next cycle again. The following periovulatory treatments were chosen:

Treatment hCG/-: preovulatory injection of hCG, but no gonadotrophin injection at detection of ovulation.

Treatment -/hCG: no preovulatory gonadotrophin injection, but injection of hCG at detection of ovulation.

Treatment eCG/-: preovulatory injection of eCG, but no gonadotrophin injection at detection of ovulation.

Treatment -/eCG: no preovulatory gonadotrophin injection, but injection of eCG at detection of ovulation.

Treatment control: no preovulatory gonadotrophin injection, no gonadotrophin injection at detection of ovulation (mares received no treatment).

Administration of hCG (1500 I.U., Chorulon 300 ad us. vet., Intervet, Vienna, Austria) was performed intravenously (i.v.; injection site: left *Vena jugularis*), administration of eCG (2000 I.U., Folligon ad us. vet., Intervet, Vienna, Austria) intramuscularly (i.m.; injection site: left *Musculus pectoralis*).

2.3. Ultrasound image of the corpus luteum

Ultrasound images of the CL were taken on the day of detection of ovulation and on Days 4, 6, 8, and 10 thereafter. They were stored and analyzed as described previously [10].