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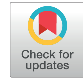
Fatty acid profile of blood plasma and oviduct and uterine fluid during early and late luteal phase in the horse



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ABSTRACT

During early pregnancy, the secretome of both oviduct and uterus serves as exchange medium for signaling factors between embryo and mother and provides the embryo with nutrients. The preimplantation embryo can utilize the fatty acids (FA) therein via direct incorporation into cell membrane lipid bilayers and for energy production via β-oxidation. The FA concentration and composition of the oviduct (OF) and uterine fluid (UF) might be regulated by ovarian hormones to meet the changing needs of the growing embryo. In our study, we analyzed the FA profile of blood plasma (BP) and reproductive fluid samples obtained *post mortem* from slaughtered mares by gas chromatography mass spectrometry. Cycle stage was determined by visual evaluation of the ovary and measurement of plasma progesterone. No major effect of cycle stage on the FA profile was observed. However, the composition of FA was different between BP and both OF and UF. While linoleic, stearic, oleic and palmitic acid were the four most prevalent FA in both BP and reproductive fluids, the latter contained higher concentrations of arachidonic, eicosapentaenoic and dihomo-γ-linolenic acid. The finding suggests selective endometrial transport mechanisms from plasma into the reproductive fluids or increased endometrial synthesis of selected FA.

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1. Introduction

During the preimplantation period, the embryo is supported by maternal secretions from the oviduct and the uterus. These secretions form a complex milieu containing e.g. proteins, amino acids, pyruvate, lactate, carbohydrates and fatty acids (FA) [1–6]. The oviduct supports gamete transportation and maturation, sperm capacitation, fertilization and development of the early embryo. Its fluid derives from blood filtrates, epithelial secretions, and, in part, follicular fluid [7]. The uterine fluid provides ongoing nutrition of the preimplantation embryo and enables the exchange of signaling factors vital for continuous embryonic growth and maternal recognition of pregnancy. It is composed of blood filtrates and secretions of endometrial luminal and glandular epithelial cells [1]. The relative quantitative contribution of FA via direct transudation from BP, uterine gland secretions and endometrial de novo synthesis are unknown. Transport of FA across membranes involves two components, passive diffusion [8] and selective protein mediated transfer [9–11] In bovine, genes related to FA uptake are upregulated in the embryonic trophectoderm during elongation, suggesting that FA from the UF are utilized by the embryo [12].

The composition of the reproductive fluids is regulated by endocrine and local hormones related to the ovarian cycle and pregnancy status [4,13–18]. The total protein content of uterine fluid in bovine [17,19] pig [20] and horse [21,22] for example, is highest in the luteal phase of the estrous cycle. Concomitantly, a higher content of free amino acids has been reported in luteal reproductive fluids of cows [23], sows [6,24] and mares [25] compared to estrus.

In contrast, cyclic effects for classical energy substrates such as lactate, pyruvate and glucose were neither evident in pig [6] nor in bovine [26]. To our knowledge, cyclic effects on the FA composition in reproductive fluids have not been investigated so far. Based on the fact that the key enzyme for FA production, FA synthase, is regulated by progesterone [27,28], a cycle effect on FA seems plausible.

The multifaceted role of FA in reproduction has only recently gained increasing attention. FA are a potent source of energy. Their

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catabolism via β-oxidation yields netto amounts of ATP 3-times higher than the oxidation of glucose [29]. Although carbohydrates are considered as the classical nutrients for the early embryo [30–33], latest studies have shown that the oocyte as well as the early embryo can contain considerable amounts of lipids providing a potential endogenous energy reserve [34]. This particularly applies to species with a long preimplantation period such as dog, pig, bovine and horse [35–38] as well as to species exhibiting delayed implantation (diapause) such as many marsupials, phocidae, mustelids, dasypodidae (armadillo) and one representative of the cervidae, the roe deer [39–41]. Interestingly, the endometrial glands of armadillo and roe deer contain lipid droplets that seem to be released at the time of reactivation from diapause [39,42] and might thereby serve as a direct energy substrate for the growing embryo and/or as a precursor for the embryonic FA synthesis.

The ability for the exogenous uptake of FA by the embryo has been demonstrated in multiple studies of various species. The rabbit embryo can absorb FA already at the zygote stage [43]. Similar observations have been made in the mouse embryo, where the rate of β-oxidation is constant from the zygote to the eight-cell stage and then increases to the blastocyst stage [44]. In bovine *in vitro* embryo culture, the addition of the β-oxidation co-factor L-carnitine supports development to the morula stage [45], most likely due to the more effective use of FA as an energy substrate. Likewise, studies in human preimplantation embryos confirmed the stage specific uptake of different FA [46]. Furthermore, the lipid profile of the oocyte and the embryo show a high plasticity dependent on the environment in which they develop [47–49]. This finding suggests that direct integration of external FA in the embryonic phospholipid layer takes place.

Apart from their role as energy substrates and membrane compounds, FA can act as signaling factors by directly altering gene expression or by mediating inflammatory responses [50–52]. By this way, maternally derived FA can influence embryonic development. These influences might not only have an immediate effect but are also thought to induce epigenetic changes relevant for the adaption of the postnatal phenotype to its environment [53].

The equine embryo has an extraordinary long preimplantation period of 40 days and is supported by the maternal secretome for an extended period of time. Up to now, comprehensive data on the nature of the FA composition of the equine secretome is lacking and a possible impact of the ovarian cycle has not been investigated yet.

In our study, we collected the fluid of oviduct and uterus and corresponding plasma samples *post mortem* from slaughtered mares. The analyses focused on the characterization of the FA profile of maternal plasma and fluid of the oviduct and uterus during the early and late luteal phase. The early luteal phase overlaps with the oviduct period of the embryo (day 0 to day 6 after ovulation) and its arrival in the uterus (reviewed by Ref. [54]). The late luteal phase corresponds to the uterine period of the embryo during which pregnancy recognition must take place at the latest.

2. Materials & methods

2.1. Animals

All samples were collected from ten healthy cyclic adult thoroughbred and warmblood mares after slaughtering (including five Franche-Montagnes breed mares that are described as "heavy warmblood") at a commercial abattoir in Switzerland between June and October 2016. The animals were aged between 5 and 22 (13.3 ± 6.3, $n = 10$ mares) years. The reproductive tract was collected immediately after slaughter and transported on crushed ice. The time span from the slaughter of mares to the sample freezing at −80 °C until further analysis was on average 4 h. The blood was collected from the jugular vein at the moment of slaughter into tubes containing EDTA, transported on ice and centrifuged (1500 × g for 10 min). Blood plasma (BP) was frozen at −80 °C until further analysis ($n = 8$ animals, 4 per group).

2.2. Collection of oviduct and uterine fluid and endometrial samples

The uterus was trimmed of excess connective tissue and the cervix was sealed off with a cable binder. The oviducts were clipped at the uterotubal junction and a blunt cannula connected to a syringe was introduced into the uterine horn contralateral to the ovary with the functional corpus luteum (CL). The uterus was flushed with 10 ml of phosphate-buffered saline (PBS) solution which was collected by gravitation from the ipsilateral horn into a petri dish. Attention was taken to strictly avoid blood contamination. The collected UF was centrifuged (800 × g for 10 min) and the supernatant was frozen at −80 °C until further analysis. OF was collected from the ipsilateral oviduct. The oviduct and infundibulum were dissected free from connective tissue. Using a stereo microscope, a rinsing cannula (0.6 × 35 mm, Provet, Switzerland) was introduced into the infundibulum and the oviduct was flushed with 0.8–1.5 ml of PBS and the retrieved volume was recorded. The collected OF was centrifuged (800 × g for 10 min) and the supernatant was frozen at −80 °C until further analysis. UF and OF was collected from 10 animals (5 animals per group). For endometrial tissue collection, the uterine body and the uterine horns were opened longitudinally. With a scissor, endometrial stripes were carefully cut out and immediately transferred into cryo-tubes. Endometrial samples ($n = 5$ mares) were frozen in liquid nitrogen and subsequently stored at −80 °C for later progesterone (P4) analysis.

2.3. Analysis of total protein

The bicinchoninic acid commercial assay (BCA assay) (ThermoFisher Scientific, USA) was used to determine the total protein (TP) content in the OF and UF, according to the provided protocol. It was assumed that the protein concentration of the retrieved fluid was dependent on the inserted volume but not on the recollected volume. Due to the different volumes used for flushing the oviduct, the TP content of the OF was corrected according to the inserted PBS volume.

2.4. Measurement of plasma and endometrial P4

P4 concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) according to Prakash et al. [55]. The lower detection limit for P4 was 0.1 ng/ml. The average intra- and inter-assay coefficients of variation (CV) were <10%. Endometrial P4 was measured after tissue extraction according to Luttgenau et al. [56].

2.5. Evaluation of cycle stage based on ovarian functional bodies and progesterone concentrations

The functional bodies of the ovaries were morphologically evaluated according to their size, texture and color. The ovaries were cut open and the size of the CL was recorded. The "early luteal phase" was defined by a large CL (4 cm, $n = 5$ mares), sometimes with a central lacuna and reddish in color, which was accompanied by smaller follicles (<2.5 cm). The presence of numerous medium sized follicles and a regressing CL, indicated by its relatively small size (2.1 ± 0.1 cm, $n = 5$ mares) and yellowish color, were characteristic for the "late luteal phase".

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