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Influence of synthetic lamprey GnRH-III on gonadotropin release and steroid hormone levels in gilts

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Abstract

Based on the supposition that lamprey GnRH-III (lGnRH-III) elicits FSH releasing activity in swine, synthetic lGnRH-III (peforelin, Maprelin® XP10) was used in puberal estrus synchronized gilts. The secretion of reproductive hormones FSH, LH, estradiol and progesterone was analyzed, and follicle growth and ovulation recorded. Altogether, 24 German Landrace gilts were treated after an 18-day long synchronization of the estrus cycle with Regumate® as follows: 48 h after the last Regumate® feeding they received im either 150 μg Maprelin® XP10 (lGnRH-III, group Maprelin, n = 6), 50 μg Gonavet Veyx® (GnRH-I agonist, group GnRH, n = 6), 850 IE Pregmagon® (eCG, group eCG, n = 6) or saline (group Control, n = 6). Additionally, in eight gilts the concentrations of FSH and LH were analyzed after treatment with 150 μg Maprelin® XP10 (n = 3), 50 μg Gonavet Veyx® (n = 3) or saline (n = 2) at mid-cycle (day 10 of the estrus cycle). Blood samples were collected via implanted jugular vein catheters. Ovarian features were judged endoscopically at the end of the Regumate® feeding and on days 5 and 6 after treatment. Maprelin® XP10 had no effect on FSH release in gilts; neither at the pre-ovulatory period or at mid-cycle. Furthermore, LH levels were unaffected. In contrast, GnRH-I agonist stimulates FSH release, however less compared to LH secretion. LH secretion was induced by GnRH-I both during the follicular phase and at mid-cycle. Equine CG did not stimulate the release of pituitary hormones FSH and LH due to its direct action on the ovary. Increased estradiol concentrations during days 2 to 5 after Regumate® in all treatment groups indicated pre-ovulatory follicle growth in gilts. Equine CG stimulated a higher ($P < 0.01$) number of ovulatory follicles compared to the other treatment groups. All together, 83 to 100 % of gilts ovulated by day 6 post treatment. In summary, results of our study on reproductive hormone secretion do not provide evidence that synthetic lGnRH-III (Maprelin® XP10) selectively releases FSH in estrus synchronized gilts.

Keywords: Follicular growth; FSH; Gilt; GnRH; LH; Ovulation

1. Introduction

Estrus synchronization in sows is an important tool of reproductive management. This kind of biotechnology is valuable to organize production of piglets, especially in large scale farms working in a batch-wise system [1–3]. Though synchronization of estrus is possible with group-wise weaning of sows or in gilts undergoing treatment with altrenogest, the onsets of estrus can spread over a week. This is partly due to insufficient follicular development. Gonadotropins may then be used after weaning or after altrenogest in order to stimulate follicular development and to achieve a better synchronization effect. The favorite gonadotropin preparation is equine chorionic gonadotropin (eCG). This gonadotropin exhibits both LH- and FSH-like activities

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[4,5] with an FSH vs. LH activity ratio that differs between 0.14–0.31 as estimated by bioassays conducted in rats and mice [6,7]. Because eCG is of biological origin, differences between batches are possible [6,8]. This may account for variations in the ovarian response seen in pigs after the use of different eCG batches [9,10]. Synthetic medications with equivalent effect to stimulate follicle growth and estrus with subsequent ovulation could reduce the variable activity of eCG.

Follicular development, including the growth and maturation of the final ovulatory follicle as well as ovulation itself, requires a series of finely-tuned hormonal events. However, it is without doubt that gonadotropin releasing hormone (GnRH) is a key regulator. GnRH stimulates both LH and, to a lesser extent, FSH. The existence of a separate, highly specific FSH-releasing hormone has been proposed [11,12]. Only recently an alternative form of GnRH, lamprey GnRH-III (lGnRH-III) [13], has been found to stimulate selectively the release of FSH in rats [14], in cattle during the luteal phase of the estrus cycle [15] and in barrows [16]. However, several *in vitro* and *in vivo* studies have questioned the selective release of FSH induced by lGnRH-III, including pigs [14,17–21].

Inasmuch in pigs lGnRH-III has only been used in barrows [16,21], we examined the effect of the recently synthesized lGnRH-III (peforelin, pGlu-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2, Maprelin® XP10, Veyx-Pharma, Schwarzenborn, Germany) in mature, estrus synchronized gilts. Here, the concentrations of the reproductive hormones FSH, LH, estradiol (E2) and progesterone (P4), and follicle development and ovulation were evaluated after Maprelin® XP10 compared to eCG and GnRH treatments.

2. Materials and methods

2.1. Animal care and use

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Agricultural Ministerial Department of Mecklenburg-Vorpommern, Germany (approval: LVL-MV/TSD/7221.3-1.1-015/09).

A total of 24 mature German Landrace gilts with a median age of 225 days at the beginning of estrus synchronization and with median weight of 136 kg were used in this study. Estrus was synchronized by an 18-day treatment with Regumate® (20 mg altrenogest/day/gilt, Janssen Cilag GmbH Neuss, Germany). Forty eight hours after the last dose of Regumate® (0800 h) gilts were treated im either with 150 μg Maprelin® XP10 (lGnRH-III, Veyx-Pharma GmbH, Schwarzenborn, Germany; group Maprelin, n = 6), 50 μg Gonavet Veyx® (GnRH-I agonist, Veyx-Pharma GmbH; group GnRH, n = 6), 850 IU Pregmagon® (eCG, IDT Biologika GmbH, Dessau-Roβlau, Germany; group eCG, n = 6) or saline (group Control, n = 6). Dosage and time of treatments were in accordance with the standard protocol. In an additional eight gilts, hormone concentrations of FSH and LH were analyzed after treatment with 150 μg Maprelin® XP10 (n = 3), 50 μg Gonavet Veyx® (n = 3) or saline (n = 2) at mid-cycle (17 d after Regumate®, corresponding to day 10 of estrus cycle).

2.2. Catheterization and blood sampling procedure

Two to three days before the last Regumate® feeding gilts were surgically fitted with an indwelling silicon catheter (ID 1.6 mm, AD 3.2 mm; AMT Düsseldorf, Germany) under ketamin/azaperon anaesthesia (0.15 ml/kg BW Ursotamin, Serumwerk Bernburg, Germany; 0.04 ml/kg Stesnil, Janssen Cilag GmbH Neuss, Germany) into the *Vena jugularis* [22]. Blood samples (each 10 ml) were collected into plastic syringes containing EDTA-potassium as an anticoagulant (KABE, Germany) once daily (0800) from the last day of Regumate® treatment (Day 0) to Day 9. On Day 2 after application of GnRH, eCG and saline blood samples were drawn in 30-min intervals for 6 h (0800 to 1400 h) and thereafter hourly up to 1400 h on Day 3. At mid-cycle, samples were collected after application in 30-min intervals (0800 to 1400 h) and hourly up to 1600 h. The samples were centrifuged immediately for 15 min at 300 × g and the plasma collected and stored at −20 °C until analysis. After each blood collection, the catheters were filled with 3 ml of 3% Na-citrate. Prior to blood collection, the citrate within the catheter together with the first 3 ml of blood was discarded.

2.3. Endoscopic monitoring of the ovaries

Follicle development was monitored endoscopically [23] at the last days of Regumate® treatment together with catheterization procedures (1st monitoring) and on Day 7 (2nd monitoring) and on Day 8 (3rd monitoring). On this occasion anaesthetized gilts were fixed in a dorsal position and a pneumoperitoneum with CO2 was automatically produced (Endo Tech, Munich, Germany). Thereafter, three trocar cannulas (Storz, Tuttlingen, Germany) were inserted into the abdomen for 0° optics (ETB, Berlin, Germany) and grasping forceps (ErgoLAP, Bowa-electronic, Gomaringen, Germany). All laparoscopic handling was observed on a video