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TREATMENT WITH GONADOTROPIN RELEASING-HORMONE IN PREPUBERTAL GILTS AT TWO DIFFERENT AGES

By

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ANDERSSON, ANNE-MARIE, STIG EINARSSON and LARS-ERIC EDQVIST: *Treatment with gonadotrophin releasing-hormone in prepubertal gilts at two different ages.* Acta vet. scand. 1983, *24*, 446—455. — To study the effect of GnRH in prepubertal gilts, seven crossbred gilts were treated with saline solution and 250 μg GnRH. In connection with saline and GnRH treatments blood was sampled every 15 min for 4 h, thereafter every 30 min for 2 h and every 60 min for 3 h, and finally every 3 h for 6 days. The ovaries were inspected by laparoscopy just before and 6 days after GnRH treatment. The first GnRH treatment was undertaken when the gilts had a mean age of 141 days and mean body weight of 66 kg. One gilt was in prooestrus at this treatment. In the other 6 gilts the mean LH level was around 0.5 μg/l during a 4 h period after the saline injection. After the GnRH treatment a LH peak was seen with a mean duration of 4 h and a mean maximum level of 9.2 ± 2.07 μg/l. None of the gilts ovulated or showed oestrus within a week after GnRH treatment, which was confirmed by laparoscopy. The seventh gilt which was in prooestrus had high levels of oestradiol-17β (> 40 pmol/l) at GnRH treatment and no LH peak was seen during a 4 h period after treatment.

Two gilts which had not shown oestrus at an age of 173 days and a mean body weight of 93 kg were treated a second time with 250 μg GnRH. The LH peak had a duration of 4 h and a mean maximum level of 5.3 ± 3.04 μg/l. Neither of these 2 gilts showed oestrus or ovulated within a week after GnRH injection. It was concluded that a single injection of GnRH results in a LH peak but is not enough to stimulate ovulation or oestrus in prepubertal gilts at a mean age either of 141 or 173 days.

GnRH-treatment; prepubertal gilts; LH; oestradiol-17β.

Several studies on the effect of treatment with gonadotropin realasing-hormone (GnRH) on LH release have been performed

in prepubertal gilts. GnRH has been administered as a single injection (*Foxcroft et al.* 1975), as repeated injections (*Chakraborty et al.* 1973: 16 injections of 25 μg LH-RH/FSH-RH with 6 h interval) or as pulsatile infusion of GnRH (*Carpenter & Anderson* 1981: 0.5 μg GnRH in 0.5 ml pulses with 0.1 ml/min at 1.5 h interval).

Foxcroft et al. (1975) and *Carpenter & Anderson* (1981) demonstrated a dose response effect of GnRH on LH release in young gilts. *Vandalem et al.* (1979) treated gilts of different weight classes (30—40, 40—50, 60—75 kg) with the same dose of GnRH (150 μg LH-RH) and found no difference in magnitude of the subsequent LH and FSH response.

Different results have been reported concerning the effect of GnRH treatment on follicular development and ovulation in prepubertal gilts. *Chakraborty et al.* (1973) found no difference in the ovarian follicle diameter between GnRH-treated and control gilts of 11 weeks of age. *Carpenter & Anderson* (1981) observed ovulation in 1 of 4 gilts (age 159 days) treated with pulsatile infusion of GnRH. *Edqvist et al.* (1978) reported that 3 of 4 gilts with delayed puberty (> 9 months old) came in oestrus and ovulated after a single injection of GnRH.

The purpose of the present experiment was to study the LH and ovarian responses following a single injection of GnRH in prepubertal gilts of two different ages (140 and 170 days).

MATERIAL AND METHODS

Seven crossbred gilts (Swedish Landrace × Swedish Yorkshire) were purchased from a breeding herd at ages of 100—102 days and with an average body weight of 32 kg (range 27—37 kg). The gilts were housed in pens, 3 and 4 per pen. Sexually mature boars were kept in adjacent pens during the entire experimental period.

The gilts were weighed once per week. They were fed according to Swedish breeding stock standards (*Eriksson et al.* 1972). The ration was calculated according to the mean body weight of animals in each pen.

Blood sampling and GnRH injection

A permanent catheter was inserted in the cephalic vein using a method described by *Karlbom et al.* (1982). Surgery was performed under general anaesthesia (pentotal-sodium, 5 % i.v.)

and strict sterile conditions. The catheter operation was done when the gilts were 137—142 days of age. On the day after surgery the animals received 20 ml of isotone saline i.v. Blood samples were drawn every 15 min starting 1 h before the saline injection and continuing for 3 h after injection. Thereafter blood samples were taken every 30 min for 2 h, then once per hour for 3 h and finally every 3 h for 15 h. Treatment with 250 μg GnRH (LH-RH, NOVO Industry A/S Copenhagen, Denmark) in 20 ml isotone saline was performed 24 h after the saline injection with the same blood sampling schedule. The sampling at 3 h intervals continued until laparoscopy was performed 6 days after the GnRH treatment. The first GnRH treatment was undertaken when the gilts were 139—144 days of age and the mean body weight was 66 kg (range 59—71 kg). A second treatment with 250 μg GnRH was given 1 month later on those gilts which had not shown oestrus.

All blood samples were collected in heparinized tubes, centrifuged as soon as possible, and the plasma was stored at —18°C until assayed.

Clinical and morphological examination

Before the catheter operation, laparoscopic inspection of the ovaries was performed under general anaesthesia by a method described by *Wildt et al.* (1973). Laparoscopy was also done 6 days after the GnRH treatment.

Checking of oestrus started after the first laparoscopy and was done twice daily during the experimental period except during the frequent blood sampling periods, when oestrus was checked on each sampling occasion.

The gilts were slaughtered during the luteal phase following their first oestrus. The genital organs were removed and examined macroscopically, especially with respect to ovarian morphology. Samples from the uterine mucosa were examined histologically.

Hormone assay

Blood plasma levels of LH were determined by radioimmunoassay. The assay system utilized an antiserum to ovine LH[1] (*Nis-*

[1] Antiserum to ovine LH was kindly donated by Dr G. D. Niswender, Colorado State University, Fort Collins, USA.

wender et al. 1969). Porcine LH (LER-786-3)[2] was used for radioiodination by the chloramine-T method and as standard. The use of these reagents for radioimmunoassay of porcine LH has been described previously (*Niswender et al.* 1970). Separation of free and antibody-bound hormone was done with a second antibody to rabbit gammaglobulin coupled to a solid phase (DASP, Organon, The Netherlands). The analyses of pooled plasma in 25 assays resulted in a mean value of 2.66 μg/l (s.= 0.47 μg/l). All LH values presented represent the mean of duplicate determinations.

Progesterone and oestradiol-17β were determined by radioimmunoassay (*Edqvist & Johansson* 1972) using antisera to 11-α-hydroxy-progesterone (*Bosu et al.* 1976) and to 6-keto-oestradiol-17β (*Boilert et al.* 1973). The analyses of pooled plasma in 16 and 25 assays resulted in mean values of 0.39 nmol/l (s. = 0.12 nmol/l) and 16.6 pmol/l (s. = 3.67 pmol/l) for progesterone and oestradiol-17β, respectively. All values of oestradiol-17β represent the mean of duplicate determinations.

All blood samples collected every third hour were analysed for oestradiol-17β and LH, while only the daily 8 a.m. samples were analysed for progesterone.

RESULTS

One gilt (no. 1) was in prooestrus at the GnRH injection and is therefore presented separately. The means ± s.e.m of LH and oestradiol-17β levels in gilts 2—7 are demonstrated in Fig. 1. The mean level of LH during a 4 h period after the treatment with saline was around 0.5 μg/l, with small fluctuations up to 1.1 μg/l. The GnRH treatment caused a sharp rise in the LH level and peak LH concentrations were found 45—60 min after the injection. The highest mean level was 9.2 ± 2.07 μg/l and the highest individual level 14.6 μg/l. The duration of the LH peak was approximately 4 h. The oestradiol-17β levels were not affected by the GnRH injection and the mean level varied between 13.4—24.8 pmol/l during a 6-day period after the GnRH treatment.

None of the gilts 2—7 showed oestrus or ovulated after the GnRH treatment. At the laparoscopy before the GnRH treatment only small ovarian follicles were found. Six days after the GnRH

[2] The authors are indebted to Dr L. E. Reichert, The Albany Medical College, Albany, New York, USA, for supplying porcine LH for iodination and as standard.

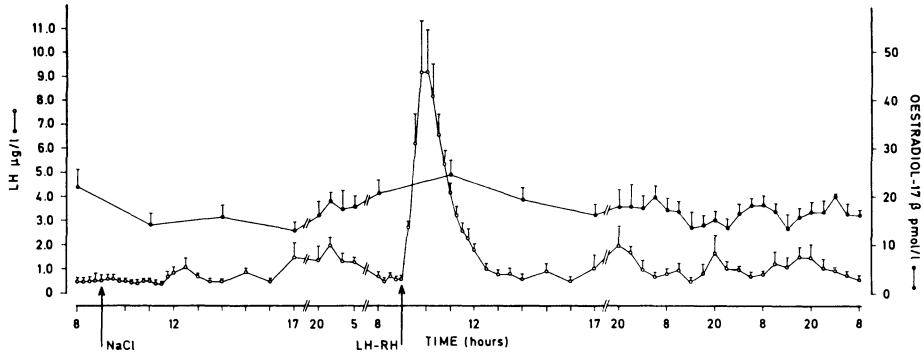

Figure 1. Mean ± s.e.m. of LH and oestradiol-17β after treatments with saline (Na Cl) and GnRH (LH-RH) in 6 prepubertal gilts at a mean age of 141 days.

injection the laparoscopic examination was repeated and then none of the gilts had corpora lutea or large follicles in their ovaries.

The LH and oestradiol-17β levels in gilt no. 1 are presented in Fig. 2. This gilt was in prooestrus at the GnRH injection, the oestradiol-17β levels being elevated (> 40 pmol/l). The LH level after the GnRH treatment varied between 0.8—1.6 µg/l during a 4 h period. The gilt showed standing reflex 8 h after the GnRH treatment and at laparoscopy 6 days later the ovaries contained 10 corpora lutea.

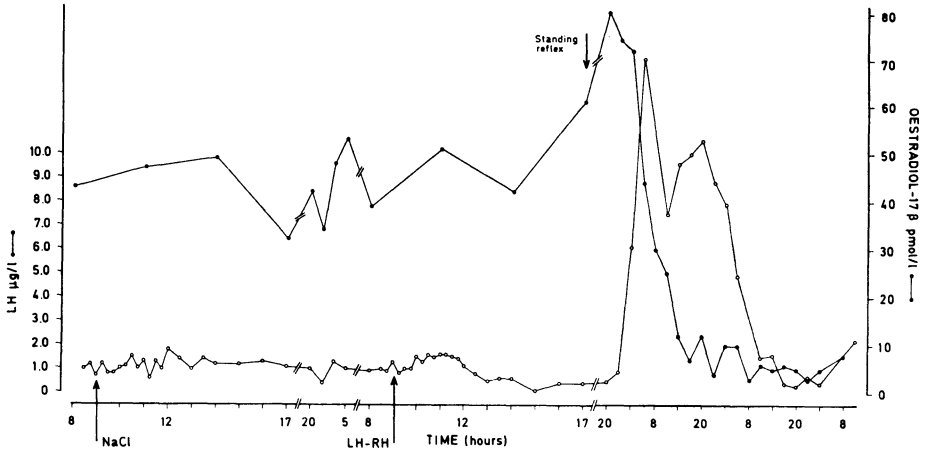

Figure 2. LH and oestradiol-17β in a prooestrual gilt (no. 1) after treatments with saline (NaCl) and GnRH (LH-RH).

Gilts 3, 5 and 6 showed oestrus spontaneously at 170, 164 and 173 days of age, respectively. Gilt no. 2 developed bronchopneumonia and was slaughtered at an age of 160 days without having exhibited oestrus. Gilts 4 and 7, which were still prepubertal, received a second GnRH treatment at an age of 173 days and a mean weight of 93 kg. The results of this treatment are presented in Fig. 3. The highest mean level of LH was 5.3 ± 3.04 μg/l, which was found 60 min after GnRH injection. The mean oestradiol-17β level varied between 9.0—23.0 pmol/l during a 6-day period. The gilts did not show oestrus and at laparoscopy 6 days after the GnRH injection no corpora lutea were observed and the size of the follicles was unchanged. These 2 gilts showed oestrus spontaneously at an age of 191 and 198 days respectively.

The mean age at first oestrus of the gilts was 173 days (range 144—198 days). The progesterone levels in these gilts were low (0—1.5 nmol/l) before their first ovulations.

The post-mortem and histological examination of the genital tract did not reveal any pathological changes. The mean number of corpora lutea recorded post mortem was 12 ± 0.9 (range 9—15).

DISCUSSION

All prepubertal gilts responded to GnRH with increased blood levels of LH. This is in agreement with earlier studies using single injections (*Foxcroft et al.* 1975, *Edqvist et al.* 1978, *Van-*

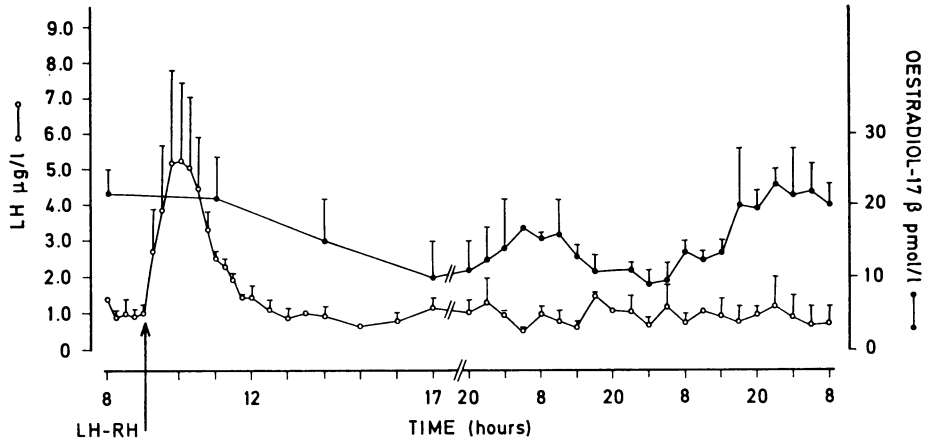

Figure 3. Mean ± s.e.m. of LH and oestradiol-17β levels after GnRH (LH-RH) treatment in 2 gilts at an age of 173 days.

dalem et al. 1979), chronic treatment (*Chakraborty et al.* 1973) or pulsatile infusion (*Carpenter & Anderson* 1981) of GnRH. When comparing the first GnRH treatment in the 6 gilts with the second treatment in the 2 gilts, the mean duration of the LH peak was the same (Figs. 1 and 3). The mean LH response, however, was somewhat lower after the second than after the first GnRH treatment. The reason for this might be the lower dose of GnRH per kg body weight at the second treatment. With a dose of 150 μg GnRH per animal *Vandalem et al.* (1979) found no significant difference in LH response between different weight classes of gilts. On the other hand, *Foxcroft et al.* (1975) and *Carpenter & Anderson* (1981) described a dose-response relationship.

The low blood levels of oestradiol-17β during a 6-day period after GnRH treatment are indicative of absence of significant follicular activity (*Karlbom et al.* 1982, *Andersson et al.* 1983).

The seventh gilt (no. 1), which was in prooestrus at the GnRH injection, did not respond with any LH peak (Fig. 2) after treatment, probably due to high levels of oestradiol-17β. During the follicular phase the plasma LH is low, concomitantly with rising levels of oestradiol-17β (*Karlbom et al.* 1982, *Van de Wiel et al.* 1981, *Andersson et al.* 1984). *Van de Wiel et al.* (1979) showed that GnRH injection during the follicular phase with high levels of oestradiol gave a minimum LH response. *Foxcroft et al.* (1975) and *Pomerantz et al.* (1975) demonstrated that chronic oestradiol treatment depresses the pituitary response to exogenous GnRH in female pigs.

None of the prepubertal gilts showed oestrus and/or ovulated within a week after the GnRH treatments. The laparoscopic inspection of the ovaries revealed no follicular development. The gilts showed oestrus spontaneously 18—34 days after GnRH injection. *Chakraborty et al.* (1973) were unable to intiate follicular development after chronic GnRH treatment (16 inj., 25 μg LH-RH/FSH-RH, 6 h intervals) in young gilts (11 weeks). *Carpenter & Anderson* (1981) only initiated ovulation in 1 of 4 150-day-old gilts by pulsatile infusion of 5 μg GnRH. *Edqvist et al.* (1978), on the other hand, induced oestrus and ovulation in 3 of 4 gilts with delayed puberty (> 9 months) 4—6 days after 1 injection of 1.0 mg GnRH per animal. The explanation of the different results of GnRH treatment in prepubertal gilts as compared to gilts with delayed puberty is not clear. One reason for the ovulation occurring in gilts with delayed puberty might be that

the ovarian follicles had reached a certain stage of maturation and therefore were able to respond to GnRH stimulation. As the cause of delayed puberty in gilts is not known, it would be of great interest to study the hormonal pattern and ovarian activity before and after GnRH treatment in these animals.

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SAMMANFATTNING

Behandling av prepuberala gyltor i två olika åldrar med Gonadotropin releasing-hormone (GnRH).

Sju prepuberala gyltor av korsningsras (lantras × yorkshire) behandlades först med isoton Na-Cl lösning och 24 tim. senare med 250 µg GnRH. Blodprov för hormonanalys uttogs var 15:e min under 1 timme före respektive behandling, därefter var 15:e min under 3 timmar, var 30:e min under 2 tim; var 60 min under 3 timmar och var 3:e timme under 15 timmar. Efter GnRH behandlingen fortsatte blodprovstagningen var 3:e timme under ytterligare 5 dagar.

Äggstockarna inspekterades med laparoskopi strax före och 6 dagar efter GnRH behandlingen. Den första GnRH behandlingen gjordes när gyltorna hade en medelålder av 141 dagar och en medelvikt

av 66 kg. En gylta var då i förbrunst. Hos de andra 6 gyltorna var medelnivån av LH ca 0.5 μg/l under en 4 timmars period efter koksaltinjektionen. Efter GnRH behandlingen steg LH nivån i blodet kraftigt till ett maximalt medelvärde av 9.2 ± 2.07 μg/l och en duration av 4 timmar. Ingen av gyltorna visade brunst eller ovulerade, vilket verifierades vid laparoskopiundersökningen. Den sjunde gyltan, som var i förbrunst, hade en hög nivå av östradiol-17β (>40 pmol/l) vid GnRH injektionen. GnRH behandlingen resulterade här inte i någon ökad frisättning av LH.

Två gyltor, som inte hade visat brunst vid en ålder av 173 dagar och en medelvikt av 93 kg, behandlades en andra gång med 250 μg GnRH. Den kraftiga LH frisättningen, som sågs därefter, pågick under 4 timmar och maximala medelvärdet var 5.3 ± 3.04 μg/l. Gyltorna visade inte brunst och ovulerade inte inom 1 vecka efter GnRH behandlingen.

Resultaten tyder på att en enda injektion med GnRH ger en kraftig frisättning av LH, men är ej tillräcklig för att framkalla brunst och ovulation hos icke könsmogna gyltor.

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