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Clinical, Morphological and Endocrinological Studies in Gilts with Delayed Puberty

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Dalin, A.-M. and L. Eliasson: Clinical, morphological and endocrinological studies in gilts with delayed puberty. Acta vet. scand. 1987, 28, 263–269. – Thirty-six gilts which had not shown oestrus at about 8 months of age or more were transported from the pig research station to the clinic, a journey of 12 km. The gilts were examined by laparoscopy and those which had only small follicles in the ovaries were catheterized and placed in pens, with sexually mature boars kept in adjacent pens. Oestrus detection was done twice daily and blood was sampled three times a day. After 7 days the laparoscopy was repeated and gilts which still had only small follicles in their ovaries were given 250 μg GnRH intravenously the following day. Blood samples were taken frequently before and after GnRH treatment. One week after administration of GnRH the ovaries were inspected by laparoscopy once more.

The first laparoscopic examination showed that 42 % of the gilts were sexually mature. One gilt had no uterus or ovaries. Twenty gilts had only small follicles in the ovaries and fourteen of these gilts showed ovulatory oestrus 5.5 days (4–7.5 days) after arrival. In these fourteen gilts a rise in the oestradiol-17ß level (>30 pmol/l) was seen at an average time of 1.9 days and a rise in LH (preovulatory peak) was seen at an averaged 4.5 days after the start of blood sampling. Six gilts were given 250 μg GnRH. An immediate rise in LH could be seen in all the gilts (mean peak level was 6.18 μg/l) and the elevated levels had a duration of 4 hours. None of the GnRH-treated gilts responded with oestrus symptoms or increased ovarian activity.

transport; oestradiol-17ß; LH; GnRH-treatment.

Introduction

In Sweden gilts normally have their first oestrus at 6–7 months of age (*Andersson et al.* 1982). If no oestrus has been observed before 8 months of age, the gilts are considered to have delayed oestrus.

Factors known to stimulate the natural attainment of puberty in gilts are, for instance, boar contact, mixing, transportation and relocation.

Anoestrus is the most common reproductive disorder in gilts. *Ehnvall et al.* (1981) showed that among culled gilts older than 9 months, 34 % of the culling cases were due to anoestrus.

Post-mortem examination of the genital organs of gilts slaughtered because of anoestrus revealed luteal tissue in the ovaries in approximately two thirds of the animals (*Einarsson et al.* 1974). The gilts had ovulated,

but the external oestrus symptoms might have been weak or lacking. Inadequate oestrus detection is another possible explanation.

The aims of the present investigation were to study:

- the ovarian status in gilts which had not shown oestrus;
- the effect of transportation and relocation on the ovarian and endocrinal status in gilts with delayed puberty;
- the LH and ovarian response to GnRH in gilts with delayed puberty.

Materials and methods

The present investigation comprised 36 anoestrous gilts of Swedish Yorkshire breed from the pig research station at the Department of Animal Breeding and Genetics (Table 1). A total of 227 Swedish Yorkshire gilts were reared at the pig research station between 1983 and 1985. The gilts were restrictedly fed. Oestrus detection was done twice daily from 160 days of age. Blood samples for progesterone determination were taken every 10 days from 170 days of age. From this age the gilts were also exposed to a boar. The boar was kept in an adjacent pen and once a day moved to the giltpens. Reproductive data from this herd are presented separately.

The gilts which had not shown oestrus at 8 months or more were taken to the Department of Obstetrics and Gynaecology, a journey of 12 km. Within 20 h after arrival to the clinic, the gilts were examined by laparoscopy (*Wildt et al.* 1973). Gilts with corpora lutea or mature follicles (about 8–10 mm in diameter) were sent back to the research station, while the gilts with only small follicles in their ovaries were catheterized. The permanent catheter was placed in the jugular vein (*Karlbom et al.* 1982, *Rodriques & Kunavongkrit* 1983). Both laparoscopy and catheterization were performed under general anaesthesia (pentol-sodium, 5 %). The gilts were placed in individual pens with sexually mature boars kept in adjacent pens. Oestrus detection was done twice daily and blood samples were taken three times daily (9 a. m., 12 noon and 3 p. m.). Laparoscopy was performed again on day 7. Gilts which still had only small follicles in their ovaries were given 250 μg GnRH (LH-RH, NOVO Industry, A/S Copenhagen, Denmark) intravenously the following day. Blood samples were taken every 15 min, starting 1 h before the injection and continuing for 3 h after. Blood samples were then taken every 30 min for 2 h and then every hour for 3 h. During the following 6 days, blood was sampled 5 times a day (7

Table 1. Description of animals used in the study.

Group	Month of arrival at the clinic	No. of gilts	Mean age at first laparoscopy (days)	No. of gilts sexually mature at laparoscopy	No. of gilts in oestrus within 8 days	No. of gilts GnRH-treated	No. of gilts with malformation
A	November 1983	11	243.3	9	1	–	1
B	April 1984	7	252.6	3	2	2	
C	September 1984	9	283.6	1	6	2	
D	December 1984	3	245.0	2	1	–	
E	May 1985	6	255.2	0	4	2	
Total		36	257.3	15	14	6	1

a. m. until 7 p. m.) and oestrous detection was done twice daily.

One week after the GnRH treatment the ovaries were inspected by laparoscopy.

All blood samples were collected in heparinized tubes, centrifuged as soon as possible and after sampling the plasma was then stored at –18°C until assay.

Hormone assay

Blood plasma levels of LH were determined by radioimmunoassay. The assay system utilized an antiserum to ovine LH (*Niswender et al.* 1969). Porcine LH (LER-786-3) was used for radioiodination by the chloramine-T method and as standard. The use of these reagents for radioimmunoassay of porcine LH has previously been described (*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 19 assays resulted in a mean value of 1.05 μg/l (SD = 0.29 μg/l). All values presented represent the mean of duplicate determinations. Progesterone and oestradiol-17ß were determined by radioimmunoassay (*Edqvist & Johansson* 1972) using antisera to a 11-α-hydroxyprogesterone (*Bosu et al.* 1976) and 6-keto-oestradiol-17ß (*Boilert et al.* 1973). The analyses of pooled plasma in 22 assays resulted in a mean value of 1.14 nmol/l (SD = 0.36 nmol/l) and 29 pmol/l (SD = 5.4 pmol/l) for progesterone and oestradiol-17ß, respectively. All oestradiol-17ß values represent the mean of duplicate determinations. Based on the analyses of plasma samples from an ovariectomized sow, we have previously defined the practical detection limit of this assay to be 24 pmol/l (*Andersson et al.* 1983a).

Results

The number of animals in the different groups, the date of arrival at the clinic and the mean age at first laparoscopy are presented in Table 1. The mean age of the 36 gilts at first laparoscopy was 257.3 days (233–289 days). The first laparoscopic examination showed 15 gilts to be sexually mature. These gilts had a mean age of 250.9 days (233–286 days). Twelve of these gilts had corpora lutea in the ovaries, the mean number of corpora lutea being 10.3 (8–13). Three gilts had mature follicles in the ovaries (diameter about 10 mm) and these gilts were in oestrus. One of the gilts lacked both ovaries and uterus, as was detected by laparoscopy. This observation of a malformation was confirmed at post-mortem examination. The vagina was small and blind at the cranial part.

Twenty gilts had only small follicles in the ovaries (<6 mm in diameter). Fourteen of the gilts showed oestrus 5.5 days (4–7.5 days) after arrival, at a mean age of 271.0 days (244–295 days). Eleven of these gilts had a normal standing reflex and 2 had a weak standing reflex, while 1 did not stand for a boar despite reddening and swelling of the vulva.

In the 14 gilts showing oestrus within 8 days a rise in the oestradiol-17ß level (>30 pmol/l) was observed at an average time of 1.9 days (0–5 days) and a rise in LH (preovulatory peak) was seen at an average of 4.5 days (3–7 days) after the start of blood sampling. The mean progesterone level was low (<0.6 nmol/l) before the first oestrus. The average duration of elevated oestradiol-17ß levels and LH levels was 4 and 1.5 days, respectively (Fig. 1).

The second laparoscopic examination performed 7 days after the first one confirmed the clinical observation of oestrus, either by newly formed corpora lutea or by mature

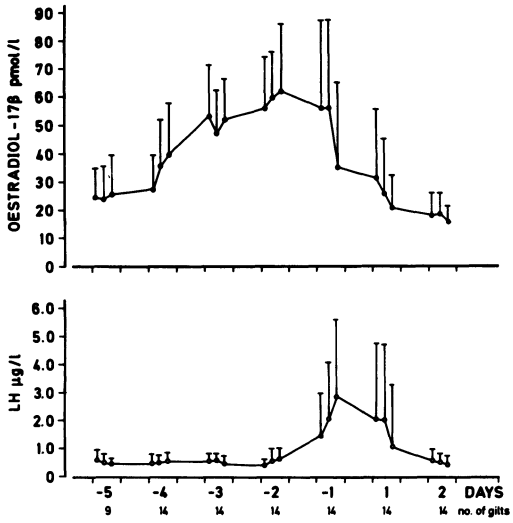

Figure 1. Plasma levels (mean ± SD) of oestradiol-17ß and LH in 14 gilts showing oestrus after arrival at the clinic. Day 1 is the first day of oestrus (standing reflex).

follicles. No change in follicular size could be observed at the second laparoscopy in six of the animals. In these gilts, 250 µg GnRH was injected on day 8 after the first laparoscopy. One gilt had to be slaughtered due to leg weakness 4 days after the GnRH injection. Post-mortem examination revealed only small ovarian follicles (≤6 mm in diameter). The other 5 gilts were examined by laparoscopy 7 days after GnRH treatment and no difference in follicular size could be discovered in these animals. No clinical signs of oestrus were observed in any of the gilts after the GnRH treatment. An immediate sharp rise of LH after the GnRH injection could be seen in all the gilts. The mean level of LH just before treatment was 0.50 ± 0.44 µg/l and the mean peak level was 6.18 ± 2.56 µg/l, observed in all animals 60 minutes after the GnRH injection. The elevated LH levels lasted for 4 h (Fig. 2). The mean oestradiol-17ß-level (<24 pmol/l, Fig. 2) as well as the mean progesterone level (<0.60 nmol/l) per day were low in all animals during the whole blood sampling period.

Discussion

The mean ages of the gilts in the 5 different groups (A, B, C, D and E) at arrival at the clinic and at the first laparoscopic examination were 243.3, 252.6, 283.6, 245.0 and 255.2 days respectively. The gilts arriving in September (group C) were the oldest, but only 1 of the 9 gilts was sexually mature at

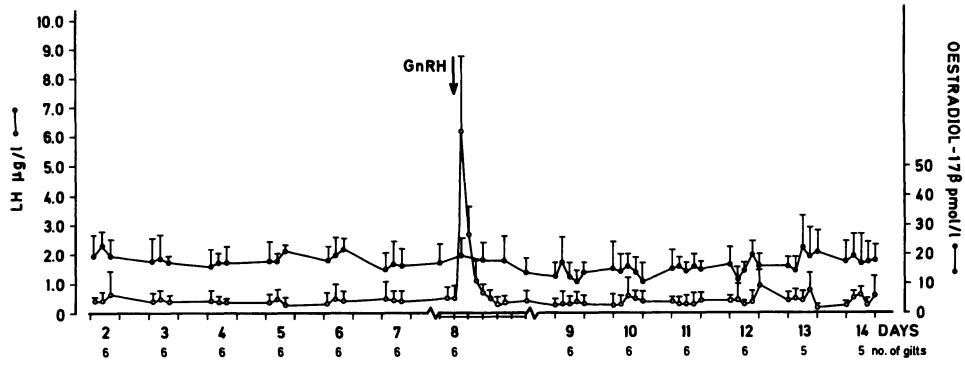

Figure 2. Plasma levels (mean ± SD) of oestradiol-17ß and LH before and after treatment with 250 µg GnRH in 6 gilts with delayed puberty.

arrival. For this reason, a seasonal effect causing the delayed puberty in this group can not be excluded (cf. *Ehnvall et al.* 1981). Twelve gilts (33.3 %) had passed their first oestrus at the research station without this being recorded. The ovulations were confirmed by the laparoscopic examination of the ovaries. The external oestrus symptoms must have been weak in these gilts. Three gilts (8.3 %) had large follicles in the ovaries at arrival at the clinic and showed standing reflex when tested with a boar. The external signs of oestrus in the form of reddening and swelling of the vulva were however weak.

The uterus and ovaries were found to be missing in 1 of the gilts. No such malformation had been found in an earlier study of the genital organs from 1 000 gilts (*Einarsson & Gustafsson* 1970).

Twenty of the gilts (55.6 %) were not sexually mature at the arrival at the clinic. Fourteen of these gilts (70 %) showed oestrus within 7.5 days. The effect of transportation and/or a change in the environment on ovarian activity has been reported earlier by e.g. *Paredis* (1961) and *du Mesnil du Boisson & Signoret* (1962). *Paredis* (1961) showed that 62 % of gilts aged 7–12 months showed oestrus within 7 days after transportation and a change in the environment. The gilts showing ovulatory oestrus had ovarian follicles ≤ 5 mm in diameter at arrival at the new place. *Du Mesnil du Boisson & Signoret* (1962) on the other hand suggested that gilts must have follicles measuring 5–8 mm in diameter to be stimulated to oestrus by transportation. In the present study no marked difference in the ovaries between gilts showing and gilts not showing oestrus within one week was seen at the arrival at the clinic.

Elevated levels of oestradiol-17ß (>30 pmol/l) were found soon after arrival at the new place in those gilts which showed oestrus during the first week. The effect of transportation/change in environment on ovarian activity must have been almost immediate. The duration of elevated oestradiol-17ß levels was approximately the same as in gilts showing their first oestrus at normal age (*Andersson et al.* 1983a).

Six gilts were given 250 µg GnRH intravenously and a rise in LH was observed immediately after the GnRH injection in all the gilts. This indicates that gilts with delayed puberty do not have an LH deficiency in their hypophyses. Five of the gilts did not exhibit oestrus within one week and the sixth, which was slaughtered 4 days after injection due to leg weakness, had immature follicles (≤ 6 mm in diameter) in the ovaries. Identical results were obtained in an earlier study (*Andersson et al.* 1983b) when prebuberal gilts (at 141 and 173 days of age) were treated with one single 250 µg injection of GnRH. *Carpenter & Anderson* (1985) studied the effects of pulsatile infusion of LHRH in prepuberal gilts during a 12-day period. Only 1 gilt (age 151 days) out of 4 ovulated but this gilt did not return to oestrus. *Lutz et al.* (1985) reported that hourly intravenous administration of GnRH (1 µg) for 7–8 days in prepuberal gilts (age 164 days) induced oestrus within 6 days. However, only 1 of the 3 gilts returned to oestrus and ovulated 3 weeks later. The results of these studies indicate that final sexual maturation occurs at the hypothalmic level. *Edqvist et al.* (1978) reported that 3 out of 4 gilts (mean age 10.8 months) with delayed puberty showed ovulatory oestrus within 7 days after one 1 000 µg injection of GnRH. None of these gilts had been exposed to transportation/change in environment before the GnRH treatment. The stress experienced by the gilts (restrained) in connection with frequent blood sampling from an ear vein may have induced the sexual maturity.

Conclusions

The present results indicate that

- what has previously been interpreted as anoestrus may in fact be weak or missing external oestrus symptoms;
- transportation/change in environment may induce ovulatory oestrus in gilts with delayed puberty;
- gilts not coming into oestrus after environmental stimulation respond to GnRH treatment with a LH surge without subsequent oestrus.

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Sammanfattning

Kliniska, morfologiska och hormonella studier av gyltor med försenad könsmognad.

Trettiosex gyltor som inte hade visat brunst vid ca 8 månaders ålder transporterades 12 km från en försöksstation till kliniken. Gyltorna undersöktes med laparoskopi. På gyltor som bara hade små folliklar i äggstockarne opererades en kateter in för blodprovstagning och därefter placerades gyltorna i boxar med könsmogna galtar i närheten. Brunstkontroll utfördes 2 gånger och blodprov togs 3 gånger per dag. Efter 7 dagar upprepades laparoskopi-undersökningen och gyltor som fortfarande bara hade små folliklar i äggstockarna behandlades med 250 µg GnRH i.v. följande dag. Frekvent blodprovstagning utfördes i anslutning till GnRH-behandlingen och 5 ggr per dag därefter. En vecka efter GnRH-behandlingen undersöktes gyltorna på nytt med laparoskopi. Den första laparoskopi-undersökningen visade att 42 % av gyltorna var könsmogna. En gylta saknade livmoder och äggstockar. Tjugo gyltor hade små folliklar i äggstockarna och av dessa visade 14 brunst 5,5 dagar (4–7,5 dagar) efter ankomsten till kliniken. Hos dessa 14 gyltor sågs en östradiol-17ß stegring (>30 pmol/l) efter i medeltal 1.9 dagar och en stegring av LH (preovulatorisk stegring) efter i medeltal 4.5 dagar från blodprovstagningens början. Hos de 6 gyltorna som behandlades med GnRH sågs omedelbart en LH-stegring. Medelvärdet för maximumnivåen var 6.18 µg/l och LH-stegringen hade en duration på 4 timmar. Ingen av gyltorna svarade med brunst eller ökad äggstocksaktivitet efter GnRH-behandlingen.

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