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Original article

Effects of gonadotrophin deprivation on follicular growth in gilts

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Summary — The endocrine and ovarian effects of hypophysectomy ($n = 5$) and gonadotrophin-releasing hormone (GnRH) antagonist administration (Antarelix) ($n = 5$) were studied in gilts by comparison with control animals ($n = 6$). All gilts were given Regumate (20 mg/d for 18 d). The last day of Regumate was day 0. Hypophysectomy and initiation of Antarelix administration (0.6 mg iv twice daily for 7 d) were performed on day 5. All ovaries were obtained at slaughter on day 12. Blood samples were obtained daily from all Antarelix-treated and control sows to measure luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations. Frequent sampling was performed on day 10 on control and Antarelix-treated gilts to assess pulsatile LH secretion. Oestrus or LH surge was initiated before the beginning of treatment in 2 hypophysectomized, 4 Antarelix-treated, and 4 control females. Gonadotropins were undetectable in the blood of hypophysectomized sows on day 6. On day 10, pulsatile LH release was blocked in Antarelix-treated gilts. At no time were FSH concentrations significantly affected. Histological observation of the ovaries demonstrated that: (i) similar populations of healthy or total (healthy + atretic) follicles < 1 mm were found in the 3 groups of females; (ii) healthy follicles 1 to 2 mm in diameter were present in Antarelix-treated but not in hypophysectomized gilts; and (iii) healthy follicles > 2 mm were absent in Antarelix-treated and hypophysectomized gilts. The present study suggests the existence of 3 subgroups amongst antral follicles (gonadotropin independent: 0.19 to 1.1 mm; FSH dependent: 1.1 to 2 mm; LH pulses dependent: > 2 mm in diameter).

gilt / gonadotrophin / hypophysectomy / GnRH antagonist / follicular growth

Résumé — Effets de la suppression des hormones gonadotropes endogènes sur les populations folliculaires de truie. *Les populations folliculaires de truie ont été comparées après hypophysectomie (n = 5), administration d'un antagoniste de GnRH (n = 5) par rapport à des truies contrôles (n = 6). De plus, les effets de l'administration de l'antagoniste de GnRH (Antarelix) sur l'hypophyse ont été caractérisés. Les 2 traitements (hypophysectomie et Antarelix) ont débuté à J5 (J0 = fin du traitement de synchronisation au Régumate). L'Antarelix a été administré biquotidiennement (0,6 mg IV par injection) de J5 à J11. Tous les animaux ont été abattus à J12. Les effets endocriniens de l'Antarelix ont été caractérisés par rapport aux truies contrôles par des prélèvements quotidiens (mesures de LH et FSH) et des prélèvements sériés à J10 (mesure de la pulsatilité de LH). L'administration d'Antarelix bloque totalement la sécrétion pulsatile de LH sans affecter les niveaux de FSH de façon significative. L'œstrus et/ou*

une montée de LH étaient détectables à J5 chez 2, 4 et 4 truies des lots «hypophysectomie» «Antarelix» et «Témoin». À J12, des corps jaunes étaient présents chez 2/5, 4/5 et 6/6 truies des lots «hypophysectomie», «Antarelix» et «témoin». Dans tous les cas, ils paraissaient fonctionnels. L'étude des ovaires montre que i) les effectifs de follicules < 1 mm sont identiques dans les 3 lots ; ii) les follicules sains de taille comprise entre 1 et 2 mm ne se rencontrent que dans les ovaires des truies «Antarelix» et «témoin» ; iii) des follicules sains > 2 mm sont totalement absents des ovaires de truies hypophysectomisées ou traitées à l'Antarelix. Ces résultats suggèrent l'existence de 3 types de follicules chez la truie : i) indépendants de l'hypophyse (et donc des gonadotropines) : taille comprise entre 0,15 et 1,1 mm ; ii) FSH dépendants : taille comprise entre 1,1 et 2 mm ; et iii) dépendants des pulses de LH : taille supérieure à 2 mm.

truie / hormones gonadotropes / antagoniste de GnRH / ovaire / follicule

INTRODUCTION

The main events of terminal follicular growth and maturation have been described in pigs (Britt *et al,* 1985; Foxcroft and Hunter, 1985; Grant *et al,* 1989; Guthrie *et al,* 1993). Furthermore, the changes in gonadotrophin secretion occurring during the follicular phase have also been documented (Van de Viel *et al,* 1981; Foxcroft *et al,* 1987; Prunier *et al,* 1987; Guthrie and Bolt, 1990; Flowers *et al,* 1991). Study of coincidental changes in follicular development and hormones levels have demonstrated that (1) an increase in follicle-stimulating hormone (FSH) secretion may not be a prerequisite for induction of follicular recruitment (Foxcroft and Van de Viel, 1982; Shaw and Foxcroft, 1985) and (2) increased basal luteinizing hormone (LH) levels and LH pulse frequency may be the stimuli for follicular recruitment towards ovulation.

However, it should be stressed that there are large between-sow variations in efficiency of these LH changes to trigger follicular recruitment (Foxcroft *et al,* 1987). Because there are also large between- and within-sow variations in features of the recruited follicles (Foxcroft and Hunter, 1985), these approaches have partly failed to identify at which size/stage of maturation follicles require FSH or LH.

Approaches whereby exogenous pure gonadotrophins are provided at times or in models where follicular recruitment is missing or incomplete have proved useful to address this question. By injecting FSH alone to prepubertal gilts, Guthrie *et al* (1990) were unable to grow large ovulatory follicles. Furthermore, stalk sectioned gilts, whose LH pulsatility is abolished and FSH concentrations are maintained, develop ovulatory follicles in response to PMSG only when they are given pulsatile gonadotrophin-releasing hormone (GnRH) to restore LH pulsatility (Kraeling *et al,* 1986, 1990). However, because ovarian status pretreatment was not closely characterized, the above studies do not conclusively identify which follicles are dependent on a specific gonadotrophin (FSH or LH). Earlier reports on the effects of hypophysectomy (Anderson *et al,* 1967) have also failed to provide detailed information on its effects on follicle numbers in specific size classes and their atresia.

Therefore, the aim of the present study was to determine the influence of gonadotrophin deprivation on antral follicular populations. Two models were used, namely hypophysectomy and GnRH-antagonist administration. To our knowledge, with the exception of a study using GnRH antagonist in neonatal pigs (Ziecik *et al,* 1989), data on the effects of GnRH antagonist administration on gonadotrophin secretion and follicular growth in mature gilts are not available.

MATERIALS AND METHODS

Animals and experimental design

Sixteen Large White gilts, with a mean body weight of 181 ± 2.6 kg, were available for this trial. All of them had demonstrated at least 3 consecutive oestrous cycles before being allocated to the experiment. All gilts had their cycles synchronized by daily feeding of 20 mg/d of Regumate (Roussel-Uclaf, Romainville, France) for 18 d. Six days before the end of Regumate administration, sows were allocated to 3 groups: hypophysectomy (n = 5), GnRH antagonist (n = 5), and control (n = 6). The GnRH antagonist had the following structure (Deghenghi *et al*, 1993): Ac-D-Nal, D-cpa, D Pal, Ser, Tyr, D-Hci, Leu, Lys-(*i*Pr), Pro, D-Ala-NH_2. On the following day, gilts from the GnRH antagonist and control groups were fitted with an indwelling Silastic R (Dow Corning, Midland, MI) catheter that was inserted into 1 jugular vein under general anaesthesia as described by Camous *et al* (1985). While anaesthesia may influence gonadotrophin secretion and hence follicular growth, it should be stressed that it was conducted at a time when gonadotrophin secretion was already low (during Regumate administration). Thus, a possible effect of anaesthesia may be masked by Regumate administration. Moreover, in control gilts, the time between the end of Regumate and the LH peak appears to be similar (4–7 d) to that of untreated gilts, showing no carry-over effect of anaesthesia. Antibiotics (9 ml Ampicilin daily, Schering Plough, France) were then given for 3 d. Oestrus was detected in all gilts twice daily as soon as day 4 (day 0 = last day of Regumate feeding). All treatments (hypophysectomy, Antarelix or saline injections) were initiated on day 5. This timing was selected to leave preovulatory follicular growth progress entirely before starting gonadotrophin deprivation. Oestrus and ovulation usually occur, 5 to 8 d after the end of Regumate administration (Kraeling *et al*, 1981; Van de Viel *et al*, 1981).

Hypophysectomy was conducted using the technique described by Du Mesnil du Buisson *et al* (1964). Anaesthesia was induced by barbiturates (Nesdonal, Rhône-Mérieux, France) and maintained by fluothane in oxygen. At the end of surgery, hypophysectomized gilts were given diurizone (Vetoquinol, France) to suppress the side effects of hypophysectomy on water metabolism. Durizone administration (5 ml daily) was continued until slaughter.

From days 5 to 11, GnRH antagonist (Antarelix, Europeptides, Argenteuil, France) was injected iv (0.6 mg per injection) twice daily at 0900 and 2100. The dosage used and the frequency of injections were based on a pretrial (see below). Blood samples were collected on these gilts once daily at 0900 from days 1 to 4 and afterward (day 5) twice daily immediately before Antarelix injection. Additionally, on day 10, treated gilts were bled at 20 min intervals for 6 h (0900 to 1600) to assess LH pulsatility. Control gilts received saline instead of Antarelix and followed the same bleeding schedule. Blood was collected by venipuncture from hypophysectomized females only on days 6 and 12 to minimize the stress on them.

On day 12, all gilts were slaughtered. The completeness of hypophysectomy was checked by examination of the skull under a dissecting microscope. The number of corpora lutea (if any) was counted and ovaries were processed for histological examination (see below). Plasma was stored at –20°C until analysis.

Histological techniques

Within 5 min of death, both ovaries were fixed in Bouin Hollande's solution. One ovary from each sow was embedded in paraffin wax, and sectioned at a 10 μm thickness. One section out of 10 was mounted and stained with haematoxylin. The population of antral follicles was counted using the oocyte as a marker to avoid counting follicles twice and checked for atresia defined by the presence of at least 5 pycnotic bodies within or along the granulosa layer of the section studied. Later stages of atresia were defined according to Driancourt *et al* (1987). Terminal stages of atresia where antrum is invaded by fibroblasts were not taken into consideration. Based on their diameter, non-atretic and atretic follicles were grouped in the following 6 size classes according to Dufour *et al* (1985): 1) 0.19–0.36 mm; 2) 0.37–0.62 mm; 3) 0.63–1.12 mm; 4) 1.13–2 mm; 5) 2–3.56 mm; and 6) more than 3.56 mm. It should, however, be recalled that histological processing induces tissue shrinkage (Driancourt and Cahill, 1984) which almost halves the *in vivo* sizes of follicles.

Pretrial to determine dose and frequency of GnRH antagonist injection

The GnRH antagonist Antarelix has been shown to be highly effective in rats (Deghenghi *et al,* 1993) and rams (Caraty, personal communication). However, an efficient regime of Antarelix had to be determined for pigs. This was achieved using 8 Large White and Pietrain gilts that had been ovariectomized 10 d before treatment, to raise endogenous gonadotropin secretion. Bilateral ovariectomy and insertion of a jugular catheter were performed simultaneously under general anaesthesia as previously described (see above). Four sows received 0.5 mg of Antarelix and the 4 others 1.5 mg. Blood samples were taken every 6 h during the 24 h preceding injection of Antarelix as well as 1, 2, 3, 4, 6, 12, 18, 24, 30, 36, 40 and 46 h after injection.

Hormone assays

Concentrations of plasma progesterone, LH and FSH were measured with validated RIA methods (Thibier and Saumande, 1975; Camous *et al,* 1985). All samples were analyzed in duplicate. Samples of the pretrial were assayed separately only for LH. Other samples were analyzed for the 3 hormones within single assays. For progesterone, sensitivity was 0.5 ng/ml and intraassay CV was 9% at 5 ng/ml. For LH, sensitivity was 0.5 ng/ml and intraassay CV was 8.8% at 9 ng/ml. For FSH, sensitivity was 1.5 ng/ml and intraassay CV was 9% at 3.5 ng/ml.

Statistical analyses

Data were analyzed using SAS procedures (1989). In the pretrial, differences between doses of Antarelix were assessed by analysis of variance using the GLM procedures (SAS, 1989). A split-plot model was used with dose, animal nested within dose, time and time x dose as sources of variation. The effect of dose was tested using animal within dose as the error term. Concentrations of LH before and after Antarelix injection were compared to LH concentrations measured immediately before Antarelix treatment with the Dunnett's *T*-test (= time 0). In the following experiment, concentrations of LH, FSH and progesterone measured at 0900 and 2100 from day 5 evening to day 11 were compared between control and Antarelix-treated females using a similar split-plot model of analysis of variance. On day 10, profiles of LH were analyzed as previously described (Prunier *et al,* 1993). Number of LH pulses, mean basal LH concentration and mean FSH concentration were compared between Antarelix and control gilts using *t*-tests. Total follicular population and follicular populations within each specific size classes were analyzed by 1-way ANOVA on raw (number) on arcsin $\sqrt{}$ transformed (percentage of atresia) data. All data are expressed as means ± sem.

RESULTS

Assessment of the dose/frequency of GnRH antagonist administration

Both doses of Antarelix induced an immediate decrease in plasma LH concentration of ovariectomized gilts (fig 1). A significant time effect ($P < 0.001$) was detected, but no dose effect and no interaction between time and dose were found. Concentration of LH was lower 1–12 h after injection and higher 36 h after injection than immediately before ($P < 0.05$). Hence, the lower dosage injected every 12 h appeared to be suitable to inhibit LH secretion and was selected for further use.

Hormonal effects of treatments

There were large individual variations in the timing of estrus and the preovulatory LH surge. Two hypophysectomized gilts had already exhibited estrus before surgery. Four control and 4 Antarelix-treated gilts had initiated their LH surge before start of treatment (fig 2). In all hypophysectomized females, gonadotrophin levels were undetectable on day 6. Concentrations of LH measured at 0900 and 2100 from day 5 in the evening to day 11 were lower in Antare-

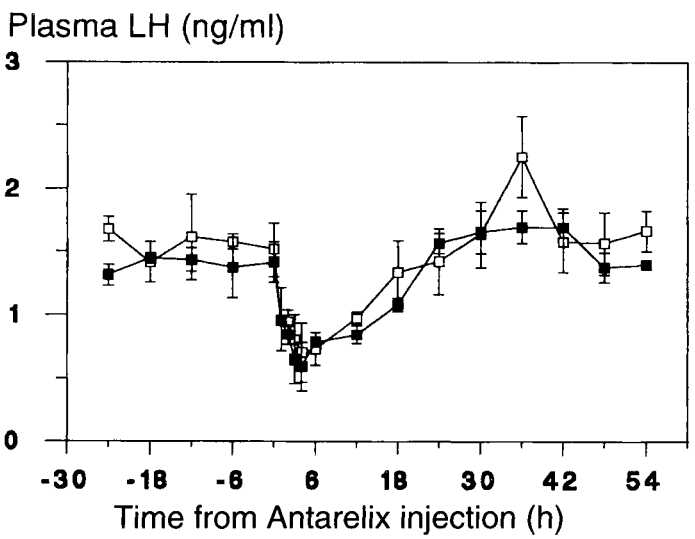

Fig 1. Changes in LH concentrations before or after Antarelix administration (0.5 ng, white squares, 1.5 mg, black squares) to ovariectomized gilts (n = 4 for each dosage).

lix-treated than in control females (0.92 ± 0.03 *vs* 1.49 ± 0.12 ng/ml, $P < 0.01$). As regards FSH, there was only a trend for lower concentrations in Antarelix-treated gilts (2.01 ± 0.07 *vs* 2.27 ± 0.09 ng/ml, $P = 0.08$).

Representative profiles of LH and FSH concentrations during the frequent sampling period on day 10 are shown in figure 3. Pulsatility of LH secretion was completely blocked (no pulse in any of the 5 gilts) in Antarelix-treated females while control females exhibited 1.8 ± 0.5 LH pulses per 6 h ($P < 0.03$). In contrast, basal LH concentration was not significantly altered by treatment (control 1.14 ± 0.08 *vs* Antarelix 0.91 ± 0.06 ng/ml). There was also no sig-

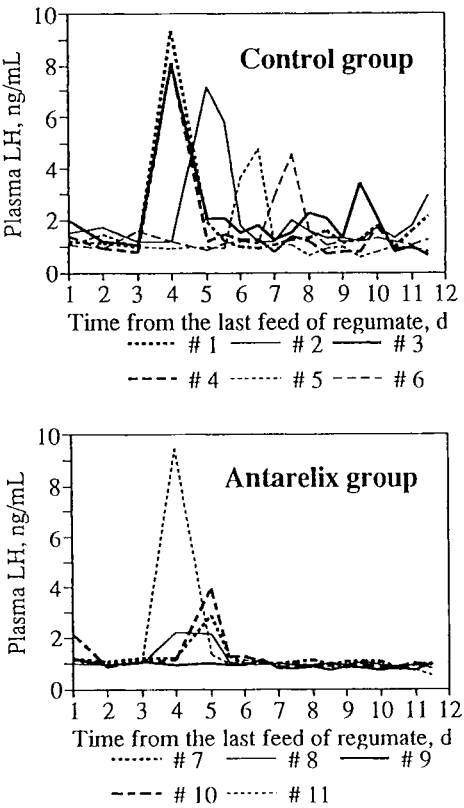

Fig 2. Changes in LH concentrations following the end of Regumate administration in the 6 control (upper panel) and the 5 Antarelix-treated gilts (lower panel). The LH peak occurred at days 4, 5, 6 and 7 in 3, 1, 1 and 1 control gilts. One Antarelix-treated gilt demonstrated an LH peak at day 4, while in 3 other gilts, Antarelix administration blocked the LH surge which was starting. One Antarelix-treated gilt did not exhibit an LH surge at all.

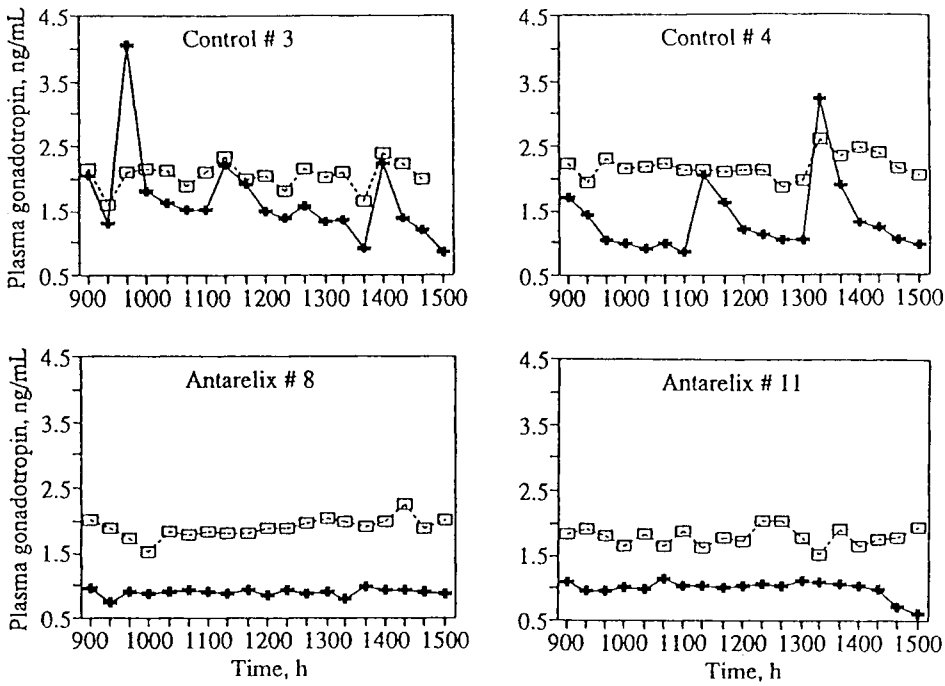

Fig 3. Representative profiles of LH (+) and FSH (□) secretion during the frequent sampling period in 2 control (upper panels) and 2 Antarelix-treated gilts (lower panels).

nificant difference in FSH concentrations measured in control (2.07 ± 0.18 ng/ml) and Antarelix-treated sows (1.91 ± 0.09 ng/ml).

Ovarian effects of treatments

All the controls, 4 out of 5 Antarelix-treated gilts and 2 out of 5 hypophysectomized gilts were found to have corpora lutea when slaughtered on day 12. The ovulatory females in the Antarelix (No 7, 8, 10 and 11) and hypophysectomized groups were those which exhibited oestrus or an LH surge before treatment or whose surge was only blunted by treatment. Mean ovulation rates of these ovulatory gilts were 18.1 ± 1.5 ($n = 6$), 18.0 ± 1.3 ($n = 4$), and 16.5 ± 0.4 ($n = 2$) in the control, Antarelix and hypophysectomized groups, respectively. These corpora lutea were all functional as shown by the similarity of the progesterone increase

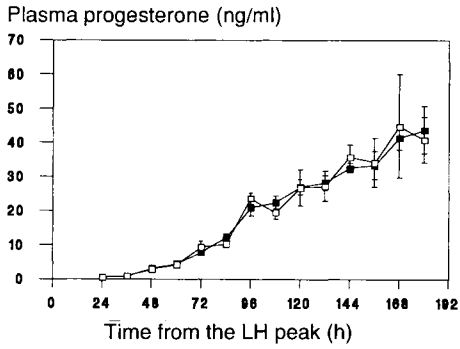

Fig 4. Changes in plasma progesterone following the LH surge in control (black squares) and Antarelix-treated (white squares) ovulating gilts.

following the peak of LH in control and Antarelix females (fig 4) and progesterone concentrations at slaughter in hypophysectomized gilts within the range of the other gilts (37 and 47 ng/ml).

There was no significant effect of treatment on the size at which an antrum was first detectable (measured by the mean size of the 5 smallest antral follicles). Hence, the number of antral follicles coud be reasonably compared between treatments. Treatment did not alter the total number of healthy antral follicles (control 316 ± 28, Antarelix 258 ± 56, hypophysectomized 219 ± 58) or the total number (healthy + atretic) of antral follicles (control 504 ± 39, Antarelix 431 ± 63, hypophysectomized 412 ± 72).

There were no treatment effects on follicle numbers in classes 1, 2 and 3 (fig 5). In contrast, large differences were induced by treatment for class 4 follicles ($P < 0.001$) with hypophysectomized gilts having hardly any healthy follicles of this size (0.8 ± 0.8), whereas Antarelix and control sows exhibited similar numbers (34.4 ± 6.8 and 26.6 ± 3.9 for control and Antarelix groups, respectively). No or very few (2.0 ± 1.0) healthy class 5 follicles were detected in hypophysectomized and Antarelix-treated gilts, respectively, as opposed to 9.6 ± 1.9 follicles present in control gilts ($P < 0.001$). Control females were the only ones which displayed class 6 follicles on their ovaries (2.4 ± 0.8). As a consequence, there were clear differences between groups in the size of the largest healthy follicle (control 3.68 ± 0.16, Antarelix 2.38 ± 0.44, hypophysectomized 1.0 ± 0.05 mm, $P < 0.001$).

Whatever the size class, treatment had no significant effect on the percentage of atresia. In class 2, 15.8, 15 and 22.6% of the follicles were atretic for control, Antarelix and hypophysectomized gilts, respectively. In class 3, these figures were 39.2, 49.4 and 51%. In class 4, these figures were 42, 45.2 and 33%. Finally, 32.8 and 47% of the class 5 follicles were atretic in control and Antarelix-treated gilts, respectively. The

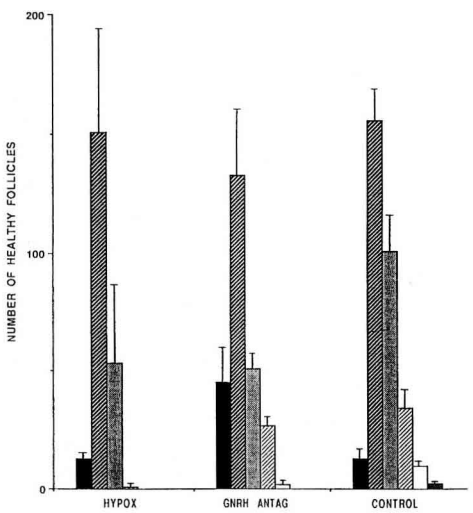

Fig 5. Mean number of healthy antral follicles in specific size classes (cl 1 ■, cl 2 ▩, cl 3 ▩, cl 4 ▨, cl 5 □, cl 6 ■) in hypophysectomized, Antarelix-treated and control gilts.

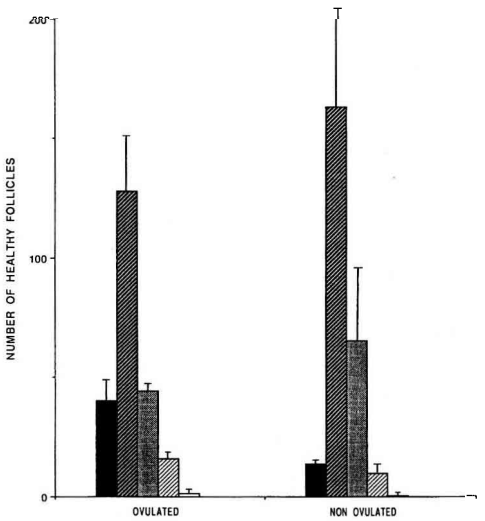

Fig 6. Mean number of healthy antral follicles in specific size classes (cl 1 ■, cl 2 ▩, cl 3 ▩, cl 4 ▨, cl 5 □, cl 6 ■) in ovulated and non-ovulated gilts.

size of the largest atretic follicle was altered by treatment (control 3.0 ± 0.1, Antarelix 2.35 ± 0.26, hypophysectomized 1.1 ± 0.06 mm) ($P < 0.01$).

Some of the females in the hypophysectomized and Antarelix groups had ovulated, and so animals could also be grouped as 'ovulated' ($n = 6$) and 'non-ovulated' ($n = 4$) and their follicular populations compared (fig 6). Whatever the size class and the follicle status (healthy/atretic), follicular populations were similar. There was also no difference between these groups for the size of the largest healthy follicle (ovulated 1.6 ± 0.2 *vs* non-ovulated 1.79 ± 1.51 mm) and of the largest atretic follicle (ovulated 1.77 ± 0.22 *vs* non-ovulated 1.66 ± 0.55 mm). Hence, it is unlikely that treatment effects are obscured by the occurrence of ovulation in some (but not all) experimental animals.

DISCUSSION

The main conclusions of this study are that 1) twice daily administration of the GnRH antagonist Antarelix can block pulsatile LH secretion, while not markedly affecting FSH levels; 2) follicular growth under 1.1 mm in diameter appears to be independent of pituitary function; 3) follicles 1.1–2 mm in diameter were present in GnRH antagonist but not in hypophysectomized gilts; 4) withdrawal of LH pulsatility blocks growth of follicles of more than 2.0 mm in diameter; 5) atresia induced by blockade of ovulation reduces size of the preovulatory follicles to less than 1 mm in 6 d; and 6) corpora lutea, despite gonadotrophin deprivation initiated around ovulation, appeared to be functional.

This is the first study describing the endocrine effects of GnRH antagonist administration to mature pigs. Antarelix was highly effective in blocking pulsatile LH release. In contrast, effects on FSH secretion were limited. Whether higher Antarelix dosages would better control FSH secretion remains to be investigated. These endocrine effects of Antarelix are in good agreement with an earlier report showing clear suppressive effects of active immunization against GnRH on LH secretion while effects on FSH were more delayed and more limited in mature gilts (Esbenshade and Britt, 1985). That manipulation of GnRH secretion/action more effectively disrupts LH than FSH secretion in mature females is also in agreement with numerous data in sheep following active immunization against GnRH (Mc Neilly *et al,* 1986), long-term GnRH agonist administration (Picton *et al,* 1990; Mc Neilly *et al,* 1992), or GnRH antagonist administration (Campbell *et al,* 1993). This underlines the different control mechanisms operating to regulate FSH *vs* LH secretion/release in both mature pigs and sheep.

This study also identified a fraction of folliculogenesis (*ie* follicles from 0.2 to 1.1 mm in diameter) which was not affected by hypophysectomy or GnRH antagonist administration. This is shown by the similar numbers of class 1, 2 and 3 follicles in the 3 experimental groups as well as the similar extent of atresia in these classes. Furthermore, the proliferation of granulosa cells in follicles of this size range was also similar between treatments (MA Driancourt, unpublished results). Hence, growth of follicles from antrum formation to 1.1 mm appears to be independent of pituitary function. Such follicles form basal folliculogenesis, as described previously for sheep and humans (Driancourt *et al,* 1992). These results contrast however with a previous report (Traywick and Esbenshade, 1988) when histological examination of the ovaries of gilts actively immunized against GnRH demonstrated that they were almost completely devoid of healthy antral follicles. The difference between our results and these could obviously be due to the short time lag between treatment and observation of the ovaries in the present study (7 d *vs* 12

weeks). However, it should be stressed that in sheep, comparison of ovaries obtained 4 or 70 d after hypophysectomy did not demonstrate major differences between these timings in numbers and atresia of antral follicles (Dufour *et al,* 1979).

Follicles with a diameter between 1.1 and 2 mm (*ie* 2–4 mm *in vivo*) in diameter were present in GnRH-antagonist-treated gilts but not in hypophysectomized ones. This could be due to a number of reasons because hypophysectomy not only removes FSH and LH but also growth hormone (GH), thyroid-stimulating hormone (TSH) and prolactin, while GnRH antagonist treatment only ablates LH pulses. Binding and action of prolactin (Veldhuis *et al,* 1980), thyroid hormones (Maruo *et al,* 1992) and of the GH/IGF (insulin-like growth factor) complex (Hsu and Hammond, 1987; Hammond *et al,* 1993) on porcine ovarian cells have been detected. However, IGFs and thyroid hormones have a limited effect *per se* and by preference affect FSH-induced cell differentiation (Maruo *et al,* 1992; Hammond *et al,* 1993). Prolactin is unlikely to explain the difference in follicular growth observed between the 2 models (hypophysectomized or GnRH antagonist), as its main effects on ovarian function are inhibitory (Veldhuis *et al,* 1980). Hence, it may be concluded that growth between 1.1 and 2 mm in diameter in gilts involves a major action of FSH (with minor modulatory effects of IGFs and thyroid hormones).

Suppression of follicles larger than 2 mm in diameter (*ie* 4 mm *in vivo*) in the GnRH antagonist-treated sows indicates that growth from that size to ovulation seems to be controlled mainly by LH pulses. This is in good agreement with observations showing that in situations where LH pulsatility is low (lactating sows, gilts given Regumate), follicular growth stops at 4 mm (Britt *et al,* 1985; Driancourt and Terqui, manuscript submitted).

Because Antarelix treatment or hypophysectomy were conducted in the presence of large preovulatory follicles, insights on the speed of atresia could be obtained by trying to identify, in non-ovulating sows, the ex-preovulatory follicles. In these sows, the largest atretic follicles were 1.7 mm in diameter (*ie* 3.4 mm *in vivo*). If it is assumed that, as in sheep, follicles become atretic around 36 h following gonadotrophin deprivation (Driancourt *et al,* 1987), then it takes 4 or 5 d for a follicle to regress from 7 to 3.4 mm. This regression rate (0.77 mm/d) is very similar to the daily growth rate calculated by Morbeck *et al* (1992) (0.69 mm/d). Such a conclusion is in good agreement with a previous report in sheep (Driancourt and Cahill, 1984).

Finally, the profile of progesterone increase found in ovulatory Antarelix-treated gilts was similar to that of the control gilts. Additionally, progesterone concentrations measured in blood of the ovulating hypophysectomized gilts were also within the normal range. This shows that early corpus luteum function in gilts may not require high gonadotrophin support, a finding which confirms an earlier report (Du Mesnil du Buisson and Leglise, 1963).

It can be concluded that the GnRH antagonist (Antarelix) used in the present study effectively controls LH pulses. If higher amounts of Antarelix or a longer duration of treatment were to be effective in blocking FSH secretion, this model could be very valuable in addressing questions regarding the hormonal control of key maturational events in gilts (aromatase, LH receptors on granulosa cells, inhibin/activin production) by immunohistochemistry, *in situ* hybridization or *in vitro* culture as well as their modulation by local mechanisms.

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