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Aberrant effects of altrenogest and exposure to exogenous gonadotropins on follicular cysts appearance in gilts

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ABSTRACT

Research was conducted to determine the effect of altrenogest and exposure to exogenous gonadotropins on ovarian function in prepubertal and mature gilts. Crossbred, presumably sexually mature gilts (n = 51), were fed with altrenogest for 18 consecutive days and the day after the last feeding with altrenogest, gilts were treated with eCG and 72 hours later challenged with hCG. Animals were slaughtered on Days 10 to 13 of their gonadotropins synchronized estrous cycle. Ovaries were examined for the number of CL, number of follicular cysts, and presence of corpora albicantia. Gilts were divided into two groups: those possessing corpora albicantia (group A—mature; n = 36) and those without corpora albicantia (Group W—prepubertal; n = 15) on their ovaries. In addition, each group was divided into two subgroups depending on the presence of follicular cysts (AC and WC) or their absence (AO and WO). There was no difference between the number of CL in group A and group W. Presence of corpora albicantia determined percentage of gilts possessing follicular cysts (13.9% group A vs. 66.7% group W). Gilts without follicular cysts (AO plus WO; n = 36) had higher number of CL ($P < 0.01$) than gilts bearing cysts (AC plus WC; n = 15). Comparison AO-AC did not show significant difference ($P = 0.075$) between CL number in mature cyst-free and cysts bearing gilts. A prepubertal gilts not bearing follicular cysts (WO) had higher ($P < 0.02$) number of CL than gilts bearing cysts. A significant negative correlation between the number of CL and number of follicular cysts was found ($r = -0.664$; $P = 0.007$). There were no differences in blood plasma progesterone and estradiol concentration between cyst-free and cyst-bearing gilts. These results indicate: (1) a higher follicular cysts appearance in prepubertal than mature gilts challenged with altrenogest and exposed to exogenous gonadotropins and (2) a negative effect of follicular cysts on the number of CL (ovulations) in prepubertal gilts.

1. Introduction

For many years, use of altrenogest alone or in combination with gonadotropins has been a common tool to synchronize estrous cycle in gilts in commercial farms. Also, the ability of eCG to stimulate follicular development and hCG to precisely control the time of ovulation in pigs has been known for many years.

Injection of eCG followed by hCG 48 to 96 hours later [1] or combined treatment with eCG and hCG [2] were also used for induction of estrus and fertile ovulation in prepubertal gilts. However, the application of gonadotropins to prepubertal gilts is not very effective [1,3] due to problems with the establishment of regular reproductive cycles or pregnancy maintenance. A wide interval from treatment to the onset of estrus after challenge with a combination of eCG and hCG [2] was also reported [2].

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A useful additional tool for more effective estrus synchronization in swine is an orally active progestagen (altrenogest, allyl trenbolone, RU-2267) which can be used alone [4,5] or in combination with gonadotropins [6,7]. The percentage of follicular cysts after estrus synchronization by using gonadotropins in gilts is about 15% [8]. Although observed from time to time, the cause of a high percentage of ovarian cysts has not been reasonably explained [9].

A possible relationship between the appearance of follicular cysts and the number of ovulations has hardly been examined to our knowledge, but the appearance of ovarian cysts can be related to the application of exogenous gonadotropins in pigs [8].

This study presents novel results of ovarian cysts appearing in prepubertal and mature gilts after altrenogest and gonadotropins treatment and their effect on the number of CL.

2. Materials and methods

2.1. Animals

Procedures performed on animals were conducted in accordance with the national guidelines for agricultural animal care and approved by the Local Animal Ethics Committee. The experiment was carried out in a commercial swine unit located in Northern Poland on 51 crossbred (landrace × large white) gilts weighting 95 to 110 kg and fed 2.5 kg of commercial developer diet daily. The prepubertal gilts were examined for outward physical signs of estrus (i.e., redness and swelling of vulva) at the morning and afternoon during 7 to 10 days. Then, gilts having red and swollen vulva were examined for behavioral symptoms of the first natural estrus by responding to the back pressure test in the presence of a mature boar. The back pressure test was performed by the experienced farm worker only once. All gilts which positively passed the back pressure test were considered as being in the first natural estrus. Ten to 15 days after estrus detection, gilts were placed in individual pens from the beginning to the end of the experiment and fed 20 mg of altrenogest (Regumate Porcine) for 18 consecutive days. The altrenogest was given in a small portion of the feed to ensure rapid and complete ingestion. The day after the last feeding with altrenogest, all gilts were treated with 750 I.U. eCG (i.m.) and 72 hours later challenged with 500 I.U. (i.m.) of hCG [1], all gilts were treated with 750 I.U. eCG (i.m.) and 72 hours later challenged with 500 I.U. (i.m.) of hCG [1].

2.2. Blood samples collection

A 10 mL of blood sample was taken from all gilts just before slaughter by jugular venipuncture into syringe containing 0.5 mL of heparin solution. Then, the blood was centrifuged at ×1600*g* for 15 minutes at 4 °C. The obtained plasma samples were stored at −20 °C until assayed for progesterone and estradiol-17β concentration.

2.3. Reproductive tracts observation

Animals were slaughtered on Days 10 to 13 of their gonadotropin synchronized estrous cycle (i.e., 10–13 days after hCG administration). Reproductive tracts were collected *post-mortem* and ovaries were examined for the number of CL (ovulations), number of follicular cysts and presence of corpora albicantia. Morphological examination of ovaries allowed to verify a sexual maturity of animals assessed on the base of the back pressure test. Considering presence or absence of corpora albicantia, gilts were divided into two groups: those possessing (group A: mature; n = 36) and those without corpora albicantia (Group W: prepubertal; n = 15) on their ovaries. Follicular structures exceeding 10 mm in diameter were classified as cysts [10] and were observed on ovaries of some mature (group A) and prepubertal (group W) gilts. Thus, each group was divided into two subgroups depending on the presence of cysts in mature (AC; n = 5) and prepubertal (WC; n = 10) gilts or cyst-free mature (AO; n = 31), and prepubertal (WO; n = 5) animals.

2.4. Determination of progesterone and estradiol-17β concentration

Concentration of progesterone and estradiol-17β in blood plasma were assessed using the RIA method and commercially available kits (DIA Source Immunoassay S.A., Belgium) in one assay as described previously [11]. The sensitivity of progesterone and estradiol-17β assay was 0.05 ng/mL and 2 pg/mL, respectively. Intraasay coefficient of variation for progesterone and estradiol-17β was 7.35% and 4.9%, respectively.

3. Statistical analysis

Because of low numbers of gilts in each group and resulting inability to verify normal distribution, the Mann–Whitney and Kruskal–Wallis nonparametric statistical tests were used. Both above mentioned tests that is, Mann–Whitney and Kruskal–Wallis are used instead of *t* Student and ANOVA tests, respectively. These nonparametric tests except mean, provide the median (Me)—central value, the first quartile (Q1), and the third quartile (Q3) instead of the commonly applied variance like standard deviation or standard error applying in the parametrical test. Q1 is the middle number between the smallest value, and Me while Q3 is the middle number between Me and the highest value of data set. The Mann–Whitney test was applied to compare numbers of CL between mature (group A) and prepubertal (group W) gilts and between gilts without cysts (AO plus WO) and those bearing cysts (AC plus WC). The Kruskal–Wallis test was applied to compare differences in numbers of CL between the four subgroups created on the basis of sexual maturity and presence of cysts (AO, AC, WO, and WC). In addition, the multifactorial model of linear regression predicting number of ovulation based on the presence of cysts and appearance of maturity was applied.