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Induction and synchronization of ovulations of nulliparous and multiparous sows with an injection of gonadotropin-releasing hormone agonist (Receptal)

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Abstract

The objective of this study was to determine if administration of a set dose (10 μg) of a gonadotropin-releasing hormone agonist, buserelin (Receptal; Rc), at set times after altrenogest (Regumate; RU) treatment or after weaning was able to induce and synchronize ovulation in female swine (gilts and sows). The pubertal (n = 187) gilts were allocated to four groups, all synchronized with RU. Group 1 (RU) was inseminated twice at detected estrus, Group 2 (RU+Rc120) and Group 4 (RU+Rc104) received 10 μg Rc at 120 or 104 h after the end of RU treatment, respectively, and Group 3 (RU+eCG+Rc104) was treated with 800 IU equine chorionic gonadotropin (eCG) at 24 h and Rc 104 h after the end of RU treatment, respectively. Gilts were inseminated twice at predetermined times, namely 144 and 168 h (Group 2), 128 and 144 h (Group 3), and 144 and 152 h (Group 4) after the end of RU treatment, respectively. Pregnant gilts were slaughtered at 30 d. Administration of Rc 104 h after the end of RU feeding synchronized ovulation over a 24-h time window in 97.9% and 100% of the gilts of Groups 3 and 4, respectively, whereas Rc administration at 120 h (Group 2) only successfully synchronized 88.9% of the gilts over 24 h. Ovulation rates of gilts of Groups 2 and 4 were similar to that of the control group. Pregnancy rates were numerically higher in Groups 2 and 3 (92% and 96%, respectively) compared with those of Groups 1 and 4 (84% and 81%, respectively). Combination of eCG with Rc administration at 104 h (Group 3) increased ovulation rate (+4 CL) but decreased embryo survival to 62% at Day 30. The weaned sow experiment involved 61 sows of a range of parities (2.7 ± 0.9), allocated to two control groups (Control 104 group and Control 94 group) and two treated groups (Rc104 group and Rc94 group), which received 10 μg Rc at 104 and 94 h after weaning, respectively. The females were inseminated at detected estrus. All pregnant sows farrowed. After treatment with Rc 94 h after weaning, 100% of sows ovulated over a 24-h time window versus only 68.7% of controls. Farrowing rate and litter size of the sows treated with Rc at that time were unaffected compared with that of control sows. In contrast, Rc administration at 104 h after weaning may have been too late; only 66.7% of the treated sows ovulated during a 24-h period. This proportion was numerically lower but not significantly different than that for control sows. Farrowing rate and litter size of treated sows were not significantly different than that of

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controls. Administration of Rc at the dose and times selected in this study tightened synchrony of ovulation in gilts and in sows after weaning. It remains to be established if such a synchrony is suitable to obtain good fertility after a single artificial insemination at a predetermined time.

Keywords: Fertility; Gilts; Ovulation; Sows; Synchronization

1. Introduction

A prerequisite for optimal sow fertility is insemination with fresh extended semen during the 24-h period before ovulation [1–8]. However, the large individual variation (both in gilts and sows) of the onset of estrus to ovulation interval [3–5] limits the possibility to always inseminate close to the optimal time. Thus, careful estrus detection together with at least two artificial inseminations with a large number of spermatozoa are generally done to obtain high fertility. Estrus detection and repeated inseminations are time consuming. Hence, many breeders are interested in shifting to timed artificial insemination (AI) ideally using only a single one. Controlling the time of ovulation is the only approach whereby an optimal time interval between ovulation and AI may be achieved.

The ability of exogenous gonadotropin-releasing hormone (GnRH) to induce luteinizing hormone (LH) release has been used to synchronize ovulation in gilts and sows [9–13] and minimize the variability in the time interval between estrus and ovulation. Several treatments using GnRH agonists were proposed for swine [11,13,14] such as buserelin (Receptal; Intervet, Angers, France).

Studies of Von Kaufmann and Holtz [14] have demonstrated that administration of 10 μg buserelin (Receptal; Rc) efficiently induces and controls the time of ovulation in gilts pretreated with equine chorionic gonadotropin (eCG).

Earlier studies [9,10,13] have previously demonstrated that inclusion of an administration of GnRH or a GnRH agonist toward the end of a synchronized follicular phase could successfully be used to time insemination. However, such studies have limitations. First, most of them included eCG administration in the synchronization regimen, which, though generally increasing ovulation rate, is not always beneficial for embryonic survival [15,16]. Second, the physiologic status of the animals and the time of administration of GnRH used in the different studies were quite different [9,14].

The main objective of this study was to expand our existing knowledge by proving that administration of 10 μg of the GnRH analogue, Rc could induce and synchronize ovulation in pubertal gilts and in weaned sows. In gilts, the timing of Rc administration (104 h) was based on the work of Brüssow et al. [9] and was associated with or without eCG. A later administration time (120 h) was also tested to determine whether late ovulations could be hastened by treatment.

In sows, administration of Rc at 104 h after weaning was selected to have a similar interval to that of gilts. However, the interval between weaning and estrus is shorter than that after altrenogest (Regumate; Janssen, Issy Les Moulineaux, France) treatment [3,17]. Thus, we also tested a shorter interval: 94 h postweaning. In addition, fertility and prolificacy after such treatments were documented in all treated females versus controls.

2. Materials and methods

2.1. Animals and management

2.1.1. Pubertal gilts

This first experiment was conducted at the experimental farm of INRA Rouillé (Rouillé, France). Gilts arrived on the experimental farm at 200 ± 13.3 (mean ± SD) days of age by batches of 8 to 19 females every 3 wk. They originated from three private farms with the same genetic scheme. Only pubertal females were included in the trial. The females were said to be pubertal if:

- they had an estrus in the 14 d that followed their arrival at the experimental farm;
- their plasma concentration of progesterone exceeded 2 ng/mL when arriving at the farm.

One hundred eighty-seven pubertal crossbred gilts were randomly distributed after first estrus among four experimental groups according to Table 1. Gilts were group penned and received 2.4 kg of a standard ration per day (3.2 Mcal Digestive Energy/kg and 15.5% proteins). During the 18-d period of altrenogest

treatment (20 mg/d Regumate; Janssen), the females received a small amount of food before the morning feeding to ensure drug uptake. Water was freely available.

2.1.2. Multiparous sows after weaning

Both experiments were conducted at the experimental farm of INRA Nouzilly (Nouzilly, France). Sixty-one hyperprolific Large White (LWh) multiparous sows of parity 1 to 5 (2.7 ± 0.9) were introduced in the experiment after weaning (12 batches every 3 wk, 4 to 6 sows per batch).

One week before their expected farrowing, all females were moved to the farrowing rooms where they stayed 5 wk until weaning. On this day at 10:30 a.m., they returned to sow pens (4 sows/pen) where they stayed for around 1 wk. After AI, they moved to gestation pens. Sows were weighed before farrowing and weaning.

Sows were fed individually twice daily. One week before farrowing, females received 2.7 kg/d (standard diet providing 3.14 Mcal DE/kg and 15.99% proteins). After farrowing, the same diet was provided in increased amounts during the first 2 wk while free access to feed was given until weaning. From weaning to AI, a standard diet (2.91 Mcal DE/kg and 14.74% proteins) was distributed according to the appetite of the sow. After AI, females were fed 3 kg/d (same diet). Water was freely available.

2.2. Experimental design

2.2.1. Pubertal gilts

All gilts (n = 187) received the recommended altrenogest (20 mg/d Regumate; RU; Janssen) treatment for 18 d [17] to synchronize estrus.

Three treatment groups received buserelin (Receptal; Rc; Intervet) intramuscularly either alone or after pretreatment with eCG (Intervet) (Table 1).

Starting 1 d after the end of RU treatment, estrus was checked three times a day (7 a.m., 12 a.m., and 5 p.m.) with a boar. The gilts were recorded in estrus when they demonstrated all behavioral characteristics (''standing'' reaction, active pursuit of the male, etc.) described by Signoret [18] and Terqui et al. [8]. Estrus duration was calculated as described by Terqui et al. [8].

Starting 3 d after the end of RU treatment until 24 h after the end of estrus, blood samples were collected twice a day (at 8 a.m. and 4 p.m.) to measure progesterone concentrations. All gilts of the four groups were submitted to estrus detection and collection of blood samples. Only some of the gilts were inseminated (see below) and fertility evaluated at Day 30 of pregnancy.

- Group 1 (RU) was the control group (n = 45). The females received RU treatment, and 24 of them were inseminated twice. The first AI was done 6 to 12 h after the onset of estrus and the second AI 6 to 12 h later.
- Group 2 (RU+Rc120). The gilts (n = 49) were treated with 10 μg Rc 120 h after the end of RU treatment. Twenty-five gilts were inseminated twice at predetermined times (144 and 168 h after the end of RU treatment; i.e., 24 and 48 h after Rc administration).
- Group 3 (RU+eCG+Rc104). Twenty-four hours after the end of RU treatment, the females (n = 45) received (by the im route) 800 IU eCG; 10 μg Rc was administered 104 h after the end of RU treatment. Among the treated gilts, 25 were inseminated twice at predetermined times (128 and 144 h after the end of RU treatment; i.e., 24 and 40 h after Rc administration).
- Group 4 (RU+Rc104). The gilts (n = 48) received 10 μg Rc at 104 h after the end of RU treatment, and all females were inseminated twice at 144 and 152 h after the end of RU treatment (i.e., 40 and 48 h after Rc administration). The AI was delayed in this group compared with Group 3 (RU+eCG+Rc104) because we anticipated a longer interval between Rc treatment and ovulation in the absence of eCG.

The timing of first and second AI was selected assuming a 24- and 42-h interval between Rc administration and ovulation, corresponding with the minimum interval and mean interval observed between the beginning of LH rise and ovulation [8]. Females were inseminated twice with seminal doses (3×10^9 spermatozoa per AI) prepared at INRA-UEICP (Rouillé, France). Six Pietrain boars produced the semen used and were allocated homogeneously to each treatment group.

Return to estrus was detected twice a day (at 7 a.m. and 5 p.m.).

Pregnant gilts were slaughtered on Day 30 ± 1 of pregnancy. Ovaries and uterine horns were weighed, and corpora lutea (CL) and embryos were dissected, counted, and weighed as described previously [19].

Weight at entry, age at entry, and age at first estrus (puberty) were not different between experimental groups (P = 0.38, P = 0.28, P = 0.55, respectively, for these parameters). The overall mean (±SD) for weight and age at entry were 200 ± 13.3 d and 108 ± 9.9 kg, respectively. The overall median age at puberty was 208

d (206 and 211 as lower and upper 0.95 confidence limits).

2.2.2. Multiparous sows after weaning

The study involved two experiments. Experiment A included:

- The Control 104 group (n = 15) in which the females received an intramuscular injection of vehicle (same volume of the solvent of Rc) at 104 h after weaning (at 6 p.m.).
- The Receptal 104 group (Rc104; n = 15) in which the females received 10 μg Rc at 104 h after weaning (at 6 p.m.).

Experiment B included:

- The Control 94 group (n = 16) in which the females received a vehicle injection at 94 h after weaning (at 8 a.m.).
- The Receptal 94 group (Rc94; n = 15) in which the sows were injected with 10 μg Rc at 94 h after weaning (at 8 a.m.).

In both experiments, the females were randomized according to parity, body weight, length of lactation, and litter size before being assigned to the control or treated groups.

Their overall parity was 2.7 ± 0.9 (mean ± SD) and did not differ between Control and Receptal groups ($P > 0.48$). Their litter size (13.7 ± 3.9 piglets) prior to inclusion in the experiment was also similar between Control and Receptal groups ($P > 0.36$). The weights of sows before farrowing and at weaning (at 287 ± 30 and 229 ± 29 kg) were also not different between experimental groups. Their overall mean (±SD) length of lactation was 28.5 ± 1.3 d and was similar between experimental groups ($P > 0.73$).

Estrus detection was carried out three times a day (at 8 a.m., 12 a.m., and 6 p.m.) with a boar. Estrus was defined and its duration computed as in gilts.

Starting at the onset of estrus until 24 h after the end of estrus, blood samples were collected twice a day (at 8:30 a.m. and 4:30 p.m.) to measure progesterone concentrations.

If a sow was not seen in estrus during the week after weaning, a blood sample was collected 14 d after weaning to measure progesterone concentrations and thus assess whether ovulation had taken place.

To avoid decreasing farrowing rate and prolificacy, sows were inseminated twice at detected estrus with seminal doses (3×10^9 spermatozoa per AI) prepared at INRA-UEICP (Rouillé, France). The first AI was done 6 to 12 h after the onset of estrus and the second 6 to 12 h later. Ten boars of the same genotype (hyperprolific Large White) were used with semen of each boar equally used in the Receptal and Control groups.

In both experiments, return to estrus was detected twice per day (at 8 a.m. and 4 p.m.).

In females that failed to display estrus after AI, pregnancy was checked by ultrasonography at 24 ± 2 d postinsemination. The codification and the interpretation of the images and the validation of the technique have been described elsewhere [20].

At farrowing, the numbers of piglets born and alive as well as their individual weights were recorded.

2.3. Detection of the time of ovulation by progesterone assay

The determination of the time of ovulation was based on changes in progesterone concentrations [8]. Jugular blood samples were collected twice a day (see Sections 2.2.1 and 2.2.2) in heparinized tubes. Plasma was collected after centrifugation and stored at –20 °C.

Plasma progesterone concentrations were determined by a validated radioimmunoassay [8]. The intra-assay and interassay coefficients of variation were 9% and 14.5%, respectively (for a Quality Control containing 2 ng mL^{-1}) [8].

The progesterone pattern during the periovulatory phase was characterized first by a period of low and steady concentrations, called basal level (nbase), until a time (taug) when progesterone concentrations started to rise exponentially (at a rate called paug). The nonlinear model can therefore be formulated as follows:

$$\text{Progesterone} = \text{nbase} \quad \text{if} \quad \text{time} \leq \text{taug}$$

else

$$= \text{nbase} \times \exp(\text{paug} \times [\text{time} - \text{taug}])$$

The parameters nbase, taug, paug, and their variance were determined from progesterone data from each individual female with the NLS2 software [21]. The SDs of all these nbase were computed.

The time of ovulation was defined as the time when progesterone increased from at least 1 SD above the basal level (nbase) determined with a nonlinear model.

As demonstrated earlier [8], the progesterone method was compared with the results obtained with five ultrasound scanning observations per day. The mean of the differences between the two methods was 3 ± 6 h, and its 95% confidence limits were respectively 0.7 and 5.7 h. Furthermore, the two methods were

Table 1
Estrus, ovulation, pregnancy rate, and number and weight of embryos in the different treatment groups of gilts.

Item	Group 1 (RU)	Group 2 (RU+Rc120)	Group 3 (RU+eCG+Rc104)	Group 4 (RU+Rc104)
Number of gilts	45	49	45	48
Occurrence of estrus, %	100[a]	100[a]	100[a]	87.5[b]
Interval end of RU treatment to onset of estrus, h*	124[a] ± 17.9	120[a] ± 17.9	105[b] ± 12.1	122[a] ± 12.7
Duration of estrus, h*	59[a] ± 16.6	60[a] ± 13.8	65[a] ± 11.7	50[b] ± 16.7
Females ovulating, %	100	100	100	100
Interval end of RU treatment to ovulation, h*	170[a] ± 14.6	165[a] ± 12.7	149[c] ± 9.1	153[c] ± 9.1
Percentage of ovulation over 24 h (144 to 168 h after the end of RU treatment), %	62.5	88.9	97.9	100
Number of gilts inseminated	24	25	25	48
Gestation rate, %	84	92	96	81.2
Number of CL*	17.1[a] ± 3	17.4[a] ± 3.2	21[d] ± 6.9	17.6[a] ± 2.9
Weight of individual CL, mg[†]	402[a] ± 17	408[a] ± 16	346[e] ± 12	394[a] ± 12
Number of live embryos*	13.6 ± 4.3	13.0 ± 4.1	14.4 ± 4.4	12.7 ± 4.0
Weight of individual live embryos, g[†]	1.55[a] ± 0.01	1.59[a] ± 0.01	1.76[f] ± 0.01	1.76[f] ± 0.1
Embryo survival, %[‡]	78.5 ± 5.6[a]	73.0 ± 5.3[a]	62.6 ± 4.1[g]	71.0 ± 4.0[a]

*Mean ± SD.
[†]lsmeans ± SEM.
[‡]Slopes × 100 of the regression with CL number.
Rows with different superscripts differ: [a] versus [b] ≠ $P < 0.002$; [a] versus [c] ≠ $P < 0.001$; [a] versus [d] ≠ $P = 0.0047$; [a] versus [e] ≠ $P = 0.018$; [a] and [f]: $P = 0.0001$; [a] versus [g] ≠ $P < 0.001$.

significantly ($P < 0.001$) correlated ($R^2 = 0.98$), and the slope of the linear regression was 0.91.

Ovulation was said to be synchronized if it took place over a 24-h period: 144 to 168 h after the end of RU treatment was chosen as the reference period for pubertal gilts, and 126 to 150 h after weaning was the reference period for sows.

2.4. Statistical analyses

Statistical analyses were performed using SAS software [22].

In the experiment on gilts, the between-group homogeneity before treatment was checked as follows. Age and weight at entry were compared with the GLM procedure while age of puberty was analyzed with a survival test (LIFEREG, LIFETEST). In experiments on sows, the homogeneity of all treatment groups before experiments was checked by the Wilcoxon test for the following variables: parity, numbers of piglets born, born alive, and weaned.

The time of estrus onset and duration in each treatment group were compared using a survival test on all animals. Mean and SD were used just as described in Table 1.

In both experiments, progesterone concentrations and logarithm of concentrations were compared between groups along time with the GLM-repeated option. The model included as factors the treatment groups, the time from the end of RU treatment or from weaning, and the interaction treatment by time. When there was a significant time by treatment interaction, the levels at each time were compared between groups with GLM.

The interval between the end of RU treatment or weaning and ovulation was displayed separately for each treatment group by descriptive means. A comparison of treatment groups was done by survival test taking into account females that did not ovulate during the experimental period.

The intervals between Rc administration and ovulation were evaluated only when the effect of Rc treatment was significant and were compared with GLM.

The synchrony of ovulation was characterized by the percentage of ovulations occurring within a 24-h time window; it was tabulated, and a Fisher's exact test was carried out to assess differences between each treatment group and the control group.

The farrowing rates or gestation rates per treatment group were displayed in a frequency table and compared using Fisher's exact test.

The weight of each corpus luteum (CL) was compared between groups with the SAS GLM

procedure; the number of CL and the interval between ovulation and slaughter, known to affect corpus luteum weight [23,24], were introduced in the model as covariates.

Wilcoxon's rank sum test was used to compare between treatment groups the number of viable embryos and the number of piglets born.

The total numbers of live embryos in the different groups were compared using the GLM procedure with the interaction between experimental groups as factor and the number of CL as covariate. The slopes of these regressions correspond with the embryo survival in each group.

The weights of individual embryos were compared between groups using a GLM mixed model; the sow effect was included as nested in experimental groups, and this effect was declared as random. The results were presented as lsmeans, adjusted for sow effect.

The heterogeneity of the variance of weight of piglets was tested with the Levene test (SAS).

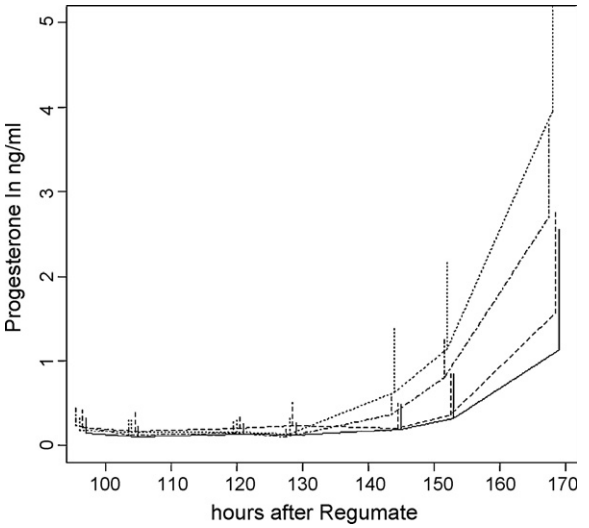

Fig. 1. Mean peripheral plasma progesterone levels + SD in the four experimental groups of pubertal gilts. Solid line, Group 1 (RU); dashed line, Group 2 (RU+Rc120); dotted line, Group 3 (RU+eCG+Rc104); dashed and dotted line, Group 4 (RU+Rc104).

3. Results

3.1. Pubertal gilts

3.1.1. Estrus

Occurrence of estrus was significantly lower ($P = 0.002$) in Group 4 (RU+Rc104). Five gilts of this group were not detected in estrus (Table 1). The time interval between the end of RU treatment and estrus was similar (around 120 h) for Groups 1, 2, and 4 but was shorter ($P < 0.002$) in Group 3 (RU+eCG+Rc104). Duration of estrus was shorter in Group 4 (RU+Rc104; $P < 0.002$) compared with that of all other groups (Table 1).

3.1.2. Progesterone pattern

Changes in progesterone concentrations were affected by treatment (Fig. 1) ($P < 0.001$). Progesterone concentrations rose earlier in Groups 3 and 4 than in Groups 1 and 2. Indeed, at 144 h after the end of RU treatment, progesterone concentrations (mean ± SD) were higher ($P < 0.003$) in Groups 3 and 4 (0.63 ± 0.77 ng/mL and 0.37 ± 0.28 ng/mL, respectively) than in Groups 1 and 2 (0.19 ± 0.29 ng/mL and 0.20 ± 0.29 ng/mL, respectively). Such differences were again significant at 152 h and 168 h after the end of RU treatment (Fig. 1).

3.1.3. Ovulation

All females ovulated (Table 1), even those not detected in estrus. However, ovulation occurred significantly earlier in Group 3 (RU+eCG+Rc104) and Group 4 (RU+Rc104) (149 and 153 h after the end of RU treatment, respectively) than in Group 1 (RU: 170 h) and Group 2 (RU+Rc120: 165 h).

The mean time of ovulation in Group 2 (RU+Rc120) was not different than that in Group 1 (RU). The interval between Rc treatment and ovulation (46 ± 9 h [mean ± SD]) observed in Group 3 (RU+eCG+Rc104) was shorter, although non-significantly, than that in Group 4 (RU+Rc104) (49 ± 9 h).

The distributions of the ovulation time in Groups 3 and 4, though similar, were significantly different than those of Groups 1 and 2 ($P < 0.001$).

The synchrony of ovulation estimated by the proportion of gilts ovulating over a 24-h time window (ovulation observed at 144, 152, and 168 h after RU treatment) was affected by treatment ($P < 0.001$). Whereas only 62.5% of gilts of Group 1 ovulated over the 24-h time window, all females of Group 4 (RU+Rc104) ovulated over 24 h (Fig. 2; $P < 0.001$). Ovulation of females of Group 3 (RU+eCG+Rc104) was also more tightly synchronized than that in Group 1 (control females; $P < 0.001$). The synchrony of ovulation was similar in Groups 1 and 2 ($P > 0.05$).

No significant effects ($P > 0.25$) of weight at entry or of age at puberty or of weight at puberty were observed on the time of ovulation.

3.1.4. Pregnancy rate

Pregnancy rate of the four groups ranged between 81% and 96% with Group 4 ($n = 48$) having the lowest

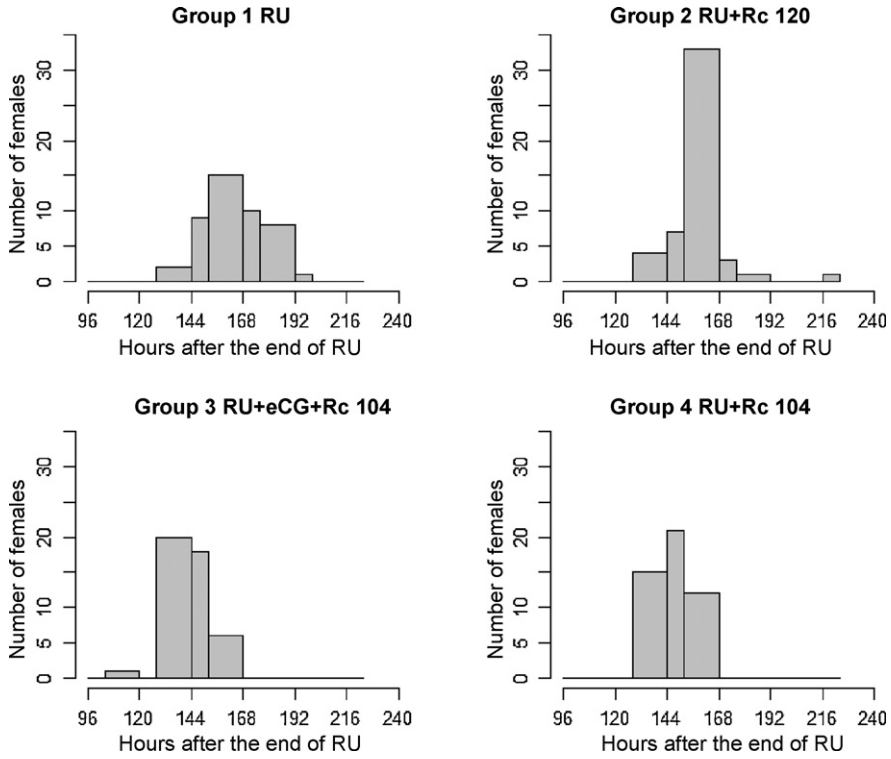

Fig. 2. Occurrence of ovulation in the different groups of pubertal gilts.

fertility (81.2%) and Group 3 (n = 25) the highest (96%). These differences between groups were not statistically different (Table 1; P = 0.27).

3.1.5. Ovaries of pregnant gilts

As indicated in Table 1, the number of CL was affected by treatment (P = 0.0047). Similar numbers of CL were found in gilts of Groups 1, 2, and 4 whereas 21 CL were present in Group 3 (RU+eCG+Rc104). Individual CL weight was related to total CL numbers per ovary (P = 0.0001). Treatment also significantly affected this relationship. The regression coefficient linking both parameters was close to 0.4 g per corpus luteum in Groups 1, 2, and 4 and only 0.34 g per corpus luteum in Group 3.

3.1.6. Embryos

Overall, there was no significant difference between groups in the number of live embryos, which ranged between 12.7 and 14.4 (Table 1). The relationship between the number of live embryos and the number of CL was significant and was affected by treatment (P = 0.0001). Embryo survival was lowest in Group 3 (62.6%) compared with that of the three other groups, which were similar (Group 1, 78.5%; Group 2, 73.0%; Group 4, 71.0%; Table 1). The adjusted weights of individual embryos of Groups 3 and 4 were higher than those of Groups 1 and 2 (P = 0.0001; Table 1).

3.2. Multiparous sows after weaning

3.2.1. Estrus

One sow of Experiment A (Rc104 group) and two sows of Experiment B (Rc94 group) were not seen in estrus and were thus not inseminated. They were however shown to have high progesterone concentrations 14 d postweaning and thus had ovulated. The number of females observed in estrus was similar in all groups, ranging between 87% (Rc94 group) and 100% (Control 104 group; Table 2).

The time interval between weaning and onset of estrus (between 96 and 115 h after weaning; Table 2) was similar in the Control and Receptal groups. In Experiment A, duration of estrus was shorter (P = 0.05) in Control sows (Table 2), but such a difference was not identified in Experiment B (Table 2).

3.2.2. Progesterone pattern

Administration of Rc did not alter the pattern of progesterone concentrations compared with that of the control group (Fig. 3). No treatment effect was identified (P = 0.08 for Control 104 group vs. Rc104

Table 2
Estrus, ovulation, farrowing rate, and litter size of the control and treated groups after weaning.

Item	Experiment A			Experiment B		
	Control 104	Rc104	P value	Control 94	Rc94	P value
Sows, n	15	15	—	16	15	—
Percentage of estrus, %	100	93	0.9	100	87	0.22
Weaning to onset of estrus interval, h*	96 ± 9.2	99 ± 14.4	0.46	107 ± 9	115 ± 10.6	0.33
Duration of estrus, h*	55 ± 10.7	64 ± 12.6	0.05	64 ± 10.6	59 ± 16.3	0.33
Females ovulating, %	100	100	—	100	100	—
Percentage of ovulation in 24 h, %†	86.6	66.7	0.71	68.7	100	0.02
Weaning to ovulation interval, h*	144 ± 3.3	148 ± 3.6	0.4	156 ± 6.9	142 ± 1.9	0.12
Sows‡ inseminated, n	15	14	—	16	13	—
Farrowing rate, %	86.7	71.4	0.39	81.3	84.6	0.9
Piglets born, total*	12.7 ± 4	15.6 ± 2.4	0.07	14.5 ± 4.5	14 ± 3.2	0.71
Piglets born alive*	11 ± 3.6	14 ± 1.6	0.06	12 ± 3.5	12.5 ± 2.5	0.58
Birth weight, kg*	1.5 ± 0.4	1.4 ± 0.4	0.23	1.3 ± 0.3	1.4 ± 0.3	0.22

*Mean ± SD.
†Within 126 and 150 h after weaning.
‡One sow of Group Rc104 and two sows of Group Rc94 were not seen in estrus and thus were not inseminated.

group and P = 0.80 for Control 94 group vs. Rc94 group). The effect of time was always highly significant (P < 0.0001).

3.2.3. Ovulation

The mean time interval between weaning and ovulation was computed for each group and shown to be similar between groups (Table 2). However, its variability was significantly reduced (P < 0.05) in the Rc94 group (142 ± 1.9 h) versus the other groups (Control 94, 156 ± 6.9 h; Rc104, 148 ± 3.6 h; Control 104, 144 ± 3.3 h).

In the Rc94 group, 100% of the sows ovulated between 126 and 150 h after weaning (Table 2; Fig. 4). In contrast, 66.7% (Rc104 group), 86.6% (Control 104 group), and 68.7% (Control 94 group) of the sows of the other groups ovulated during the same 24-h time period (Fig. 4; P < 0.048).

No significant effects (P > 0.2) of parity or of litter size or of weight before and after farrowing were observed on the time of ovulation.

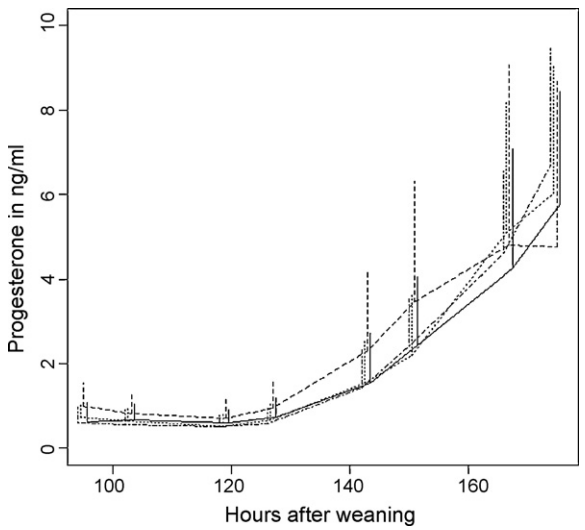

Fig. 3. Mean peripheral plasma progesterone levels + SD in the four experimental groups of sows after weaning. Solid line, Control 104; dashed line, Rc104; dotted line, Control 94; dashed and dotted line, Rc94.

3.2.4. Farrowing rate and litter size

Farrowing rate was not different between treatment groups and ranged between 71.4% (Rc104 group) and 86.7% (Control 104 group) (Table 2).

Treatment with Rc at 104 h postweaning tended to increase the total number of piglets born (P = 0.07) or born alive (P = 0.06) compared with the other groups. The variance of litter size tended to be smaller (P = 0.08) in the Rc-treated groups compared with that of the control groups. The weight of piglets was unaffected by treatment (Table 2).

4. Discussion

As the optimum breeding time of gilts and sows is difficult to predict, controlling the time of ovulation is the only approach whereby an optimal time interval between AI and ovulation may be achieved.

The main conclusions of this study are that (1) Rc administration in the 104- to 120-h time window efficiently induces and synchronizes ovulation in gilts

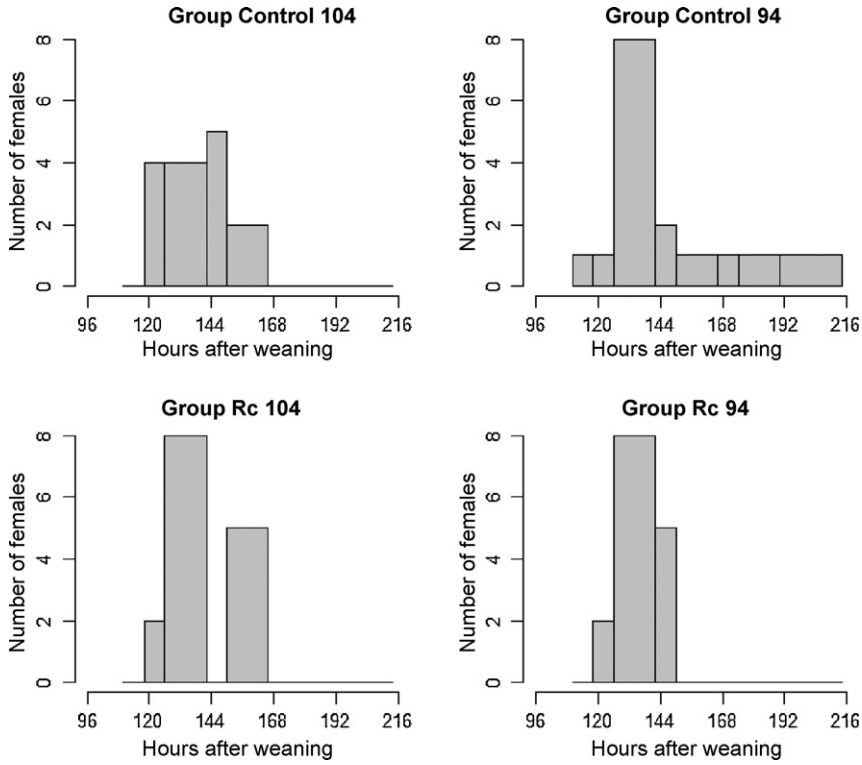

Fig. 4. Occurrence of ovulation after weaning in the different treatment groups of sows.

not pretreated with eCG; (2) the window of insemination generating normal fertility ranged between 24 and 40 h after Rc administration; (3) in sows, administration of 10 μg Rc at 94 h after weaning tightly synchronized ovulation without reducing farrowing rate. Such conclusions confirm and expand results of earlier studies [9,10].

Several GnRH analogues have been evaluated for ovulation induction in swine [11,13]. Among these, Rc has been used successfully in prepubertal gilts [14,25,26] and multiparous sows [27–30]. However, in each animal type, the time and/or dose of Rc administered differed between studies. In addition, eCG was commonly injected (in variable amounts) in most studies, precluding extrapolation of conclusions to untreated cycling gilts. In our experiments, we consistently injected 10 μg Rc at specific intervals after the end of RU treatment (104 or 120 h) or weaning (94 or 104 h). Such a dose was selected based on the earlier studies in swine of Von Kaufmann and Holtz [14] and Möller-Holtkamp et al. [31]. The different times of Rc administration were selected based on results of earlier studies [10,11,28].

The time of ovulation was determined by progesterone measurements as validated previously [8]. The validity of this approach was confirmed by the finding that, in our control groups (pubertal gilts experiment and multiparous sows after weaning), the mean time of ovulation was 47 h after the onset of estrus, a value very close to the one measured by ultrasonography [2]. Otherwise, in ours groups treated with Rc, the mean time of ovulation was 39 h after the onset of estrus, a value similar to those measured by laparoscopy or ultrasonography after stimulation with exogenous GnRH [32].

In gilts, administration of Rc at 104 h after the last dose of RU (Group 4) tightly synchronized ovulation (100% of ovulations in 24 h) and allowed us to obtain a similar ovulation rate (17 CL), pregnancy rate (81%), and number of live embryos on Day 30 of gestation (12.7) after two fixed-time AIs as that in control gilts (Group 1) after double AI at detected estrus (ovulation rate, 17 CL; pregnancy rate, 84%; number of live embryos, 13.6). A later administration of Rc (120 h in Group 2) was associated with a numerically higher pregnancy rate (92%) and unaltered ovulation rate (17 CL) and number of live embryos on Day 30 (13.0) despite a slightly reduced tightness of synchronization (89% of the gilts synchronized over 24 h). Group 3 evaluated the benefits of administration of 800 IU eCG before administration of Rc 104 h after the last RU dose, together with two fixed-time AIs performed as

recommended by Brüssow et al. [9]. This schedule significantly reduced the following intervals: end of RU treatment to onset of estrus (105 h vs. 124 h for control, $P < 0.002$) and end of RU treatment to ovulation (149 h vs. 170 h), in good agreement with earlier reports [9,10,13,14]. Administration of eCG, though significantly increasing ovulation rate (21 CL, $P = 0.0047$), resulted in a much smaller number of live embryos on Day 30 of pregnancy (14 vs. 13 in control gilts). Embryo survival was reduced in Group 3 (62.6%) compared with that in other groups (78.5%, 73%, and 71% in Groups 1, 2, and 4, respectively). The intervals between the second AI and mean ovulation time were 3, –5, and –1 h for Groups 2, 3, and 4, respectively. Hence, it is unlikely that such intervals may be responsible for the reduction of embryo survival in Group 3 compared with that of the other groups. These results confirm earlier studies showing that administration of eCG after RU treatment simultaneously increases ovulation rate, number of early embryos, and embryonic losses, therefore resulting in minimal (if any) increases in the number of live embryos [15,16].

We conclude that eCG administration 24 h after the end of RU feeding has no beneficial effects in the ovulation induction program tested in the current study in gilts.

Data documenting induction and synchronization of ovulation in sows are more limited than that in gilts as neither the optimal time of administration of Rc after weaning nor a clinical dose of Rc has been identified. Two treatment strategies have been tested in weaned sows, with administration of the GnRH agonist being set either at a specific time after an eCG administration or at the time of the first AI.

In the first strategy, involving eCG treatment at 24 h after weaning, Bergfeld et al. [33] showed that treatment with a GnRH agonist (D- Phe6 - LHRH, Gonavet, Berlin-Chemie, Berlin, Germany) 56 h after eCG administration successfully induced ovulation between 113 and 122 h after weaning. Schäfer et al. [28] and Rosales et al. [30] applied a treatment combining administration of eCG (24 h after weaning) and 5 μg Rc (56 to 58 h after eCG) together with two fixed-time AIs (between 102 to 104 and 116 to 120 h after weaning) and observed an improved farrowing rate and litter size compared with that of their control groups.

In the other strategy investigated by Peters et al. [29] and Martins et al. [25], Rc was administered (8 to 10 μg) at the time of the first AI. No significant effects of treatment on farrowing rate or litter size were demonstrated suggesting that administration of GnRH agonist at the onset of estrus or at the time of AI may therefore be too late.

In our study, we investigated the efficacy of the 10 μg Rc dose and at novel and well-defined timings of administration and showed that this dose of Rc administered 94 h postweaning considerably reduced the spread of the time interval between weaning and ovulation while maintaining fertility and prolificacy. Administration of Rc at 104 h after weaning may have been too late as the time interval between weaning and ovulation was unaffected (versus the controls) and fertility numerically decreased.

Comparison of our data with those obtained in eCG-GnRH agonist treated sows [28,30,33] suggests that injection of the GnRH analogue must be done later (around 90 h postweaning) in untreated weaned sows than in sows pretreated with eCG (around 80 h) to obtain a tight synchronization of ovulation. These observations confirm those of Knox et al. [11] and Baer and Bilkei [12] who reported that the interval from weaning to ovulation was reduced after intravaginal application of triptorelin (a GnRH agonist) at 96 h after weaning. Farrowing rates and litter size were similar to those of sows that were inseminated based on estrus.

In conclusion, Rc administration at 94 h after weaning (sows) or 104 to 120 h after the last dose of RU (gilts) synchronizes ovulations over a narrow 24-h time period. This tight synchronization of ovulation would allow the use of one fixed-time AI after administration of Rc. Further investigations are needed to check whether this will result in acceptable fertility and prolificacy results in gilts and sows of different genetics, housing, seasons, and management. A major advantage of such a protocol would be that estrus detection could be avoided therefore saving the cost of labor for estrus detection and one insemination, together with the cost of one semen dose.

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