

A BLOCK OF THE LUTEINIZING HORMONE AND OF OVULATION DESPITE OESTRUS IN ICI 33828-TREATED GILTS

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Summary. Thirty-five sexually mature gilts were fed either the compound ICI 33828 (100 mg/day) or a control ration on Day 18 of the oestrous cycle (Day 1 = oestrus). At 2, 4 or 6 days after oestrus, gilts were killed and ovarian and pituitary data collected. Treated gilts not showing an oestrus were killed 10 days after treatment was started. Sixty-seven per cent of the treated gilts showed the forthcoming oestrus. A high percentage (75%) of the treated gilts which exhibited oestrus failed to ovulate. The number of follicles was less than would normally be present before ovulation, and pituitary FSH and LH levels were greater in the ICI 33828-treated (non-ovulating) gilts than in controls. Gilts killed 10 days after initiation of treatment had high levels of pituitary FSH and LH, which may explain the occurrence of superovulation in one gilt (> 100 corpora lutea). These gilts, which did not ovulate except for one pig, also had no follicles greater than 6 mm in diameter, indicating that FSH release was blocked by the drug. The lower ovulation rate in the three ICI 33828-treated gilts which ovulated, compared to the controls, was thought to be the result of decreased follicular growth in the treated gilts.

INTRODUCTION

It has been shown that a high percentage of gilts started on ICI 33828 (an oestrus synchrony drug) during the late follicular phase of the oestrous cycle (Days 18 to 21) will exhibit the forthcoming oestrus (Polge, 1965; Stratman & First, 1965; Garbers & First, 1967). Based on oestrous cycle lengths, Polge (1965) suggested that some of the gilts exhibiting the forthcoming oestrus when treatment was started during the late follicular phase may not have ovulated or formed active corpora lutea (CL). In the same study, he found two gilts out of 100 that received 100 to 250 mg of ICI 33828/day showing oestrus while under treatment. Neither of these two gilts formed active CL. Garbers & First (1969a), in a dose level study in gilts, further reported that gilts on a lower drug level (40 mg/day) tended to show oestrus but only partial ovulation occurred.

It was the purpose of this study specifically to design an experiment which would test whether the ICI 33828 compound could allow oestrus to occur in some gilts but inhibit ovulation. Since a high percentage of gilts show oestrus if treatment is started during the late follicular phase of the cycle, it was

decided to use gilts at this stage of the cycle to study ovarian and pituitary changes in ICI 33828-treated gilts compared to control gilts.

MATERIAL AND METHODS

Thirty-five, sexually mature, crossbred gilts were assigned to ICI 33828 at 100 mg/day or to control treatments. All gilts started treatment at Day 18 of the oestrous cycle (Day 1 = oestrus). Gilts were penned separately and given 1.8 kg of feed daily. The ICI 33828-treated gilts had 100 mg of the drug added per 1.8 kg feed daily. The gilts were checked daily for oestrus with a boar and randomly assigned to be killed at 2, 4 or 6 days after onset of oestrus. Since the pig ovulates 25 to 50 hr after oestrus, the span of 2 to 6 days enabled determination of the relative time of ovulation and CL development. The ICI 33828-treated gilts not showing an oestrus were killed 10 days after the beginning of ICI 33828 treatment. All control gilts showed an oestrus.

At autopsy, follicle diameters were measured, CL were counted and weighed, uteri were flushed for ova, and follicular fluid and stromal weights were taken. The pituitary glands were collected and immediately frozen at -10° C. The stromal weight was adjusted by co-variance for follicle size by the method described by Short (1967).

Pituitary LH was estimated by the method of Parlow (1958) and FSH by the method of Steelman & Pohley (1953). Both methods were modified by the methods described by Short (1967) with the following exceptions. Doses of 0.15, 0.30 and 0.60 mg dry pituitary powder were used in a three-dose (two rat/dose) assay for LH. In the HCG-synergism (HCG-s) bio-assay for FSH, doses of 2 and 4 mg of pituitary powder from ICI 33828-treated gilts and 4 and 8 mg from control gilts were used. NIH-LH-s11 and NIH-FSH-s4 standards were also run at 0.4, 0.8 and 1.6 μ g and 60 and 120 μ g respectively. Because of unequal subclass numbers, the overall assay regression was tested against the standard regression by the method of Steel & Torrie (1960). The regressions were not different ($P > 0.10$). An analysis of variance was accomplished on each treatment subclass to determine if a significant dose effect occurred. In the OAAD bio-assay, all subclasses had a significant dose effect, but in the HCG-s bio-assay, the controls at Day 4 and the ICI 33828-treated gilts at Day 4 showed no significant dose response in the test rats. The ICI 33828-treated gilts at Day 4 were further separated into those which ovulated and those which did not. It was found that the gilts with a block to ovulation gave a significant dose response, whereas the other gilts did not respond. The standard LH and FSH dose responses were also significant. The ICI 33828-treated gilts which ovulated were not further analysed although the relative potency was calculated and is presented. This value probably over-estimates the actual pituitary potency, since the combined within-treatment regression was calculated using only those subclasses with a significant dose effect. The controls at Day 4 were used in the analysis of variance. The mean relative potency of these controls is also over-estimated. Because of this, interactions may exist and not be declared, or significant interactions may not be real. Relative LH and FSH potencies were calculated before statistical analysis by the method of Finney (1964). Least squares analyses

were accomplished on all the data from the control gilts and on the ICI 33828-treated gilts which did not ovulate. This resulted in data from fourteen control and seven drug-treated gilts in the analyses. Means of ICI 33828-treated gilts which ovulated are presented separately in Table 3, but statistical analyses were not attempted.

RESULTS

Of the eighteen gilts treated with ICI 33828, 67% showed the forthcoming oestrus. The mean length to oestrus after treatment initiation was 1.9 days for the treated gilts and 2.0 days for the controls. These values were not different. Two treated gilts showed oestrus on the day treatment was started (Day 18) and both had a block to ovulation. No CL were present in the majority of the treated gilts showing oestrus (75%), whereas 94% of the control gilts had CL at autopsy. The one control gilt without CL was killed on the 2nd day after oestrus and had pre-ovulatory-size follicles. A 72% ova recovery in the

TABLE 1

LEAST SQUARES ADJUSTED MEANS FOR CONTROL AND ICI 33828 (NON-OVULATING) GILTS

	No. of gilts ovulating/ No. showing oestrus	No. of gilts analysed*	No. of follicles			Follicular fluid wt (g)	Anterior pituitary wt	LH relative potency	FSH relative potency	Stromal wt	
			4 mm	4 to 7 mm	7 to 10 mm						
Control	2	5/6	5	28.4	0.2	2.2	0.9	60.6	0.32	0.78	4.5
	4	6/6	5	49.3	0.8	0.0	1.8	56.9	0.36	0.45†	4.2
	6	5/5	4	56.6	12.5	0.5	3.2	59.5	0.42	0.62	4.6
ICI	2	1/3	2	17.4	0.0	6.0	3.6	69.6	1.22	0.94	3.5
	4	2/5	3	20.7	1.0	6.3	3.8	71.5	1.61	0.96	4.4
	6	0/4	2	87.0	10.2	4.8	4.0	58.1	1.29	1.61	4.7

* Pituitary glands were not collected or were lost for five gilts on which ovarian data was collected. The data from these gilts were not included in the least squares analysis.

† The test rat dose response was not significant and therefore this estimate of relative potency is probably overestimated.

controls and in those ICI 33828-treated gilts which had CL as against no ova recovery in the ICI 33828-treated gilts which showed oestrus but lacked CL demonstrated that ovulation had occurred in animals with, but not in animals without CL.

The least square means and the analysis for the control gilts and for the ICI 33828-treated gilts which showed oestrus but did not ovulate are presented in Tables 1 and 2, respectively.

Follicle growth and ovulation rate

Follicular fluid weight and the number of follicles 7 to 10 mm in diameter were greater in the ICI 33828-treated gilts (non-ovulating) than in the controls ($P < 0.05$). The numbers of follicles ≤ 4 mm and 4 to 7 mm increased from Days 2 and 4 to Day 6 in both treated and control gilts ($P < 0.01$; Table 2). At Day 2

TABLE 2

LEAST SQUARES ANALYSIS WITH PRESENTATION OF MEAN SQUARES OF OVARIAN AND PITUITARY DATA

Source	df	LH relative potency	FSH relative potency	Follicular fluid wt	No. of follicles			Anterior pituitary wt	Adj. stromal wt
					≤ 4 mm	4 to 7 mm	7 to 10 mm		
Treatment	1	4.59**	1.37**	9.01*	171	25	74*	248	0.33
Day	2	0.08	0.26	0.63	4202**	137*	10	65	0.71
2+4 versus 6	1	0.00	0.42*	0.53	8405**	273**	17	129	1.14
2 versus 4	1	0.15	0.08	0.82	35	2	3	3	0.37
Day × treatment	2	0.07	0.24	4.00	700	24	8	99	0.68
2+4 versus 6 × trt	1	0.04	0.41*	7.75	1260	46	9	163	0.29
2 versus 4 × trt	1	0.10	0.10	0.48	85	0	5	25	1.14
Within	15	0.34	0.09	1.90	431	29	15	111	0.44

* $P < 0.05$; ** $P < 0.01$.

after oestrus, the ICI 33828-treated gilts had a mean of 6.0 follicles of ovulatory size. The one control gilt that had not ovulated on Day 2, had twelve follicles of ovulatory size. A comparison of mean CL numbers in the controls and ICI 33828-treated gilts which ovulated revealed 11.3 (94% in 10 to 15 range) and 7.7 (100% in 7 to 8 range) mean numbers of CL for controls and ICI 33828-treated gilts respectively.

In the gilts inhibited from showing oestrus and ovulation, and killed at 10 days, follicle growth was suppressed. No follicles of over 7 mm diameter were present. The one gilt not showing oestrus and presented separately in Table 3, superovulated and had more than eighty ova in the upper uterus. A superovulation has also been reported after treatment-withdrawal by Polge (1965).

A tendency for a Day 2+4 versus Day 6 × treatment interaction ($P < 0.1$) existed in the analysis of follicular fluid weight. The controls increased in

TABLE 3

OVARIAN AND PITUITARY MEANS OF GILTS OVULATING OR NOT SHOWING OESTRUS WHILE BEING TREATED WITH ICI 33828

Day	No. of gilts	Follicular fluid wt (g)	Follicle size			LH relative potency	FSH relative potency
			< 4 mm	4 to 7 mm	> 7 mm		
2	1 (ovul.)	0.8	33	0.0	0	2.07	1.09
4	2 (ovul.)	3.2	55	0.5	0	0.39	0.81†
10*	5 (no oestrus)	1.3	83	4.8	0	2.22	1.93
	1 (superovul.)	> 40	0	11.0	16	0.14	0.84

* These gilts did not show oestrus and were killed 10 days after treatment began. One gilt super-ovulated and is presented separately.

† The test rat dose response was not significant and therefore this value is probably overestimated.

follicular fluid weight between Days 4 and 6, whereas the ICI 33828-treated gilts remained constant in follicular fluid weight.

Pituitary and adjusted stromal weights

Neither anterior pituitary nor adjusted stromal weight was affected by treatment nor the day after oestrus on which the gland and organs were recovered ($P > 0.1$). Stromal weight was not significantly correlated with any other variable studied.

Pituitary gland FSH and LH levels

LH and FSH levels were greater in ICI 33828-treated gilts than in the controls ($P < 0.01$). FSH levels were also greater on Day 6 than on Days 2 and 4 after oestrus ($P < 0.05$), but no differences due to days existed in LH levels. A Day 2+4 versus Day 6 × treatment interaction existed in the analysis of relative FSH potency ($P < 0.05$). The ICI 33828-treated gilts appeared to have increased in pituitary FSH at Day 6, whereas the controls remained nearly the same as at Days 2 and 4 after oestrus.

Table 3 presents the means of the gilts which ovulated while under ICI 33828 treatment, and of gilts inhibited from showing oestrus and killed 10 days after treatment was started. In general, it appears that if a gilt ovulated while under ICI 33828 treatment, the LH and FSH levels were lower than in suppressed gilts. The one exception is the single gilt which ovulated and was killed 2 days after oestrus. The suppressed gilts killed at Day 10 had very high levels of FSH and LH whereas the gilt which superovulated had relatively low levels of pituitary FSH and LH. The correlation between FSH and LH was 0.47 which corresponds closely to the value of 0.52 reported by Kirkpatrick, First & Casida (1963).

DISCUSSION

The LH surge necessary for ovulation was blocked by the ICI 33828 compound in gilts in oestrus while being treated. This is supported by the higher LH levels in drug-treated gilts not ovulating compared to the controls. Since two of the treated gilts (not ovulating) showed oestrus on the day treatment was started, the release of LH necessary for ovulation must not occur too early during oestrus. This is especially interesting since du Buisson & Leglise (1963) reported that pigs hypophysectomized just a few hours after the first signs of oestrus formed CL. The time required after ICI 33828 feeding, until biological activity exists, is not known and therefore direct comparison is not possible, although it might be expected that the action of ICI 33828 would occur later than the hypophysectomies. Other explanations for the differences in effect may be a subtle effect of ICI 33828 on the ovary, liberation of LH due to the hypophysectomy procedure, or large variations between pigs as to the time of the critical ovulatory discharge of LH from the pituitary. Since all the gilts which ovulated while on ICI 33828 treatment showed oestrus 1 to 3 days after drug treatment started, the drug apparently is either not an effective inhibitor of gonadotrophin release in some gilts at this period of the cycle, or the time of critical LH release is quite variable. Brinkley, Norton & Nalbandov (1964) reported that pro-

gesterone given on the day of oestrus, the day of ovulation or one day after ovulation does not inhibit formation of CL. The same authors further reported that of five gilts started on 400 mg of progesterone the day before oestrus, two ovulated and three did not. Corpora lutea were formed in those gilts ovulating. Their hypothesis, that the only apparent way to prevent CL formation is by blocking ovulation, is verified by the data in this experiment.

Although follicle growth and oestrus occurred in the gilts prevented from ovulating by ICI 33828 treatment, the pituitary FSH levels were also greater in the treated gilts. This could be explained if there was less follicle growth than would normally occur. This, in fact, is the case. The mean ovulation rate in the controls was 11.3 versus 7.7 in ICI 33828-treated gilts. The one control gilt failing to ovulate had twelve follicles of ovulatory size (> 7 mm) at 2 days after oestrus, whereas those treated with ICI 33828 had a mean of six follicles at 2 days after oestrus which were capable of ovulating. Two conclusions can be drawn:

(1) Less follicular development occurred in treated gilts and therefore higher FSH levels were present. This was due to inhibition of some FSH release by the ICI 33828 compound.

(2) When ovulation occurred in ICI 33828-treated gilts, the ovulation rate was less due to the reduction in follicle growth.

The significant Day 2+4 versus Day 6 \times treatment interaction in the analysis of FSH levels can also be explained. Anderson (1966) suggested that FSH release must occur since follicles developed during the early part of the oestrous cycle. Between Days 4 and 10, FSH levels increase in the pituitary gland (Parlow, Anderson & Melampy, 1964). If some FSH release begins between Days 4 and 6 in the normal gilt, the following events would be expected to occur after Day 4: (1) an increase in follicular fluid weight and follicle development in controls, but no increase in ICI 33828-treated gilts, if FSH release is blocked by the drug; (2) a marked increase in pituitary FSH in treated gilts, if synthesis of FSH is not affected, and a maintenance of FSH levels if synthesis is affected.

If follicular fluid weight is taken as a measure of follicular development, then the hypothesis of a block to FSH release but not to FSH synthesis by ICI 33828 fits the data. The numbers of follicles < 4 mm and 4 to 7 mm in diameter in the treated gilts, however, increased to equal those of the controls. Another problem associated with the hypothesis is the mechanism by which the follicles formed at oestrus were maintained. Although the controls increased in follicular fluid weight between Days 4 and 6 and the ICI 33828-treated gilts did not, the absolute value of follicular fluid weight was still greater in the treated gilts. If these follicles require FSH, and if the growth of small follicles is indicative of the presence of FSH, the effect of the ICI 33828 compound was to enhance FSH synthesis. The theory of increased FSH synthesis due to the ICI 33828 compound seems unreasonable, since Brown (1963) has shown decreased FSH production in the rat, and follicle development in the cycling pig is suppressed by the drug (Polge, 1965; Stratman & First, 1965). The suppression of follicle growth at Day 10, except in the one pig which superovulated, and the high levels of pituitary FSH at Day 10, support the hypothesis that the ICI 33828 compound blocks LH and FSH release, but not their synthesis (Garbers & First, 1969b).

A final problem is that the within-treatment correlation between follicular fluid weight and numbers of follicles is very low and, because of this, the variable most indicative of FSH release is debatable.

The very high levels of FSH and LH in those gilts killed at 10 days probably reflects residual levels of FSH and LH not released and, since one of these gilts superovulated, the levels may reflect some continued synthesis of FSH and LH in the treated gilts. Because this single gilt did not show oestrus, levels of progesterone from previous CL may have caused the superovulation.

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