FERTILITY AFTER ESTROUS CYCLE CONTROL USING GONADOTROPIN AND PROSTAGLANDIN $F_2\alpha$ TREATMENT OF SOWS

H. D. Guthrie[1,2]

Science and Education Administration
U. S. Department of Agriculture
Beltsville, MD 20705

Summary

Two experiments were conducted to determine farrowing rates in sows after estrus and ovulation were controlled by the use of gonadotropins and prostaglandin $F_2\alpha$ ($PGF_2\alpha$). Accessory corpora lutea (CL) were induced in 74 Yorkshire and Duroc sows by an injection of 1,500 IU of pregnant mare serum gonadotropin (PMSG) followed 72 hr later by 750 IU of human chorionic gonadotropin (HCG). Thirty-eight sows (Experiment 1) were injected on days 13 and 14 after HCG with 10 mg and 5 mg of $PGF_2\alpha$, respectively. Sows were artificially inseminated on the second day of estrus after $PGF_2\alpha$ treatment with 6 billion spermatozoa in the Beltsville L-1 extender. Three groups of 12 sows each were used in Experiment 2. Group 2-1 sows were treated and inseminated as described for sows in Experiment 1. Groups 2-2 and 2-3 sows were injected on days 13 and 14 after HCG with 10 mg of $PGF_2\alpha$ and were injected at the time of a second $PGF_2\alpha$ injection with 1,000 IU of PMSG followed by 500 IU of HCG either 72 hr later (Group 2-2) or 96 hr later (Group 2-3). Groups 2-2 and 2-3 sows were artificially inseminated 30 to 32 hr after the 500 IU of HCG. Plasma progesterone was quantified in blood plasma to estimate whether corpora lutea were present at the time of blood collection.

At the time of the first $PGF_2\alpha$ injection, plasma progesterone concentrations indicated that 89% of all sows had functional CL. The CL had regressed after $PGF_2\alpha$ treatment in 87% of the sows in Experiment 1 and Group 2-1 and in 100% of the sows in Groups 2-2 and 2-3. In Experiment 1 and Group 2-1, 70% of sows had a synchronized estrus 4 to 7 days after $PGF_2\alpha$ treatment; in Groups 2-2 and 2-3, 33% and 83%, respectively, had such an estrus. Percentage of inseminated sows that farrowed in Experiment 1 and Group 2-1 combined, in Group 2-2 and in Group 2-3 was 65.5, 25 and 75, respectively. These results showed that the treatment protocol used for sows in Group 2-3 effectively controlled ovulation and might facilitate the efficient use of artificial insemination in the pig.

(**Key Words**: Pig, Prostaglandin $F_2\alpha$, Estrous Synchronization, Fertility.)

Introduction

When prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or 16-aryloxy-prostaglandin $F_2\alpha$ analogs were injected into cycling gilts or sows, luteolysis was induced only in animals that were on days 11 to 14 of their estrous cycles (Gleeson, 1974; Hallford *et al.*, 1975; Guthrie and Polge, 1976a). However, if natural luteolysis was delayed by estrogen treatment (Guthrie, 1975; Kraeling *et al.*, 1975), by gonadotropin induction of accessory corpora lutea (CL) (Guthrie and Polge, 1976b) or by conception (Diehl and Day, 1974; Guthrie and Polge, 1978), then $PGF_2\alpha$ or the 16-aryloxy analogs induced luteolysis synchronously. Gilts were in estrus 4 to 7 days after initiation of $PGF_2\alpha$ treatment, and 75% to 80% of the gilts were pregnant at

[1] Reproduction Laboratory, Animal Physiology and Genetics Institute, Beltsville Agricultural Research Center, Beltsville, MD 20705. The author expresses appreciation to The Upjohn Co., for their generous gift of prostaglandin $F_2\alpha$ (Prostin F_2 Alpha), to Kathy Ogle and Dan Deaver for their technical assistance, and to L. T. Frobish, Chief of the Non-ruminant Nutrition Laboratory, Nutrition Institute, SEA, for use of the sows.

[2] Mention of a trade name, product of specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

about 4 weeks after artificial insemination (Guthrie, 1975; Guthrie and Polge, 1976b, 1978).

Effective methods of estrus and ovulation control for cycling gilts or sows are required for the practical use of stored or frozen semen for artificial insemination. In these two experiments, the results of Guthrie and Polge (1976b) were extended to evaluate (1) luteolysis by a different $PGF_2\alpha$ compound, (2) ovulation control by gonadotropin injections after $PGF_2\alpha$ induced luteolysis, and 3) pregnancy rate at term of inseminated sows.

Materials and Methods

Experiment 1. Thirty-eight Yorkshire sows at random days of an estrous cycle and weighing an average of 152 kg were injected subcutaneously with 1,500 IU of pregnant mare serum gonadotropin (PMSG, Equinex, Ayerst Research Laboratories) in 2 ml of Sodium phosphate-buffered (pH 7.4; .01 M) saline (PBS). Sows were injected intramuscularly 72 hr later with 750 IU of human chorionic gonadotropin (HCG, APL, Ayerst Research Laboratories) to induce ovulation and the formation of accessory CL. Thirteen and 14 days after the HCG injection, 10 and 5 mg of $PGF_2\alpha$ (Prostin F_2 Alpha, The Upjohn Co.), respectively, in 2 ml of PBS were injected intramuscularly to induce luteolysis. Sows were checked for estrus daily with a boar. Sows with a synchronized estrus (4 to 7 days after the first $PGF_2\alpha$ injection) were artificially inseminated with 6×10^9 spermatozoa in 60 ml of the Beltsville L-1 (BL-1) extender (Pursel *et al.*, 1973) at 2 to 4 pm on the second day of estrus. Blood was collected on the day of the PMSG injection, the day of the first $PGF_2\alpha$ injection, 4 days after the first $PGF_2\alpha$ injection and 21 to 23 days after insemination. Plasma progesterone concentrations were quantified by radioimmunoassay (Guthrie, 1977) to monitor luteal function. Plasma progesterone in the peripheral blood of sows has been shown to vary in a cyclic pattern with peaks separated by a 5-day "trough" in which plasma progesterone was 1.0 ng/ml or less (Stabenfeldt *et al.*, 1969; Guthrie *et al.*, 1972). Assuming a 20-day cycle, this repeatable pattern in plasma progesterone was used to estimate whether the luteal phase of the experimental cycle was extended in sows that had been artificially inseminated. A decline in plasma progesterone concentration to less than 2.5 ng/ml, by 4 days after the first $PGF_2\alpha$ injection characteristic of plasma progesterone concentrations after $PGF_2\alpha$ analog treatment on day 12 of the cycle (Guthrie and Polge, 1976a), was defined as induced luteolysis.

Experiment 2. After accessory CL were induced by PMSG and HCG treatment as described in Experiment 1, 36 Yorkshire and Duroc sows weighing an average of 175.6 kg were randomly assigned to one of three treatment groups. Sows were injected 13 days after the HCG injection with 10 mg of $PGF_2\alpha$; 24 hr later, they were injected again with 5 mg (Group 2-1) or 10 mg (Groups 2-2 and 2-3) of $PGF_2\alpha$. In an effort to more exactly control the time of ovulation after $PGF_2\alpha$ treatment, sows in Groups 2-2 and 2-3 were injected at the time of the second $PGF_2\alpha$ injection with 1,000 IU of PMSG, followed by 500 IU of HCG either 72 (Group 2-2) or 96 hr later (Group 2-3). Sows in Group 2-1 were artificially inseminated as described in Experiment 1 and sows in Groups 2-2 and 2-3 were artificially inseminated 30 to 32 hr after 500 IU of HCG without regard to estrus. Blood samples were collected from all sows on the day of PMSG treatment, on the first day of $PGF_2\alpha$ treatment and 4, 15 and 28 days after the first $PGF_2\alpha$ injection. An additional blood sample was collected from sows in Groups 2-2 and 2-3 on the day of the second $PGF_2\alpha$ injection. Luteal function was monitored by plasma progesterone concentrations. The proportion of sows in estrus 4 to 7 days after the first $PGF_2\alpha$ injection and the farrowing rates were compared among groups by chi square analysis. Plasma progesterone concentrations on the day of the first PMSG injection, on the day of the first $PGF_2\alpha$ injection and on days 4 and 15 after the first $PGF_2\alpha$ injection were analyzed as separate variables by least squares analysis of variance. Duncan's Multiple Range Test was used to compare group means when significant group effects were detected.

Results

Plasma progesterone was quite variable on the day of the first $PGF_2\alpha$ injection ranging from .5 to 122.1 ng/ml in the 74 sows. Seven (9.4%) of the sows had plasma progesterone concentrations of 2.5 ng/ml or less on the day of the first $PGF_2\alpha$ injection (table 1). Six other sows, five in Experiment 1 and one in Group 2-1, had intermediate values ranging from 4.1 to 8.4 ng/ml; the others were greater than 9.0 ng/ml. Four days after the first $PGF_2\alpha$ injec-

TABLE 1. ESTRUS AND FERTILITY AFTER PROSTAGLANDIN $F_2\alpha$ ($PGF_2\alpha$)-INDUCED LUTEOLYSIS IN SOWS WITH GONADOTROPIN-INDUCED ACCESSORY CORPORA LUTEA

Sows	Experiment 1	Groups in experiment 2			Total
		1	2	3	
	(Number)				
Treated	38	12	12	12	74
Plasma progesterone (P_4) ≤2.5 ng/ml[a] at:					
1. First $PGF_2\alpha$ injection	3	1	1	2	7
2. 4 days after $PGF_2\alpha$	35	8	12	12	67
With synchronized estrus[b]	29	6	4[c]	10	49
Bred by artificial insemination (AI)	29	6	12	12	59
With nonsynchronized estrus	8	5	0	0	13
With no estrus detected	1	1	3	1	6
Ovulating after $PGF_2\alpha$ injections[d]	29	8	8	12	57
P_4 ≤ 2.5 ng/ml 28 days after PGF	4	1	4	3	12
Estrus 18 to 28 days after AI	8	1	6	2	17
Pregnancy loss 28 days after $PGF_2\alpha$ to term[e]	7	0	1	0	8
Farrowing	18	5	3[f]	9	35

[a]Defined as complete luteolysis.

[b]Estrus 4 to 7 days after the first $PGF_2\alpha$ injection.

[c]Smaller proportion of treated sows in estrus in Group 2 than Group 3 ($P<.05$, chi square).

[d]Judged by plasma progesterone >9.1 ng/ml or detection of estrus.

[e]Failure to farrow.

[f]Smaller proportion of inseminated sows farrowed in Group 2 than Group 3 ($P<.05$, chi square).

tion, 43 (86%) of 50 sows in Experiment 1 and Group 2-1 and 24 sows (100%) in Groups 2-2 and 2-3 had plasma progesterone concentrations of 2.5 ng/ml or less (table 1). The mean plasma progesterone concentration 4 days after the first $PGF_2\alpha$ injection was less for sows in Groups 2-2 and 2-3 than for sows in Group 2-1 (table 2); however, only the difference between Groups 2-1 and 2-2 was statistically significant.

Thirty-five of 50 sows (70%) had a synchronized estrus after $PGF_2\alpha$ treatment in Experiment 1 and Group 2-1 (table 1). Percentage of sows with a synchronized estrus was 38 and 83 ($P<.05$) for Groups 2-2 and 2-3, respectively. Thirteen sows, all in Experiment 1 and Group 2-1, were in estrus outside the 4- to 7-day

TABLE 2. PLASMA PROGESTERONE CONCENTRATIONS IN SOWS AT THE TIME OF THE FIRST PREGNANT MARES SERUM GONADOTROPIN (PMSG) INJECTION AND AT VARIOUS TIMES AFTER THE FIRST $PGF_2\alpha$ INJECTION IN EXPERIMENTS 1 AND 2

Experiment	Group	No. of sows	Day of PMSG injection	Plasma progesterone: Days after first PGF injection 0	1	4	15
			ng/ml (mean ± SEM)				
1		38	11.1 ± 2.0	39.3 ± 5.0		3.4 ± 1.6	
2	1	12	9.4 ± 3.4[a]	32.7 ± 7.6[a]		3.4 ± 1.5[a]	15.8 ± 2.9[a]
	2	12	9.5 ± 3.1[a]	36.1 ± 7.2[a]	16.3 ± 4.8	.6 ± .2[b]	16.6 ± 3.3[a]
	3	12	17.5 ± 6.9[a]	27.0 ± 4.3[a]	13.8 ± 3.4	.9 ± .2[ab]	38.8 ± 10.9[b]

[a,b]For Experiment 2, means in a column with no common superscript letter differ significantly by Duncan's Multiple Range Test ($P<.05$).

interval after the first $PGF_2\alpha$ injection. Six sows failed to be detected in estrus during the experiments. Three sows in Group 2-2 became anovulatory and one in Group 2-3 was pregnant; whether the other two were also anovulatory was not determined.

In Experiment 2, 28 of 36 sows were assumed to have ovulated after $PGF_2\alpha$ treatment based on by the concentration of plasma progesterone 15 days after the first $PGF_2\alpha$ injection (about 10 days after the sows should have ovulated). Plasma progesterone in sows judged to have ovulated ranged from 10.8 to 157.2 ng/ml. The seven sows judged not to have ovulated had plasma progesterone concentrations ranging from .1 to 4.2 ng/ ml. The mean plasma progesterone concentration for sows in Group 2-3 was significantly greater than that for sows in Groups 2-1 and 2-2.

As indicated in table 1, 24 of 59 inseminated sows failed to farrow pigs. In Group 2-2 four sows that were inseminated did not ovulate. Twelve sows had plasma progesterone concentrations of 2.5 ng/ml or less 28 days after the first $PGF_2\alpha$ injection; an additional eight sows presumed pregnant at that time did not farrow. Of the 20 nonfarrowing sows 17 were detected in estrus 18 to 28 days after insemination. The proportion of inseminated sows farrowing in Group 2-2 was less ($P<.05$) than that for sows in Group 2-3. Eighteen sows in Experiment 1 farrowed 10.1 ± .9 (mean ± SEM) live pigs per sow and 17 sows in Experiment 2 farrowed 9.1 ± .6.

Discussion

Plasma progesterone concentrations in the present experiments and in those of Guthrie and Polge (1976b) indicated that PMSG and HCG induced accessory CL or otherwise delayed natural luteolysis of the estrous cycle in 95% of the treated gilts and sows. Luteolysis was induced in at least 90% of the animals when sufficient amounts of $PGF_2\alpha$ or a 16-aryloxy-prostaglandin $F_2\alpha$ analog were given. The dose of $PGF_2\alpha$ required to induce luteolysis in sows in the present experiments was about 10-fold greater than the amount of the 16-aryloxy-prostaglandin $F_2\alpha$ analogs (ICI 79939 and 80996) used in the experiments of Guthrie and Polge (1976a, b, 1978) for gilts with CL of comparable stages of development.

In Group 2-3, 75% of 12 sows farrowed; in Group 2-2, only 25% of 12 sows farrowed. The reduced incidence of estrus and lower farrowing rate for sows in Group 2-2 may have been caused by the injection of HCG 72 hr after the PMSG injection. Moor *et al.* (1975) proposed that increased estradiol secretion before estrus in ewes induces the pre-ovulatory luteinizing hormone (LH) surge that, in turn, acts on the follicle to inhibit estradiol production and initiate luteinization. Injection of HCG at 72 hr may have terminated the increase in follicular estradiol secretion in many Group 2-2 sows that failed to express estrus. Other effects of a premature injection of HCG on oocyte maturation, fertilization and gamete transport are not known, but could interfere with conception.

In Experiment 1 and Group 2-1, 86% of the sows inseminated had plasma progesterone concentrations indicative of functional CL 3 weeks after insemination. Subsequently, only 66% of inseminated sows farrowed which may indicate that enough embryos were present in only partially or temporarily block luteolysis. When estrus was checked only once daily and when low numbers of spermatozoa were inseminated as in the present experiments, a single insemination 24 to 30 hr after the onset of estrus as advocated by Polge (1970) may not place adequate numbers of spermatozoa in the oviducts at the right time to maximize the chances of fertilization. A similar conclusion could be drawn from the results of Pursel *et al.* (1973).

To artificially inseminate only once at estrus, certain assumptions have been made (Polge, 1970). The assumption that the time of ovulation is about 40 hr after the endogenous preovulatory LH surge is probably valid. The timing of insemination in relation to estrus has been based upon the assumption that the onset of the preovulatory LH surge coincided with the onset of estrus. This assumption is now open to question because the preovulatory LH surge in many gilts and sows starts 24 to 36 hr before estrus is detected (Findlay *et al.*, 1974; Aherne *et al.*, 1976). Therefore, a single insemination on the second day of estrus may have been made after the animal had already ovulated.

Hunter (1967) reported that artificial insemination of gilts 10 to 14 hr after ovulation resulted in fewer gilts with fertilized ova than did artificial insemination in gilts 6 hr before through 2 hr after ovulation and also resulted in normal fertilization of only 50% of the ova recovered and a high incidence of unilateral

fertilization. Thus, in Experiment 1 and Group 2-1, late insemination could have resulted in some sows' having no fertilized ova and some sows' having too few embryos in the uterus to block luteolysis and maintain pregnancy (Polge *et al.*, 1966; 1967).

Use of only a single insemination of low numbers of spermatozoa (3 to 6 billion) will probably require ovulation control with gonadotropins or gonadotropin-releasing hormone as in Group 2-3. Alternately, if precise control of ovulation is not possible, two inseminations, one on the afternoon of the first day of estrus and one 24 hr later, should improve the chances of fertilization.

Literature Cited

Aherne, F. X., R. J. Christopherson, J. R. Thompson and R. T. Hardin 1976. Factors affecting the onset of puberty, post-weaning estrus and blood hormone levels of Lacombe gilts. Can. J. Anim. Sci. 56:681.

Diehl, J. R. and B. N. Day. 1974. Effect of prostaglandin $F_2\alpha$ on luteal function in swine. J. Anim. Sci. 39:392.

Findlay, J. K., J. M. Buckmaster, I. A. Cumming, J. R. Goding, H. A. Jonas and R. A. S. Lawson. 1974. Gonadotrophin release in the sow after weaning and after treatment with gonadotrophin-releasing hormone (GnRH). J. Reprod. Fertil. 36:476 (Abstr).

Gleeson, A. R. 1974. Luteal function in the cyclic sow after infusion of prostaglandin $F_2\alpha$ through a uterine vein. J. Reprod. Fertil. 36:487.

Guthrie, H. D. 1975. Estrous synchronization and fertility in gilts treated with estradiol-benzoate and prostaglandin $F_2\alpha$. Theriogenology 4:69.

Guthrie, H. D. 1977. Induction of ovulation and fertility in prepuberal gilts. J. Anim. Sci. 45:1360.

Guthrie, H. D., D. M. Henricks and D. L. Handlin. 1972. Plasma estrogen, progesterone and luteinizing hormone prior to estrus and during early pregnancy in pigs. Endocrinolgoy 91:675.

Guthrie, H. D. and C. Polge. 1976a. Luteal function and oestrus in gilts treated with a synthetic analogue of prostaglandin $F_2\alpha$ (ICI 79,939) at various times during the oestrous cycle. J. Reprod. Fertil. 48:423.

Guthrie, H. D. and C. Polge. 1976b. Control of oestrus and fertility in gilts with accessory corpora lutea by prostaglandin analogues, ICI 79,939 and ICI 80,996. J. Reprod. Fertil. 48:427.

Guthrie, H. D. and C. Polge. 1978. Treatment of pregnant gilts with a prostaglandin analogue, Cloprostenol, to control oestrus and fertility. J. Reprod. Fertil. 52:271.

Hallford, D. M., R. P. Wettemann, E. J. Turman and I. T. Omtvedt. 1975. Luteal function in gilts after prostaglandin $F_2\alpha$. J. Anim. Sci. 41:1706.

Hunter, R. H. F. 1967. The effects of delayed insemination on fertilization and early cleavage in the pig. J. Reprod. Fertil. 13:133.

Kraeling, R. R., C. R. Barb and B. J. Davis. 1975. Prostaglandin induced regression of porcine corpora lutea maintained by estrogen. Prostaglandins 40:604.

Moor, R. M., M. F. Hay and R. F. Seamark. 1975. The sheep ovary: regulation of steroidogenic, haemodynamic and structural changes in the largest follicle and adjacent tissue before ovulation. J. Reprod. Fertil. 45:595.

Polge, C. 1970. Developments in the artificial breeding of pigs. Proc. S. Africa Soc. Anim. Prod. 9:13.

Polge, C., R. M. Moor, B. N. Day, W. D. Booth and L. E. A. Rowson. 1967. Embryo numbers and luteal maintenance during early pregnancy in swine. J. Anim. Sci. 26:1499 (Abstr.).

Polge, C., L. E. A. Rowson and M. C. Chang. 1966. The effect of reducing the number of embryos during early stages of gestation on the maintenance of pregnancy in the pig. J. Reprod. Fertil. 12:395.

Pursel, V. G., L. A. Johnson and L. L. Schulman. 1973. Fertilizing capacity of boar semen stored at 15C. J. Anim. Sci. 37:532.

Stabenfeldt, G. H., E. L. Akins, L. L. Ewing and M. C. Morrissette. 1959. Peripheral plasma progesterone levels in pigs during the oestrous cycle. J. Reprod. Fertil. 20:443.