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Suppression of LH Concentration by Difluoromethylornithine in Gilts

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Gilt에 있어서 Difluoromethylornithine에 의한 LH분비 억제

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적 요

Ornithine decarboxylase(ODC)가 polyamine 생합성에 주요 효소로 역할을 하지만 difluoromethylornithine(DFMO)은 polyamine 합성에 억제자로 작용하고 있다. Cycling crossbed gilt들을 무작위로 두 그룹으로 분배하였다(6/group). Indwelling silicone catheter를 모든 동물의 경정맥에 수술을 통해 이식하였다. DFMO는 생리식염수(20 mg/ml)에 용해하여 매일 80 mg/kg을 i.m.으로 주사하였으며 대조구에게는 같은 양의 생지식염수만을 주사하였다. DFMO는 estrous cycle day 16일 부터 21일 혹은 발정때까지 하루 3번(08 : 00, 16 : 00, 24 : 00h) 주사하였다. Day 14일 부터 마지막 DFMO주사 이틀 후까지 하루 한번씩 10 ml의 혈액을 채취하였다. Day 16일 부터 21일까지 매일 다른 gilt로 부터 8시간 동안(08 : 00~16 : 00h) 15분 간격의 주기로 window 혈액을 채취하였다. Serum으로 부터 progesterone(P_4), Estradiol(E_2), LH 및 FSH를 측정하였다. P_4와 E_2는 DFMO처리에 관계없이 follicular phase동안에 전형적인 profile을 보였다. DFMO처리는 발정직전의 LH농도를 저하시켰지만($p<0.01$), FSH에는 아무런 영향을 미치지 않았다.

결론적으로 gilt에 있어서 DFMO는 LH분비에 억제적인 영향을 미치는 한편, P_4, E_2 및 FSH에는 별로 영향을 미치지 않음이 나타났다.

Ⅰ. INTRODUCTION

The polyamines are a group of polycationic molecules found ubiquitously in mammalian cells and tissues(Pegg and McCann, 1982; Aslam et al., 1987). They play important roles in cell proliferation, differentiation(Persson et al., 1985; Pegg and McCann, 1988). Ornithine decarboxylase(ODC), the enzyme catalyzing the conversion of ornithine into putrescine, is the rate-limiting step in the biosynthesis of polyamines. Controlling the availability of the enzyme has the potential for exerting control over some aspects of reproduction by blocking the activity of ODC(Persson et al., 1985; Nicholson

et al., 1988). The highly specific, irreversible inhibitor difluoromethylornithine(DFMO) can block the activity of ODC.

Studies utilizing DFMO to block ODC activity
and polyamine formation in the pituitary have been limited mainly to rodents. Both ODC activity and amount of polyamines increased in the pituitary gland during proestrus in the rat (Persson et al., 1985; Nicholson et al., 1988). This increase suggests an involvement of ODC and polyamines in LH secretion. The administration of DFMO to rats during the afternoon of proestrus resulted in a dose-related reduction in the concentration of LH in plasma (Aslam et al., 1987; Nicholson et al., 1988; Nicholson and Wynne-Jones, 1989).

There are no known studies which define the relationship between administration of DFMO and the reproductive hormone profiles in domestic species. Therefore, our hypothesis is that administration of DFMO during follicular phase of the cycle in the pig does not alter the concentration of progesterone(P_4), estradiol(E_2), LH and FSH.

Ⅱ. MATERIALS AND METHODS

1. Experimental animals

Twelve crossbred gilts, 8 to 9 mo of age, were checked once daily for estrous in the presence of mature boar. Gilts that had exhibited at least two consecutive estrous cycles were randomly assigned to one of two treatment groups (6/treatment). Before day 14 of the estrous cycle(day zero being the first day of estrus), Silastic^{TM}(Dow Corning, Midland, MI) catheters were surgically implanted in the jugular vein of the gilts.

Fifteen cm of a 90 cm catheter(O.D. 3.18 mm, I.D. 1.57 mm) were inserted into a jugular vein via the cephalic vein. Gilts were maintained on individual 46 cm catheters under standard management conditions. All gilts received 2.3 kg/day of a standard corn-soybean with water allowed *ad libitum*.

2. Treatment and sample collection

DFMO(MDL 71,782A, Merrell Dow Research Institute, Cincinnati, OH) was dissolved in saline(200 mg/ml) and administered by i.m. injection at a dose of 80 mg/kg/day. The DFMO was administered via three injections per day (08 : 00, 16 : 00, 24 : 00h) from estrous cycle days 16 to 21 or until estrous. The control animals received an equivalent saline injection as the treatment group.

Blood smples(10 ml) were collected from all gilts once daily from day 14 until two days after the last DFMO treatment. In addition, one gilt/treatment group was assigned to a blood collection window(6 gilts/treatment, 6 windows/treatment). Blood samples(10 ml) from each window interval were collected every 15 min for 8 h(from 08 : 00 to 16 : 00h) starting on day 16 and continuing until day 21. All blood samples were allowed to clot for 30 min, then placed on ice until centrifugation at 4℃ for 25 min at 1900 × g. Serum samples were stored at −20℃ until assayed for hormones.

3. Radioimmunoassay of progesterone

Serum samples were assayed for P_4 in duplicate by using a specific, solid-phase ^{125}I-radio immunoassay kit(Coat-a-Count^{TM}). All samples were analyzed in a single assay(C.V. = 8.5%) with an assay sensitivity of 0.034 ng/ml.

4. Radioimmunoassay of estradiol-17β

Serum concentrations of E_2 were measured using antiserum(Gift from Dr. Norm Mason,

Indianapolis, Indiana) which was diluted 1 : 30, 000. The procedure was based on that of Breuel et al.(1988) with some modifications. The average recovery was 82.5% and the intraassay and interassay coefficients of variation were 10.6% and 17.9%, respectively with an assay sensitivity of 0.2 pg /ml. We have presented values corrected for recovery losses.

5. Radioimmunoassay of LH

Serum porcine LH(pLH) concentrations were determined in duplicate by a specific double-antibody method(Akinlosotu et al., 1986). The intraassay and interassay coefficients of variation were 6.7% and 12.8%, respectively with an assay sensitivity of 0.4 ng /ml.

6. Radioimmunoassay of FSH

Serum porcine FSH(pFSH) concentrations were determined in duplicate by a specific double-antibody method. The procedure was previously described by Guthrie and Bolt(1983), modified by Esbenshade and Britt(1985) with some modifications. Three serum pools across three assays had intraassay and interassay coefficients of variation of 9.3% and 14.1%, respectively.

7. Statistical analyses

The experimental design used in this study was a completely randomized split plot(Steel & Torrie, 1980). The LH pulse frequency(peaks /8h) and mean LH pulse amplitude(ng /ml) were calculated by the method of Christian et al.(1978). Probability values less than 0.05 were considered significant.

Ⅲ. RESULTS

The profile of jugular vein serum P_4 during the follicular phase of the cycle is presented in Fig. 1. Seventy-six percent of the decrease occurred during the first control sampling day. The level was below 1 ng /ml for the next two days prior to the first day of post-DFMO estrous. After estrous, the P_4 concentration increased rapidly. Treated gilts showed a similar pattern throughout the sampling period. Serum P_4 concentration(mean±SE) was not different between treatments.

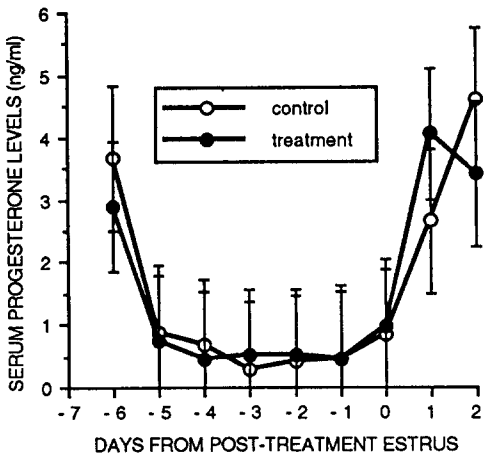

Fig. 1. Mean serum progesterone concentrations on day-6 through 2 days after estrous(day 0).

The mean serum E_2 concentrations(pg /ml) are presented in Fig. 2. Serum E_2 concentrations began to rise coincident with the decrease of serum P_4. When E_2 concentrations were compared between treatments, no differences were found.

The serum LH profiles are presented in Fig. 3. The peak concentration occurred on day −1 in both groups. When the two treatments were compared by day, no differences were found($p>0.05$) except on day −2(one day before LH peak). Statistical analysis of LH concentration across days indicated a definite treatment effect($p<0.01$). LH pulse patterns from sampling windows showed no difference($p>0.05$) between control and treatment group for frequency

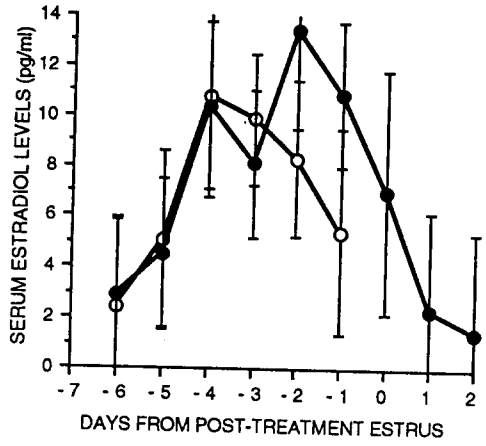

Fig. 2. Mean serum estrdiol on day-6 through 2 days after estrous(day 0).

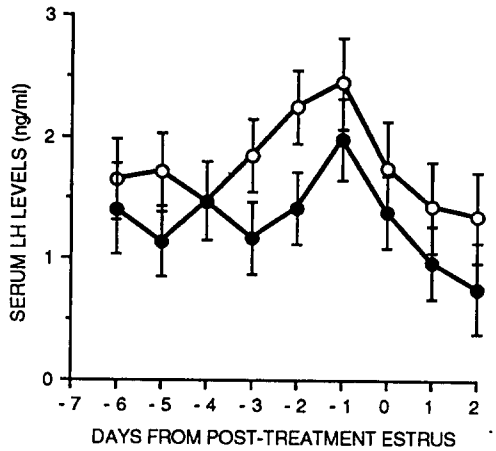

Fig. 3. Mean serum LH concentratrations on day-6 through 2 days after estrous(day 0). Mean concentration averaged across all days was different among treatments($p< 0.01$)

and mean amplitude of LH secretory spikes. However, mean LH pulse concentration in the control group increased ($p<0.001$) in both the morning(0~240 min) and afternoon(240~480 min) compared to treatment group(Fig. 4).

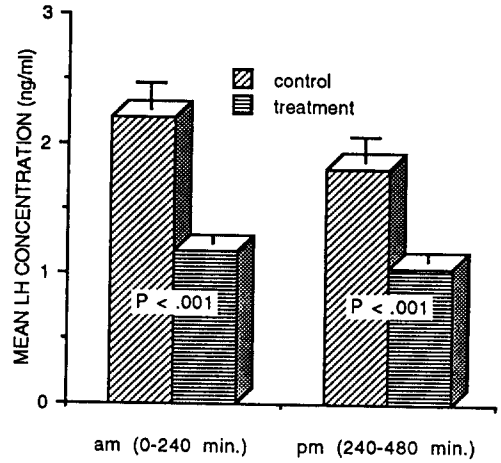

Fig. 4. Mean serum LH concentrations averaged across all window days.

Two periods of distinctly elevated FSH concentrations were observed during the periovulatory period in the control group(Fig. 5). Following the preovulatory FSH peak, mean FSH declined during the next 3 days. The second FSH surge(postovulatory elevation) began approximately 48 hr after the LH peak. Serum concentrations of FSH were not affected ($p>0.05$) by DFMO.

Ⅳ. DISCUSSION

The dosage of DFMO at 80 mg /kg /day was adjusted for the pig body weight and metabolic rate(Blaxter, 1989) based on the dosage previously shown to be effective in rats(Aslam et al., 1987). Since DFMO has a relatively short half-life of 3 or 4 h(Haegele et l., 1981), the daily dosage was administered in three equal portions at 8 h intervals. Also, since it is not known when ODC activity might be greatest in the pig, treatment was begun on day 16 in an effort to include a physiological state equal to that occuring on day of proestrous in rats.

Both P_4 and E_2 profiles were norml during the follicular phase regardless of DFMO treatment. Maximal value of serum P_4 was reached at day 14 of the cycle before drastically falling on day 15, which corresponds with the demise of luteal function. Serum concentrations of E_2 did not begin to increase during proestrous until serum P_4 fell, which confirmed an earlier study(Henricks et al., 1972). The rapidly increasing P_4 concentration after estrous was a strong indication of a normal CL development. Results of our study suggest that 80 mg /kg /day of DFMO has little or no effect at the ovarian level during the follicular phase of the cycle of the pig. This is in contrast with previous work in rat(Nicholson et al., 1988) in which DFMO blocked a rise in P_4 levels and caused the decline in plasma E_2 concentrations. It might be due to differences in frequency of drug administration, drug dosage or physiological differences between pigs and rats.

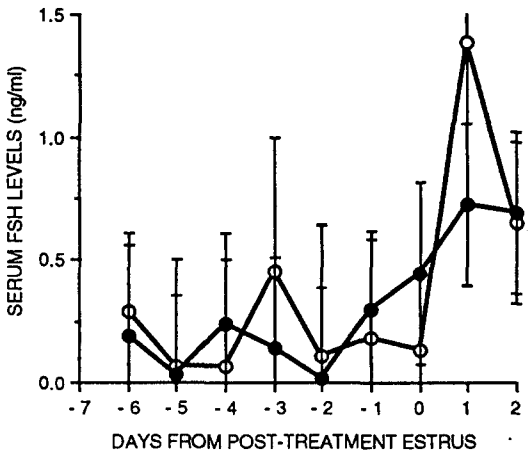

Fig. 5. Mean serum FSH concentrations on day-6 through 2 days after estrous(day 0).

The significantly lower LH concentration in the treatment group as compared to the controls of the current work clearly demonstrate that DFMO suppressed LH secretion during the proestrous /estrous periods. Since DFMO is specific for ODC, the lower LH at this time suggests that a positive relationship exists between ODC activity and LH concentration. Hence, LH concentration was decreased by inhibiting ODC activity. Reports by Aslam et (1987), Nicholson et al.(1988) and Nicholson and Wynne-Jones(1989) showed lower plasma LH levels in the DFMO-treated rats.

Two elevated FSH concentrations(preovulatory peak and postovulatory elevation) were seen in control group, which is in agreement with Kelly et al.,(1988). Higher FSH concentration during the secondary surge may be responsible for the recruitment of greater numbers of follicles(Knox et al., 1991). Although no significant effect of DFMO on FSH was detected, the two FSH peaks did not occur in the treatment group. This confirms the study showing no effects of DFMO on FSH in rats (Nicholson & Wynne-Jones, 1989). It is possible that DFMO acts on separate releasing factors or mechanisms for two pituitary hormones, resulting in dissociated release of LH and FSH.

In conclusion, DFMO had a selective inhibitory effect on LH secretion in the pig, but did not affect P_4, E_2 and FSH release. However, it would be premature to suggest a mechanism of the role of ODC on the pituitary-ovarian axis.

V. SUMMARY

While ornithine decarboxylase(ODC) is considered a key enzyme in the biosynthesis of polyamines, difluoromethylornithine(DFMO) acts as an inhibitor of polyamine synthesis. Cycling crossbred gilts were randomly assigned to one of two(treatment and control) groups(6 /group). An indwelling silicone catheter was surgically implanted in the jugular vein of each animal. DFMO was dissolved in saline(200 mg /ml) and

adminstered by i.m. injection at a dose of 80 mg/kg/day. The control group received an equivalent volume saline injection. DFMO was injected 3 times daily(08 : 00, 16 : 00, 24 : 00h) from day 16 of estrous cycle to 21 or until estrus. Once daily blood samples(10 ml) were taken from day 14 until two days after the last DFMO treatment. Window blood samples were collected every 15 min for 8 h(from 08 : 00 to 16 : 00h) starting on day 16 and continuing until day 21 from one gilt per day. Serum progesterone(P_4), estradiol(E_2), LH and FSH were measured. Typical concentration profiles for P_4 and E_2 were seen during the follicular phase regardless of DFMO treatment. Injection of DFMO suppressed the preovulatory LH concentration in the serum($p<0.01$) while having no effect on FSH profile. The present results indicate that DFMO had an inhibitory effect on LH secretion in the pig, but did not affect P_4, E_2 or FSH release.

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