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GONADOTROPHIN RELEASING HORMONE TREATMENT OF HOLSTEIN COWS WITH FOLLICULAR CYSTS MONITORED BY SKIM MILK PROGESTERONE DETERMINATION

By

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SUMMARY

Progesterone was assayed in skim milk fraction of 38 cystic cows, 26 (68.4%) of which had basal (<0.5 ng/ml) progesterone concentration and were diagnosed as having follicular cysts. These cows were allotted at random to one of 3 treatments: (1) a sham injection of sterile water in 7 cows as controls; (2) a single injection (1 mg) of GnRH analog in 8 cows; (3) double injections (1 mg) of GnRH analog at 7-day interval in all cows. According to rectal palpation confirmed by skim milk progesterone determination, the double injection gorup responded well ($P < 0.05$) compared to the single injection and control groups (81.8% vs 62.5% vs 42.9%). The interval from calving to 1st insemination, interval from treatment to conception and interval from calving to conception were significantly ($P < 0.01$) shorter in the double-injection group than in the other 2 groups. Moreover, the conception rate to first insemination was significantly ($P < 0.05$) higher (63.6% vs 37.5% vs 42.9%). The data provide an evidence for reduction in infertility and reproductive disorders in cystic cows given double injections of GnRH.

INTRODUCTION

Cystic ovarian degeneration associated with infertility has been reported in cows for more than a century. Despite considerable efforts by clinicians and researchers to discover the etiology and successful methods for treatment of this syndrome is responsible for considerable economic loss in dairy herds. The incidence of this condition may increase to 47% (Nessan et al., 1977; Peralta & Ax, 1982; Marcek et al., 1985).

The routine therapy employed for the treatment of cystic ovarian degeneration consists of the injection of a single dose of either HCG (Nessan et al., 1977; Kesler and Garverick, 1982), $PGF_2\alpha$ (Dobson et al., 1977; Eddy, 1977 and Humblot & Thibier, 1980) or GnRH (Manns and Richardson, 1976; Nessan et al., 1977; Kesler et al., 1981; Kesler and Garverick, 1982 and Dinsmore et al., 1987 & 1989). The results of these treatments in terms of fertility were not satisfactory and this may be due either to the improper regimens used in these treatments or to the incorrect pre-treatment differentiation between follicular and luteal cysts by rectal palpation of the ovaries (Nakao et al., 1983 and Dinsmore et al., 1989). Therefore, the differential diagnosis of the ovarian cysts by determination of progesterone concentration in blood or milk followed by using an appropriate method of GnRH treatment may have an important role in the improvement of fertility of the affected cows.

The purpose of the present investigation was to evaluate what improvement in terms of fertility

would be brought about if skim milk progesterone values were monitored before and after GnRH treatment which was used in a manner diferent from that has been previously reported.

MATERIAL AND METHODS

Animals:

A total of 38 Holstein cows, maintained at the dairy barn of Michigan State University, were diagnosed via rectal palpation as having ovarian cysts. Findings were carefully recorded as to location, size, number and characteristics of the cysts. Oestrual behaviour of the cows prior to treatment was also recorded as anoestrus or nymphomanic. Age of the 38 cows averaged 6.2±1.8 years and the mean interval between the last calving and the start of the experiment was 95±10.8 days. None of these animals had been given any treatment for the ovarian dysfunction. Pre-treatment skim milk progesterone concentration was measured 3 times every 72 hours to differentiate between the different types of the cysts. Twenty-six cows had a base-line (<0.5 ng/ml) progesterone concentration and were judged to have follicular cysts. The other 12 cows had a higher (>1 ng/ml) 12 cows were not included in this study. The selected 26 cows were assigned to one of the following treatment regimens: (a) a sham injection of sterile waters in 7 cows as controls; (b) a single injection (1 mg) of GnRH analog (Receptal, Hoechst) in 8 cows; (c) double injections (1 mg) of GnRH analog at a 7-day interval in 11 cows. Approximately, 10 days after the start of treatment all animals were re-examined rectally for morphologic changes in the ovaries confirmed by changes in skim milk progesterone levels. Animals establishing a normal post-treatment oestrous cycle were inseminated at the observed oestrus and pregnancy was verified by rectal examination 2 months later.

Sampling:

For the correct judgement of luteiniza following treatment, progesterone values determined in milk samples collected daily for days from both GnRH treatment groups (day treatment = day 0) and in milk smaples collec for the same interval during the fi spontaneously recovered oestrous cycle of control gorup (day 0 = day of the obser oestrus).

Stripping samples (samples taken by ha immediately after machine milking) we collected into sterile plastic bags (Whirl-Pa Nasco) and kept at -15°C before transferring the laboratory within 2 hours. The samples we cooled in the refrigerator for one hour a centrifuged at 4229g for 10 minutes. The ski milk was removed and stored at -20°C un assayed. Three samples were separated into flocculent precipitate and a clear fluid on thawi and were rejected from the hormonal assay.

Radioimmunoassay of progesterone:

Progesterone (P4) was assayed without extracti from skim milk by using the FAO/IAE solid-phase RIA kit (Coat-a-Count, Diagnos Products Corporation). The intra-ass coefficients of variation averages 3.8% at 1 mg/ml and 3.4% at 5.3 ng/ml (n=9). T inter-assay coefficients of variation averag 9.6% at 1.2 ng/ml and 5.7% at 5.3 ng/ml (n= The sensitivity, specificity and accuracy of th method have been previously described (Eissa al., 1994).

Statistical analyses:

Hormonal variations were tested by least-square analysis of variance using the general linea models procedures of the Statistical Analysi System (SAS, 1990). Differences between group

concerning fertility data and conception rates were respectively, measured by one way analysis of variance and Chi-square analysis (Snedecor and Cochran, 1967). The data were expressed as mean ± SE.

RESULTS

Clinical Findings:

Oestrous behaviour of the cows prior to treatment was similar in all cows with 85% (n = 22) of the cows being anoestrus. Thirty-two percent of the cysts were 2.5-3 cm in diameter, 57% were 3-4 cm and 11% were over 4 cm. Single cysts were predominant (74%) with the highest number occurring on the right ovary. Multiple cysts were detected with lower frequency and occasionally cases were found to involve both ovaries.

Clinical response and post-treatment fertility parameters are indicated in Table 1.

Of the 19 treated cows, 14 (73.7%) responded well, as indicated by the increased skim milk progesterone concentrations, within 30 days of GnRH injection compared to 3 (42.9%) control cows which recovered spontaneously within 90 days after the treatment.

The mean interval between parturition and 1st insemination (1st oestrus) as well as between treatment and conception was 34 and 29 days, respectively, shorter ($P < 0.01$) in cows with double GnRH injections than those with a single injection. Moreover, the interval between calving and conception was significantly ($P < 0.05$) shorter (22 days) in the first than in the latter gorup. The conception rate was significantly ($P<0.05$) higher in the double injection gorup. On the whole, the previously mentioned intervals were significantly shorter in the treated than in the control animals and the conception rate was higher ($P < 0.05$) in the former gorup.

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Table 1: Comparative reproductive performances of cystic cows treated with GnRH analog (single or double injections) or untreated (controls).

Treatment	No. of cows responded	Interval calving to 1st A.I. (days)	Interval treatment to conception(days)	Interval calving to conception (days)	No. of cows conceived on 1st A. I.
Single injection (n = 8)	5 (62.5%)$^{(a)}$	103.4±9.5$^{(a)}$	57.6±4.2$^{(a)}$	104.3±9.2$^{(a)}$	3 (37.5%)$^{(a)}$
Double injections (n = 11)	9 (81.8%)$^{(b)}$	69.1±3.2$^{(c)}$	28.3±1.8$^{(c)}$	82.6±5.3$^{(b)}$	7 (63.6%)$^{(b)}$
Single + double injections combined (n = 19)	14 (73.7%)$^{(a)}$	86.5±5.2$^{(b)}$	43.2±3.3$^{(c)}$	93.5±6.4$^{(c)}$	10 (52.6%)$^{(b)}$
Control (n = 7)	3 (42.9%)$^{(c)}$	108.6±8.6$^{(a)}$	72.5±6.3$^{(a)}$	131.7±11.3$^{(a)}$	3 (42.9%)$^{(a)}$

a & b : Significant at 5 % level

a & c : Significant at 1 % level

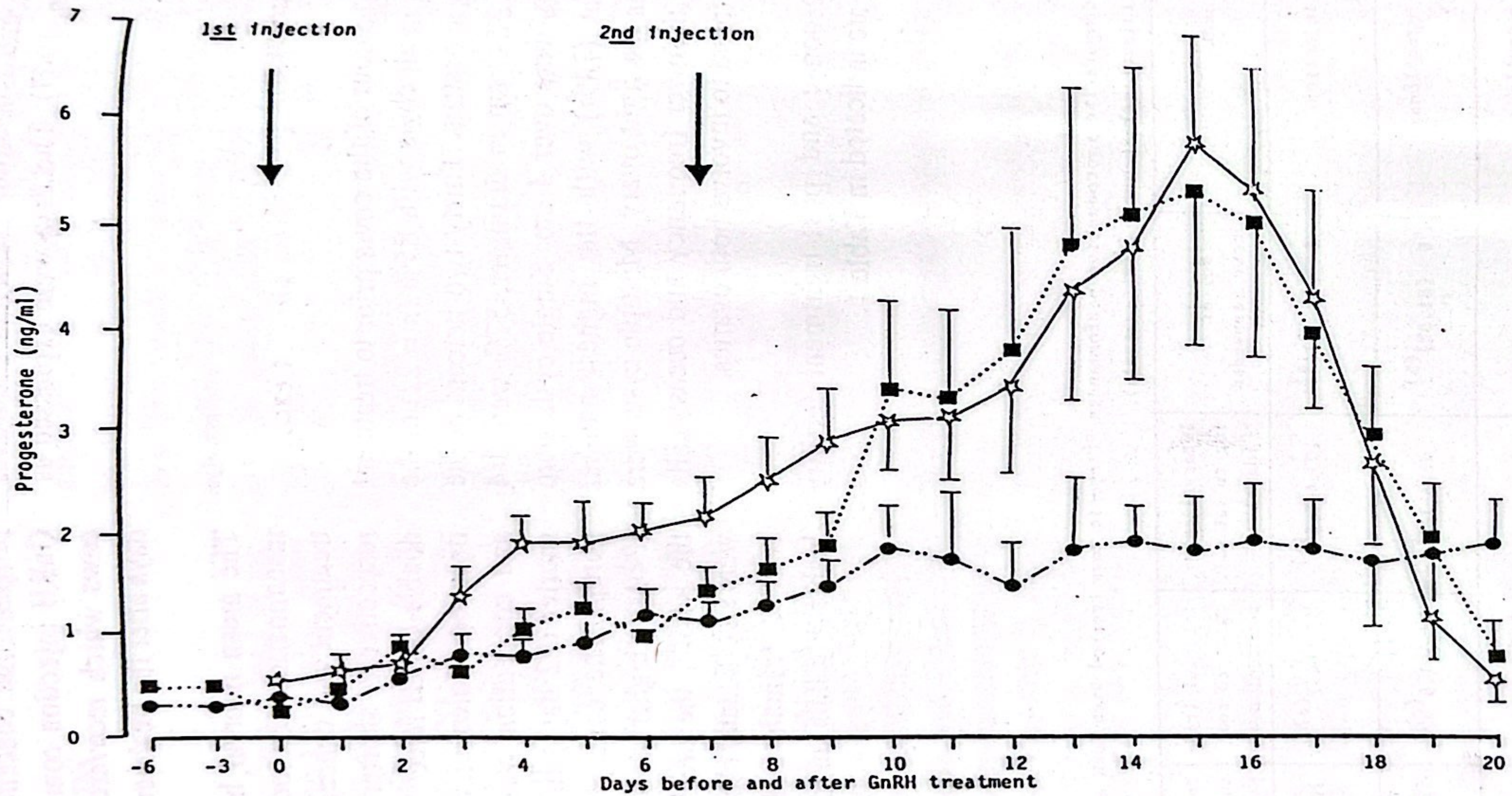

Fig. 1: Skim milk progesterone concentrations (mean ± SE) in cystic cows treated with a single (●) and double (■) injections of GnRH or untreated controls (☆). The point designated 0 represents the day of GnRH injection in the treated animals and represents the day of observed oestrus of the spontaneously recovered oestrous cycle in control cows.

Hormonal concentrations:

Skim milk progesterone values for the treated and control cows with ovarian cysts before and after treatment are shown in Fig. 1.

The cows with a single injection showed slow increase in progesterone concentrations 3 days after treatment and the values did not exceed 3 ng/ml until the end of sampling period. On the other hand, animals with double injections showed a significant ($P < 0.05$) increase in progesterone concentration in excess of 3 ng/ml 3-4 days after the second injection. Progesterone values in the latter gorup increased progressively thereafter to reach over 5 ng/ml 4-6 days later on and decreased significantly ($P < 0.01$) at the end of sampling period. Progesterone concentration of the double injection group approximated those of the control animals during the second half of the sampling period.

DISCUSSION

Clinical findings of cows diagnosed as having ovarian cysts in this experiment were similar to previous studies with regard to size of cysts, occurrence of multiple cysts and location (Bierschwal, et al., 1975; Marcek et al., 1985; Dinsmore et al., 1989). The percent of anoestrous cows was slightly higher than those reported by Bierschwal et al. (1975), Nessan et al., (1977), Humblot and Thibier (1980).

The response to treatment obtained with double injections of GnRH was better than after a single one both in this study and those reported by Kesler et al. (1981), Nakao et al. (1983) and Dinsmore et al. (1987). Moreover, double GnRH injections resulted in a shorter non-pregnant period and 64% of these animals could be inseminated and diagnosed pregnant to 1st A.I. Before 50 days, compared to only 37% of the single injection and 43% of the control group. This way of using double GnRH injections seemed more efficient than did the reported by Manns and Richardson (1976), Humblot and Thibier (1980) and Dinsmore et al. (1989) who recorded a conception rate ranging from 50-55% following a single GnRH injection.

The higher success rate of double GnRH injection in this study can be explained after skim milk progesterone monitoring. Five cows (62.5%) of the single injection group displayed slight increases in progesterone concentrations and were considered as having follicular cysts in which luteinization was insufficient and the cysts remained after treatment. In contrast, complete luteinization had occurred after the 2nd GnRH injection in the other gorup of cows with regression of lutein tissues after the end of sampling period as did the control animals.

Although the mechanism by which double treatments reestablish ovarian cycle with higher conception rate have not been fully established; yet extensive investigation has provided considerable insight regarding the mechanism of response to GnRH treatment. The higher skim milk progesterone concentration secreted by the luteinized ovarian cysts following the 2nd injection play a major role in post-treatment concentrations of LH and oestradiol-17β (Grunert, 1972; Grunert et al., 1974; Seguin et al., 1976; Kesler et al., 1981). Progesterone appears to have a negative feed back on the hypothalamus and pituitary on LH release (Kesler et al., 1980; Zaied et al., 1981; Kesler and Garverick, 1982) and content of progesterone in ovarian cyst fluid is negatively correlated with plasma oestradiol-17β levels (Hernandez-Ledezma et al., 1982). Therefore, the higher skim milk progesterone concentrations following the second GnRH injection resulted in the decrease in pulsatile LH concentration and slow down progressive follicular development as well as oestradiol synthesis with subsequent normal follicular

growth in conjunction with normal ovulation occurring at the post-treatment oestrous cycle.

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