DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(20), 2377-2384 (1995)

COMMUNICATIONS

THE EFFECT OF CONSTANT DELIVERY OF GONADOTROPIN RELEASING HORMONE ON FERTILITY OF CATTLE

R.J. Favero, L.C. Cruz[a] and D.J. Kesler[b]

Department of Animal Sciences
University of Illinois
Urbana, IL 61801

ABSTRACT

Four hundred and three (403) postpartum beef cows, synchronized with the norgestomet and estradiol valerate bovine estrus synchronization procedure, were included in three experiments. In the first experiment, 78 of the 178 cows were administered biodegradable microcapsules containing 180 μg GnRH manufactured to biodegrade (and release GnRH relatively constantly) in approximately three days. In the second experiment, 38 of the 90 cows were implanted with osmotic pumps designed to deliver 2.5 μg of GnRH per hour for six days. The third experiment consisted of two trials. In the first trial, 21 of the 41 cows were implanted with biodegradable implants manufactured to delivery 250 μg of GnRH (relatively constantly) over a four day period. In the second trial, 46 of the 94 cows were implanted with same implant as in trial 1 except the implants were coated so that there was a slight delay in initiating delivery after implantation. The constant delivery of GnRH during the proestrus period reduced pregnancy rates when GnRH was administered via osmotic pumps, microcapsules, and implants. Constant delivery implants with a delay in release after implantation, however, had no effect on pregnancy rates. In summary, constant delivery of GnRH, via all of the delivery systems, was not considered a valid method of enhancing fertility in proestrus cattle.

---

[a] Philippine Carabao Center, DCIEC Building, NIA Complex, EDSA, Diliman, Quezon City, Philippines.
[b] Correspondence: 1207 W. Gregory Dr., Urbana, IL 61801.

INTRODUCTION

Various programs have been developed to synchronize estrus in beef females. Estrus synchronization programs can be separated into two categories: luteolytic agents or combinations of progestins and luteolytic/anti-luteotropic agents. Programs using luteolytic agents (prostaglandin $F_{2\alpha}$; $PGF_{2\alpha}$) hasten estrus in females with mature corpora lutea (day 5 or greater of the estrous cycle). The problems with $PGF_{2\alpha}$ are it's ineffectiveness in anestrous females and the variable interval from injection to estrus. Therefore, estrus detection or two timed artificial inseminations are required to achieve acceptable pregnancy rates (8).

Programs utilizing progestins and luteolytic/anti-luteotropic agents are effective in inducing ovulation in anestrous females and have more predictable intervals from progestin withdrawal to estrus allowing for a single timed artificial insemination. Hixon et al. (4) found that following the Syncro-Mate B (SMB) treatment (7), 60% of the cows included in the study experienced spontaneous preovulatory luteinizing hormone (LH) surges 30 to 34 hours after implant removal. Also the administration of gonadotropin releasing hormone (GnRH) between the time of norgestomet implant removal and timed artificial insemination, during the anticipated time of the LH surge, has been shown to increase pregnancy rates in females treated with norgestomet and $PGF_{2\alpha}$ (10, 11, 12). However, this program required additional animal handlings. Troxel et al. (10) treated cows with the conventional SMB procedure followed by GnRH 30 hours after implant removal and this procedure increased the pregnancy rate in both cyclic and anestrous GnRH treated beef cows, but again required additional animal handlings for treatments as compared to SMB alone.

Capel et al. (2) demonstrated that luteal activity could be induced in anestrous cows by administration of GnRH (0.5 to 2.5 μg/hour) either by constant infusion or by pulses every 2 hours with a total treatment duration of 48 hours. Similarly Bishop et al. (1) were able to overcome a nutritionally induced anestrus in beef cows by pulsatile infusion of GnRH at a rate of 0.5 to 2.0 μg GnRH per hour.

The purpose of the experiments reported herein were to investigate the effect of GnRH administered in constant release formulations at low levels beginning at the time of norgestomet implant removal. Previous experiments in this laboratory have demonstrated the

efficacy of GnRH administration to synchronized cows between the time of norgestomet implant removal and artificial insemination. Administration of GnRH in manners similar to those reported herein would allow the administration of GnRH, without increasing the number of animal handlings.

MATERIALS AND METHODS

Three experiments with crossbred beef females maintained at the Dixon Springs Agricultural Center at Simpson, IL.

Experiment 1. One hundred and seventy eight (178) cows were synchronized with SMB. The SMB[c] treatment consists of an intramuscular injection of norgestomet (3.0 mg) and estradiol valerate (5.0 mg) in sesame oil/benzyl alcohol and a hydron ear implant that contains 6.0 mg norgestomet. The implant was subcutaneously inserted into the convex surface of the middle or distal one-third of the ear. At the end of 9 days the norgestomet implants were removed. At SMB implant removal cows were assigned to one of two groups. Seventy-eight (78) of the cows were administered biodegradable microcapsules containing GnRH. The other one hundred (100) cows served as controls. There were fewer cows in the treated group than the control group because of a limited availability of microcapsules. The microcapsules consisted of GnRH entrapped in poly (DL-lactide-co-glycolide) by a phase separation process. They were sterilized with gamma irradiation and stored in a free-flowing powder of spherical particles at room temperature until used. For injection, the microcapsules were suspended in a solution of 2 % (by weight) carboxymethylcellulose and 1 % (by weight) tween 20 in saline. Each treated cow received a single intramuscular injection of 100 mg of microcapsules containing 180 μg of GnRH. All females were artificially inseminated approximately 48 hours after SMB implant removal.

Experiment 2. Ninety (90) cows were synchronized with SMB. At the time of implant removal the cows were assigned to one of two groups. Thirty eight (38) of the cows were implanted with osmotic pumps[d] containing GnRH. The remaining cows (n = 52) served as controls and received no further treatment. There were fewer cows in

---

[c] Sanofi Animal Health, Overland Park, KS.
[d] Alza Corporation, Palo Alto, CA.

the treated group than the control group because of a limited availability of osmotic pumps. The GnRH was administered at a rate of approximately 2.5 μg/hr for 6 days via primed osmotic pumps (Alzet; model 2001). The osmotic pumps were primed by placing them in a room temperature (21°C) saline solution for 12 hours prior to administration to the animal. By being primed in this manner, the osmotic pumps began delivering GnRH upon implantation. The osmotic pumps were subcutaneously implanted over the rib cage at the time of SMB implant removal. All cows were artificially inseminated approximately 48 hours after SMB implant removal.

The osmotic pumps were surgically implanted without anesthesia with a scalpel, a hemostat to separate the skin from the subcutaneous tissue, and a suture was used to close the wound. The implantation area was cleaned and disinfected both immediately before and after implanting the osmotic pumps. Osmotic pumps were surgically removed with a scalpel after cleaning and disinfecting the area.

Experiment 3. Experiment 3 consisted of two trials with cows synchronized with SMB. In trial 1, 41 cows were divided into two groups after SMB implants removal. Twenty one (21) of the cows were administered a GnRH implant and twenty (20) served as controls. The GnRH implant was subcutaneously implanted on the convex surface of the ear with a trocar. Implants contained 250 μg of GnRH in a biodegradable lipid based matrix that eroded relatively constantly, making GnRH available, over approximately 4 days. All females were artificially inseminated approximately 48 hours after SMB implant removal. In trial 2, ninety four (94) cows were assigned to one of two groups. Forty-six (46) of the cows were administered a GnRH implant and the remaining forty-eight (48) served as controls. The GnRH implants were subcutaneously implanted on the convex surface of the ear with a trocar. The implants were identical to those used in trial 1 except they were coated so that there was a slight delay in initiating delivery after implantation. The outer layer did not contain GnRH and only after erosion of that layer was GnRH capable of being released. All females were artificially inseminated approximately 48 hours after SMB implant removal.

Six days following artificial insemination the females from all studies and all treatment groups were exposed to bulls for the remainder of the 63 day breeding season. Calving rates to the various treatments were based on calving dates the following calving season.

Comparisons of calving rate were made between the GnRH treated groups and the control groups using Chi-square analysis (3).

RESULTS AND DISCUSSION

We hypothesized that the constant delivery of GnRH to cattle during the proestrus interval from SMB implant removal to artificial insemination would enhance fertility. In consideration of future application it was decided to use biodegradable microcapsules first because of their ease of delivery and the extensive development work previously conducted. Upon obtaining the results from experiment 1 and from data that was subsequently published about the microcapsules, we then decided to test the hypothesis with an established constant delivery system: osmotic pumps. However, again based on results from experiment 2 and more data from the literature we made one last attempt and conducted experiment 3.

In experiment 1, the administration of GnRH microcapsules decreased ($P < .05$) synchronized pregnancy rates. In data published elsewhere we demonstrated that the non-encapsulated GnRH or the GnRH in the outer layers of the microcapsules that was released soon after administration triggered a preovulatory LH surge (6). The stores of LH in the anterior pituitary gland were then depleted and the anterior pituitary was incapable of synthesizing enough LH for another LH surge at the appropriate time to induce a synchronized ovulation.

In the second experiment, the administration of GnRH via osmotic pumps tended to decrease ($P = .08$) synchronized pregnancy rates. Other investigators (9) working with the same hypothesis demonstrated that the GnRH treatment initiated a premature preovulatory-like LH surge which peaked 7.2 ± .5 hours following the initiation of GnRH treatment.

One possible reason for the decrease in fertility that was encountered when osmotic pumps were used, might be because of the pliable nature of the external case of the pre-incubated osmotic pumps. The osmotic pumps were inserted by grasping them with forceps and inserting the pump into a small incision in the skin. During this process the outer walls of the osmotic pumps may have been compressed and this increased pressure may have caused the release of a small volume (but high concentration) of GnRH. This GnRH could have been enough to cause the premature LH surge that was described earlier. Further, variability in delivery from osmotic pumps has been previously observed (5).

Table 1. The effect of continuous administration of gonadotropin releasing hormone (GnRH) on pregnancy rates.

Implant	Untreated	GnRH Treated
Experiment 1: Biodegradable Microcapsules	18/100 (18%)[a]	6/ 78 (8%)[b]
Experiment 2: Osmotic Pumps	13/ 52 (25%)[c]	4/ 38 (11%)[d]
Experiment 3: Biodegradable Implants-1[e]	7/ 20 (35%)[a]	2/ 21 (10%)[b]
Biodegradable Implants-2[f]	17/ 48 (35%)	13/ 46 (28%)

[a,b] Groups with different superscripts differ ($P < .05$).
[c,d] Groups with different superscripts tended to differ ($P = .08$).
[e] Implants were manufactured to release GnRH upon implantation.
[f] Implants were manufactured to have a delay in releasing GnRH after implantation.

In order to understand these results, the authors developed a novel lipid based ear implant that delayed release of GnRH for a few hours after administration. The implant was manufactured so that there was an outer layer that did not contain GnRH. After, and only after, erosion of that layer was GnRH capable of being released. Results from trial 2 of experiment 3 demonstrate that when the initial release of GnRH was eliminated there was no longer a detrimental effect ($P > .25$) of GnRH on synchronized pregnancy rates. If the coating that delayed release was not included, pregnancy rates were decreased ($P < .05$) as for the microcapsules and for the osmotic pumps. However, even though the technical problem of reduced fertility was corrected, our original hypothesis that a constant delivery of GnRH would enhance fertility in proestrus cattle was incorrect and the concept was abandoned.

REFERENCES

1. Bishop, D.K., R.P. Wetteman, and C.M. O'Connell. Pulsatile infusion of GnRH initiates luteal activity in

nutritional anestrous beef cows. J. Anim. Sci. 68 (Suppl. 1):416 (1985).

2. Chapel, J.P., A.R. Peters, and G.E. Lamming. Pulsatile vs continuous infusion of GnRH to induce LH release in the post-partum beef cow. Tenth Int. Cong. Anim. Reprod. AI, 2:6 (1984).

3. Cochran, W.G., and G.M. Cox. Experimental Designs (2 nd ed.). John Wiley and Sons, New York, NY (1957).

4. Hixon, D.L., D.J. Kesler, T.R. Troxel, D.L. Vincent, and B.S. Wiseman. Reproductive hormone secretions and first service conception rate subsequent to ovulation control with Syncro-Mate B. Theriogenology 16:219-229 (1981).

5. Kesler, D.J., L.C. Cruz, and J.A. McKenzie. The effect of constant delivery of GnRH on the episodic secretion of equine LH. J. Cont. Release 8:55-61 (1988).

6. Kesler, D.J., L.C. Cruz, J.A. McKenzie, and E.A. Henderson. The effect of the GnRH burst release on the efficacy of GnRH microcapsules. Proc. 13th Int. Symp. Cont. Rel. of Bioactive Materials, pp. 54-55 (1986).

7. Kesler, D.J., R.J. Favero, and T.R. Troxel. A comparison of hydron and silicone implants in the bovine norgestomet and estradiol valerate estrus synchronization procedure. Drug Development and Industrial Pharmacy 21:475-485 (1995).

8. Lauderdale, J.W., B.E. Sequin, J.N. Stellflug, J.R. Chenault, W.W. Thatcher, C.K. Vincent and A.F. Loyancano. Fertility of cattle following PGF,α injection. J. Anim. Sci. 38:964-967 (1974).

9. McVey, W.R. and G.L. Williams. Effects of temporary calf removal and osmotic pump delivery of gonadotropin-releasing hormone on synchronized estrus, conception to a timed artificial insemination and gonadotropin secretion in norgestomet-estradiol valerate-treated cattle. Theriogenology 32:969-978 (1989).

10. Troxel, T.R., L.C. Cruz, R.S. Ott, and D.J. Kesler. Norgestomet and gonadotropin releasing hormone enhance corpus luteum function and fertility of postpartum suckled beef cows. J. Anim. Sci. 71:2579-2585 (1993).

11. Valle, E.R., L.C. Cruz, G.F. Cmarik, R.S. Ott, L.A. Peterson, and D.J. Kesler. The effect of GnRH and its method of administration on ovulation response, corpus

luteum function and fertility of beef heifers synchronized with norgestomet and $PGF_2\alpha$. J. Anim. Sci. 63 (Suppl. 1):132-133 (1986).

12. Valle, E.R., L.C. Cruz, G.F. Cmarik, R.S. Ott, L.A. Peterson, and D.J. Kesler. The effect of short term calf removal, GnRH and its method of administration on ovulation response, corpus luteum function and fertility of postpartum suckled beef cows synchronized with norgestomet and $PGF_2\alpha$. J. Anim. Sci. 63 (Suppl. 1):132 (1986).