

**OVULATION CONTROL
IN CATTLE WITH PROGESTERONE
INTRAVAGINAL DEVICE (PRID)
AND GONADOTROPIN RELEASING HORMONE (GnRH)**

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SUMMARY

A technique for chronic application of progesterone intravaginally in cattle is described. When used in conjunction with gonadotropin releasing hormone (GnRH), synchronized ovulation is achieved for timed artificial insemination. Progesterone is released from a silicone rubber matrix attached to a stainless steel spiral. Plasma progesterone concentration reaches luteal phase levels shortly after insertion and falls to 1 to 3 ng per ml of plasma by day 21 when the device is removed. An intramuscular injection of 100 µg of GnRH 28 to 30 hours after device removal induces an LH surge comparable to the normal preovulatory LH surge. Conception rates in dry cows and heifers have been comparable to controls.

We have developed and described in this presentation the adaptation of a sustained drug release system to administer progesterone for estrous control in cattle. Following estrous inhibition with progesterone, gonadotropin releasing hormone (GnRH) was injected to precisely control the time of luteinizing hormone (LH) release and ovulation. This system eliminates the need for detection of behavioral estrus and permits appointment of the time of insemination.

Progesterone was administered intravaginally with a progesterone releasing intravaginal device (PRID) consisting of a stainless steel spiral coated with progesterone impregnated silicone rubber. The application of silicone rubber for delivery of steroids was described by DZIUK and COOK (1966). The progesterone in silicone rubber suspension was either coated or molded on the spiral, or slipped over the spiral as a sleeve. The PRID was administered by winding it on a rod and inserting it into the vagina as close to the cervix as possible. A nylon cord was attached to the

posterior end of the spiral and remained exposed through the vulva for removal of the PRID. The PRID was removed following 18 to 21 days to permit spontaneous luteal regression. GnRH, the decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) described by MATSUO *et al.* (1971), diluted in saline was injected intramuscularly 28 to 30 hours after PRID removal to induce ovulation at a predicted time.

Progesterone dosage was controlled by varying the concentration of the drug in silicone rubber or by varying the surface area of the device exposed to the vagina. Several concentrations of progesterone and sizes of devices have been tested with two formulations selected for extensive testing. These are referred to as type A PRID and a type B PRID.

Type A consists of a 1.5 mm thick, 16 700 mm² surface area layer of silicone rubber containing 7.5 p. 100 progesterone affixed to nylon fabric. This fabric was in the form of a sleeve which was slipped over the stainless steel spiral. The average daily release rate was 78 mg or a total of 1 600 mg during a 21 day treatment. Estimated daily release, based on *in vitro* determinations, declined from 150 mg on day 2 to 40 mg on day 21.

The type B device was prepared by dipping the steel spiral into a liquid suspension of progesterone and silicone rubber. Approximately 22 gm of silicone rubber containing 10 p. 100 progesterone was applied to the device. The total amount of progesterone released was 1 200 mg during a 21 day treatment.

Plasma progesterone levels in cows treated for 21 days with these devices are shown in figure 1. Progesterone levels were elevated to luteal phase levels of 5 to 8 ng/ml by 24 hours after insertion and then declined to 1 to 3 ng/ml by day 14,

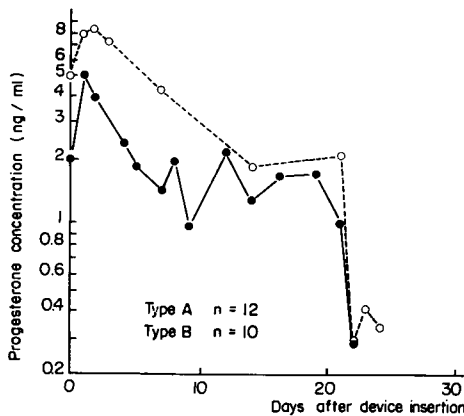


FIG. 1. — Plasma progesterone levels in cows treated with type A (●—●) or B (○----○) intravaginal progesterone releasing device for 21 days

remaining stable until day 21. Progesterone levels declined to 0.5 ng/ml by 24 hours after PRID removal. Preliminary data indicated a higher plasma progesterone level on day 21 with the type B than with the type A device. Further studies shown in figures 2 and 3 with frequent blood collection demonstrate that the elevation in progesterone levels occurred within 60 to 90 minutes after PRID insertion.

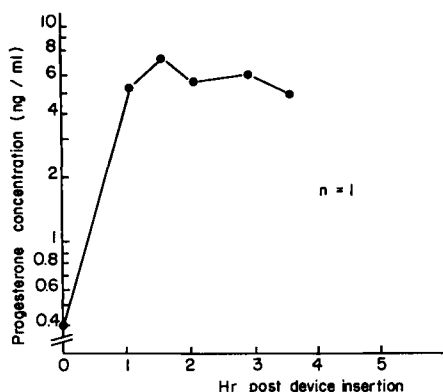


FIG. 2. — Plasma progesterone level in a follicular stage animal for the first 4 hours after insertion of PRID into the vagina demonstrating the rapidity with which progesterone is absorbed from the device into the system

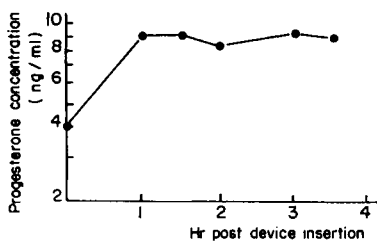


FIG. 3. — Plasma progesterone level in luteal stage animals for the first 4 hours after PRID insertion into the vagina demonstrating the rapid absorption of progesterone in the vagina and levels reached in these animals

Plasma LH levels near the onset of estrus are shown for non-treated cows, PRID-treated cows and PRID + GnRH-treated cows in figure 4. The LH peak is normally closely associated with the onset of behavioral estrus. In four cows, estrus was detected 30 to 40 hours, while the LH peak occurred 35 to 40 hours, after PRID removal. The LH profiles were similar in non-treated and PRID-treated cows but were of shorter duration in PRID + GnRH-treated cows, lasting 4 to 6 hours compared to 6 to 10 hours in the untreated cows.

The time and magnitude of LH peaks induced by GnRH depend on the dose, route of administration and time of injection relative to PRID removal (Mauer, unpublished). Intramuscular injection induced a higher peak response than an i. v. injection presumably due to a somewhat longer absorption time. A 100 µg dose given at the time of PRID removal induced a smaller LH peak than one given 24 hours later. The pituitary appeared to be increasingly sensitive as the cow approached estrus. When GnRH was injected 30 hours after PRID removal some cows had already initiated the endogenous LH surge and showed an exaggerated response.

The proportion of cows exhibiting estrus and the time of estrus were influenced by the level of plasma progesterone at PRID removal. In a preliminary study with 44 cows receiving differing doses of progesterone but no GnRH, the plasma level varied from 0.5 to 3.9 ng/ml on day 21 when the PRID was removed.

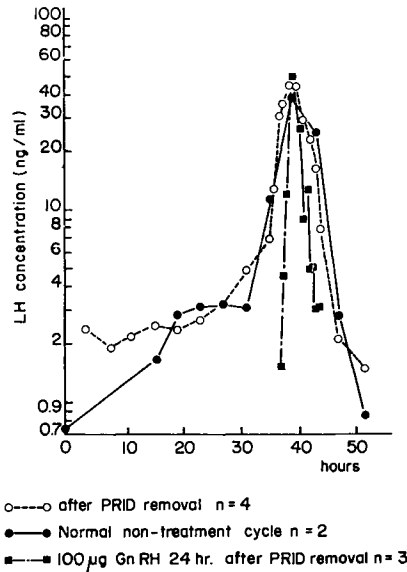


FIG. 4. — Pre-ovulatory LH levels at a normal estrus, in an estrus synchronized with PRID, and when 100 µg GnRH was injected 24 hours after PRID removal. All surges have been normalized to the peak LH value

The time to estrus was correlated ($r = .46$, $P < 0.01$) with plasma progesterone levels at PRID removal. When circulating levels of progesterone were relatively high (over 3 ng/ml serum) at the end of treatment the interval from PRID removal to estrus was extended and more variable (70 ± 30 hours). Conversely, if the progesterone dosage was low and near threshold (approximately 1 ng/ml) some animals came into estrus during treatment while others came into estrus in the first 24 hours after PRID removal (average 30 ± 7 hours). Animals with a systemic level of 1 to 3 ng/ml of serum at the time of PRID removal had a synchronized estrus 48 ± 16 hours after PRID removal. Cows not exhibiting synchronized estrus after PRID treatment apparently ovulated since these animals returned to estrus in 21 days. The incidence of silent estrus was increased when GnRH was injected in another study with similar cows.

The time from PRID removal to estrus in cows treated with type A and B devices is shown in table 1. This interval was greater in cows with type B than in cows with type A devices. There was an apparent association between interval to estrus and plasma progesterone levels at PRID removal since type B cows had higher levels on day 21 (fig. 1). With type A PRID 32 p. 100 of the cows had shown standing heat by the time GnRH was injected at 28 hours post PRID removal while with type B PRID only 6 p. 100 were in standing heat at this time. The incidence of silent estrus was greater in cows with type B (35 p. 100) than in cows with type A (15 p. 100) PRID. This was apparently due to LH release and ovulation sooner than would normally occur in type B cows eliminating the normal estrogen rise and behavioral estrus. The induced ovulation was apparently fertile since conception rate in cows inseminated at a predetermined time was not different in cows exhibiting behavioral estrus and those having a silent estrus.

TABLE I

Time from prid removal to estrus

Treatment (1)	Proportion of cows in estrus (%)			
	26 to 30 hr	31 to 38 hr	39 to 46 hr	Silent
Type A PRID (2)	32	41	12	15
Type B PRID (3)	6	26	32	35

(1) GnRH injected 28 hr after PRID removal.

(2) Thirty-four cows.

(3) Thirty-one cows.

Two types of devices were used in a program to synchronize breeding in large groups of cows. The experimental design was as follows : The devices were randomly inserted in half of the cows in a herd and removed 21 days later. GnRH was injected 28 to 30 hours after PRID removal. All treated cows were bred with frozen semen 18 to 24 hours after injections. Controls were bred at detected estrus with similar semen. The breeding period for controls began 2 weeks prior to and extended 2 weeks after date of breeding for treated cows. The conception rates are based on non-return or palpation at 60 days and expressed as the proportion of cows pregnant of the total bred.

Three hundred and eighty seven heifers and dry cows have been treated with the type A and 108 with the type B device. Retention of the devices was 96 p. 100 and 99 p. 100 for A and B, respectively in non-lactating cows and heifers. Retention for type A devices was 75 p. 100 in 177 cows suckling calves. Conception rates with the two formulations in dry cows and heifers were 49 p. 100 of 342 with the type A device and 50 p. 100 of 54 with the type B device. Control conception rates for the two treatments were 58 p. 100 and 41 p. 100 respectively in 420 and 58 cows.

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RÉSUMÉ

UTILISATION COMBINÉE D'UN DISPOSITIF INTRAVAGINAL IMPRÉGNÉ DE PROGESTÉRONE ET DE L'HORMONE LIBÉRANT LES GONADOTROPINES (GnRH) POUR LE CONTRÔLE DE L'OVULATION CHEZ LA VACHE

Une technique pour l'application chronique de progestérogène par voie intravaginale est décrite chez la Vache. Son emploi en combinaison avec l'hormone libérant les gonadotropines (GnRH) provoque une ovulation synchronisée permettant l'insémination artificielle à temps prédéterminé. La progestérogène est libérée à partir de la matrice de caoutchouc silicone fixée à une spirale d'acier

inoxydable. La concentration plasmatique de progestérone atteint des taux comparables à ceux de la phase lutéale peu après l'insertion et tombe à 1-3 ng par ml de plasma au 21^e jour ; le dispositif est alors enlevé. Une injection intramusculaire de 100 µg de GnRH 28 à 30 heures après le retrait du dispositif détermine une forte sécrétion de LH, comparable à sa sécrétion préovulatoire normale. Les taux de conception obtenus après ce traitement chez les vaches sèches et les génisses sont comparables à ceux des témoins.

REFERENCES

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