

SYNCHRONIZATION OF ESTRUS AND OVULATION IN BOVINE EMBRYO TRANSFER RECIPIENTS

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INTRODUCTION

Traditional methods of estrus synchronization developed over the past 60 yr have involved controlling bovine estrous cycle length by administering a progestin and/or regressing the corpus luteum with luteolytic drugs. The synchrony of estrus and timing of ovulation following traditional estrus synchronization treatments has been too variable to allow the transfer of a fresh or frozen/thawed embryo without estrus detection. Synchronization treatments developed more recently, however, combine traditional methods of controlling cycle length with the manipulation of follicular development in order to “program” or “select” the ovulatory follicle. The new methods synchronize estrus more precisely and control the time of ovulation more exactly. These treatments may control ovulation with enough repeatability and precision to insure synchrony of estrous cycles of ET recipients without the need for visual estrus detection.

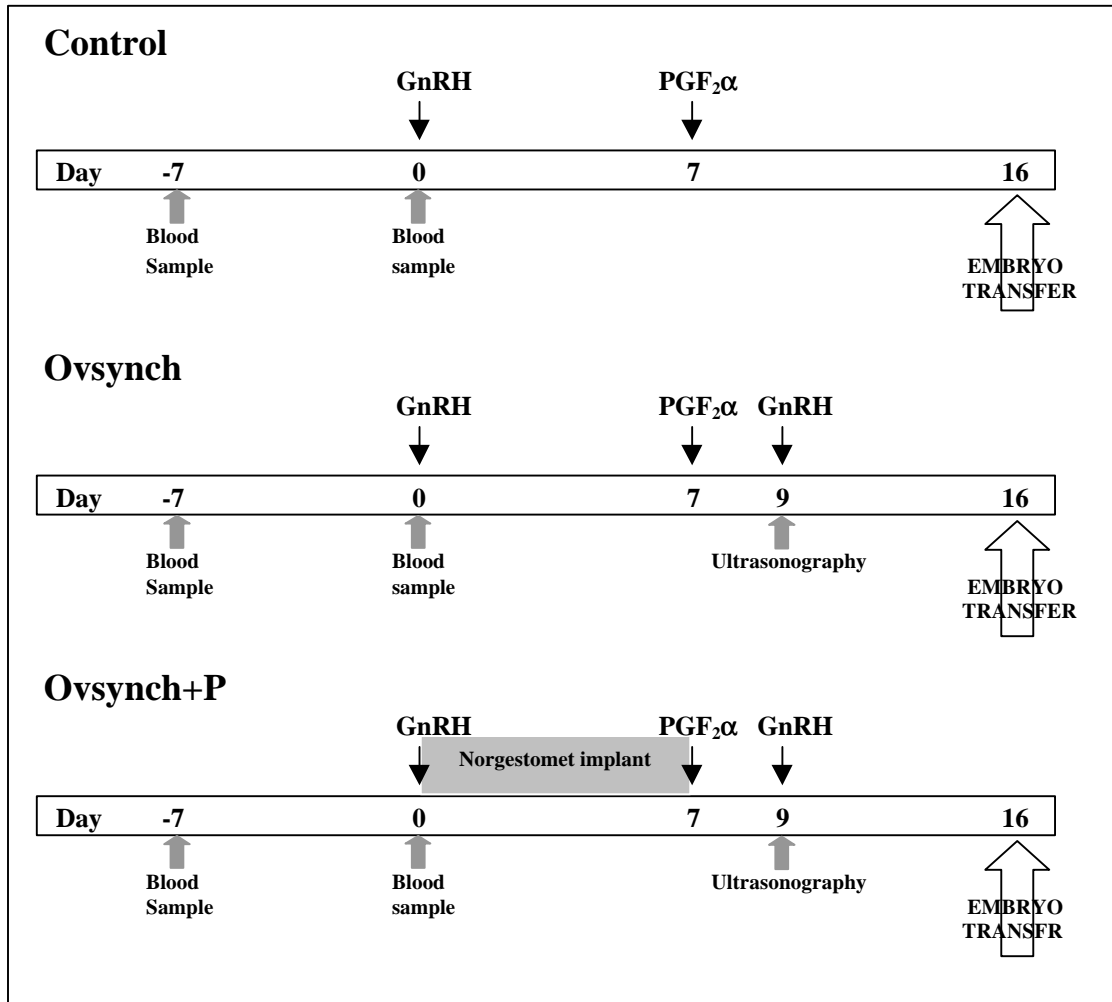
Maintaining a herd of recipients is perhaps the greatest cost associated with the on-farm use of bovine embryo transfer. Efficient use of recipients is critical to managing the cost of an ET program. The basis for efficiently utilizing recipients is to select a consistent method of estrus/ovulation control, employ reliable estrus detection and to have a system in place that allows repeated use of recipients. The research described below was aimed at improving the methodology for controlling the estrous cycles of recipient cows and eliminating the need for visual estrus detection. In addition, we investigated a method to resynchronize recipients that fail to conceive to an initial ET such that they could be reutilized immediately.

MATERIALS AND METHODS

Initial Synchronization of Estrus and Ovulation in Recipients

The experiment was conducted at Mossy Creek Farm, Mt Solon, VA. Four hundred ninety-nine primiparous (n=90) or multiparous (n=409) beef cows nursing calves (28-92 d postpartum) were assigned by age and d postpartum to one of three treatments to synchronize estrus or to synchronize estrus and control ovulation (Figure 1). Approximately one-third of the animals received 100 µg of gonadotropin-releasing hormone (GnRH, Cystorelin®, Merial, Islin, NJ) via intramuscular injection followed 7d later by 25 mg of prostaglandin F₂α (PGF₂α, im, Lutalyse®, Upjohn Co., Kalamazoo, MI). This group (Control, n=169) was monitored continuously for signs of behavioral estrus using an electronic heat detection system (HeatWatch™; DDx Inc., Boulder, CO) beginning at the time PGF₂α was injected. Recipients were assigned for ET 6-8 d following detection of estrus and embryos were assigned based on the stage of embryo development (stage 3-6) in an effort to maximize pregnancy rate.

Figure 1. Diagrammatic representation of initial synchronization treatments



The second one third of the animals (Ovsynch, n=165) were treated as described above, but received a second injection of GnRH 48 h after the administration of PGF₂α to control the timing of ovulation (Figure 1). Animals in this group were monitored continuously for signs of behavioral estrus using HeatWatch™ beginning at the time PGF₂α was injected and ovarian structures were recorded by ultrasonography at the time of the second GnRH injection. However, all cows in the Ovsynch group were presented for ET 9 d after the injection of PGF₂α, without regard to the detection of estrus. Embryos frozen at stage 4-6 of development were thawed and transferred to suitable recipients (see below) in this group without regard to the occurrence of estrus.

The final one-third of the animals (Ovsynch+P, n=165) were injected with 100 μg of GnRH and fitted with a subcutaneous hydron implant containing 6 mg Norgestomet (Merial, Islin, NJ) at the beginning of treatment (Figure 1). Seven days later the subcutaneous implant was removed and each cow received 25 mg of PGF₂α. A second injection of GnRH was administered 48 h after implant removal to control the timing of

ovulation. Animals in this group were monitored continuously for signs of behavioral estrus using HeatWatch™ beginning at the time PGF₂α was injected and ovarian structures were recorded by ultrasonography at the time of the second GnRH injection. However, all cows in the Ovsynch+P group were presented for ET 9 d after the injection of PGF₂α, without regard to the detection of estrus. Embryos frozen at stage 4-6 of development were thawed and transferred to suitable recipients (see below) in this group without regard to the occurrence of estrus.

Radioimmunoassay of serum progesterone - Blood samples were collected from each animal by venipuncture 7 d prior to the initiation of treatment and on the d that treatment began in each group. After separation of serum, radioimmunoassay of progesterone content in serum was conducted to retrospectively determine if each cow was cyclic or anestrus prior to treatment. Cows with serum progesterone concentrations >1 ng/ml at the time of either blood collection were considered to be cyclic.

Ultrasonography of ovarian structures - Transrectal ultrasonography of ovarian structures was performed at the time of the second GnRH injection in cows in the Ovsynch and Ovsynch+P groups. The presence or absence of luteal structures and the position of the dominant follicle was recorded.

Evaluation of recipients at the time of ET - Six to 8 d following detection of a synchronized estrus (Control) or 9 d following administration of PGF₂α in groups treated to control ovulation (Ovsynch and Ovsynch+P), recipients were presented for embryo transfer. Rectal palpation to verify the presence of a corpus luteum was performed by a single practitioner. The practitioner had access to heat detection information recorded for animals in the Control group, however, the practitioner was unaware of estrous activity or the results of ultrasonography of animals in the ovulation control groups. If the practitioner deemed a recipient in the ovulation control groups suitable for ET based on the presence of a palpable corpus luteum (>12 mm diameter), an embryo was transferred.

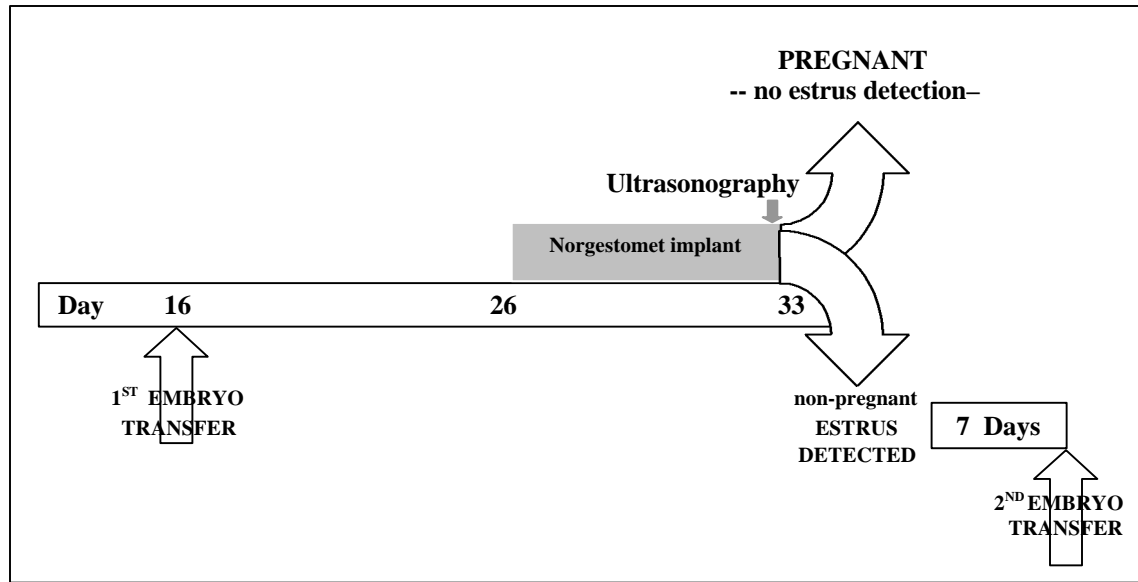
Embryo transfer was performed by personnel from Ashby Embryos (Harrisonburg, VA). Grade 1 or 2 frozen embryos were thawed by one technician and transferred to suitable recipients by a single practitioner. Stages of embryo development (stages 4-6) and the time of estrus detection were used to assign embryos to recipients in the Control group. Embryos frozen at different stages of development were assigned for transfer to recipients in the ovulation control groups (Ovsynch and Ovsynch+P) based on an “assigned” time of estrus that was coincident with the second injection of GnRH.

Resynchronization of Estrus and Ovulation in Recipients

All recipients were fitted with a subcutaneous Norgestomet implant 10 d after receiving a frozen/thawed embryo (equivalent to d 16-18 of the cycle or pregnancy; Figure 2) to control the timing of the return to estrus in recipients that failed to become pregnant to the initial embryo transfer. Seven days after insertion of the implant, each recipient was examined by ultrasonography for diagnosis of pregnancy (equivalent to d 23-25 of cycle or pregnancy) and implants were removed. Non-pregnant recipients were monitored continuously for signs of behavioral estrus using HeatWatch™ beginning at the time of

implant removal. Recipients detected in estrus received another frozen/thawed embryo 6-8 d after estrus detection using the procedure described above for Control animals. Sixty-three days after the second embryo transfer (89 d after the initial transfers) pregnancy was diagnosed via ultrasonography in all recipients.

Figure 2. Diagrammatic representation of resynchronization method



Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System (SAS Inst. Inc., Cary, NC). Estrus response and pregnancy rates (to first ET) of recipients in the Control, Ovsynch and Ovsynch+P groups were compared by ANOVA with working location, treatment and cycling status prior to treatment as main effects. The interval from calving to the beginning of the experiment was included as a covariate in the analysis. Estrus responses and pregnancy rates (to second ET) of non-pregnant recipients treated to resynchronize estrus were compared to the same response of Control animals following the initial synchronization and first embryo transfer. If main effects or interactions with more than one degree of freedom were significant, differences among means were determined using the PDIFF option of SAS.

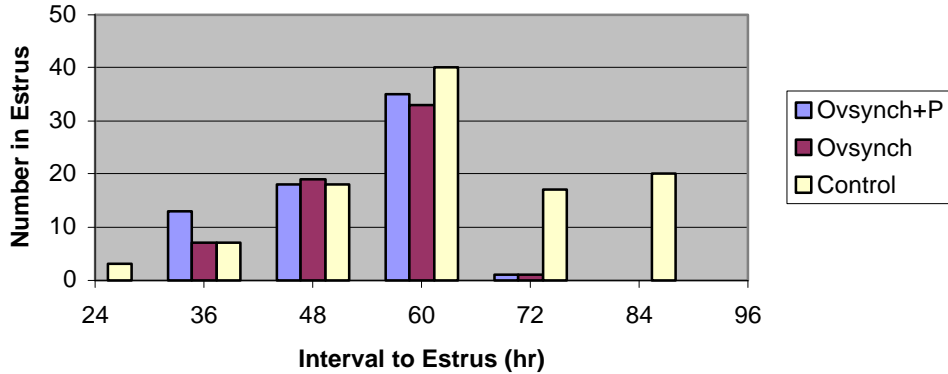
PRELIMINARY RESULTS AND DISCUSSION

Initial Synchronization of Estrus and Ovulation in Recipients

Estrus was detected in 64% of the cows in the Control group within 4 d after the injection of PGF₂α. Although detection of estrus was not considered in the selection of suitable recipients or in assigning embryos, 38 and 44% of the cows in the Ovsynch and Ovsynch+P groups, respectively, were detected in estrus.

The distributions of the intervals from administration of PGF₂α to estrus are depicted in Figure 3. Cows in the Control group exhibited estrus on each of the 4 d following PGF₂α treatment and the greatest number of cows were detected in estrus between 48 and 60 h after PGF₂α. Cows in the ovulation control groups exhibited estrus in a pattern similar to that of the Controls until 12 h after the administration of the second GnRH injection. Beginning at that time, essentially no additional cows in the ovulation control groups were detected in estrus.

Figure 3. The distribution of estrus following the initial synchronization treatments



There was a significant interaction between the effects of treatment and cyclic status prior to treatment on transfer rate (embryos transferred/recipients; Table 1). Fewer ($P < .01$) non-cyclic cows in the Control group exhibited estrus and received embryos than cows that were cyclic prior to the beginning the Control treatment. This trend was not evident in the ovulation control groups and regardless of cyclic status, more cows in the Ovsynch and Ovsynch+P groups received embryos ($P < .01$) than cows in the Control group. This result indicates that the second injection of GnRH administered to cows in the Ovsynch and Ovsynch+P treatments may have caused ovulation and development of a corpus luteum in cows that were not destined to exhibit estrus or ovulate following the administration of PGF₂α.

Table 1. The Rate of Embryo Transfer in Cyclic and Non-cyclic Recipients Treated to Synchronize Estrus and Control the Timing of Ovulation

Treatment	Cyclic Status	No.	Transfer Rate ¹
Control	Cyclic	102	73/102 = 72% ^a
	Non-cyclic	67	35/67 = 52% ^b
Ovsynch	Cyclic	92	82/92 = 89% ^c
	Non-cyclic	72	67/72 = 93% ^c
Ovsynch+P	Cyclic	113	104/113 = 92% ^c
	Non-cyclic	52	48/52 = 93% ^c

¹ significant treatment x cyclic status interaction ($P < .06$).

^{a,b,c} means with different superscripts are significantly different ($P < .01$).

The pregnancy rate of cows that received an embryo (pregnancies/transfers; Table 2) was greater ($P<.05$) among cows in the Control group than among cows in either ovulation control group. The higher pregnancy rate per transfer in the Control group may have been due to the assignment of embryos to recipients based on stage of embryo development and the timing of estrus in that group. The synchrony between embryo development and the stage of the estrous cycle is likely to have been less precise in Ovsynch or Ovsynch+P recipients that received an embryo based on an “assigned” time for the onset of heat coincident with the timing of the second GnRH injection.

Table 2. The Pregnancy Rate in Recipients Treated to Synchronize Estrus and Control the Timing of Ovulation

Treatment	No.	Pregnancy Rate/ Transfer¹	PregnancyRate/ Recipient
Control	169	67/108 = 62%^a	67/169 = 40%
Ovsynch	165	72/150 = 48%^b	72/165 = 44%
Ovsynch+P	165	82/152 = 54%^b	82/165 = 50%

¹ significant treatment effects ($P<.05$).

^{a,b} means with different superscripts are significantly different ($P<.07$).

The overall pregnancy rates (pregnancies/recipients; Table 2) were not significantly different among the three treatment groups. The higher transfer rate among cows in the ovulation control groups compensated for the lower pregnancy rate per transfer recorded in those groups. Therefore, the use of an ovulation control treatment in addition to a method for controlling corpus luteum function and synchronizing estrus was successful in enabling the transfer of embryos without the need for estrus detection in this experiment.

Resynchronization of Estrus and Ovulation in Recipients

One hundred sixteen cows fitted with Norgestomet implants 10 d after receiving an embryo were determined to be non-pregnant at the time of implant removal. These cows were considered “eligible” for resynchronization. Sixty nine percent of the eligible cows were detected in estrus within 4 d following implant removal (Table 3). The greatest number of cows that responded to the resynchronization were in estrus beginning 36 to 48 h after implant removal (Figure 4).

Figure 4. The distribution of estrus following the resynchronization treatment

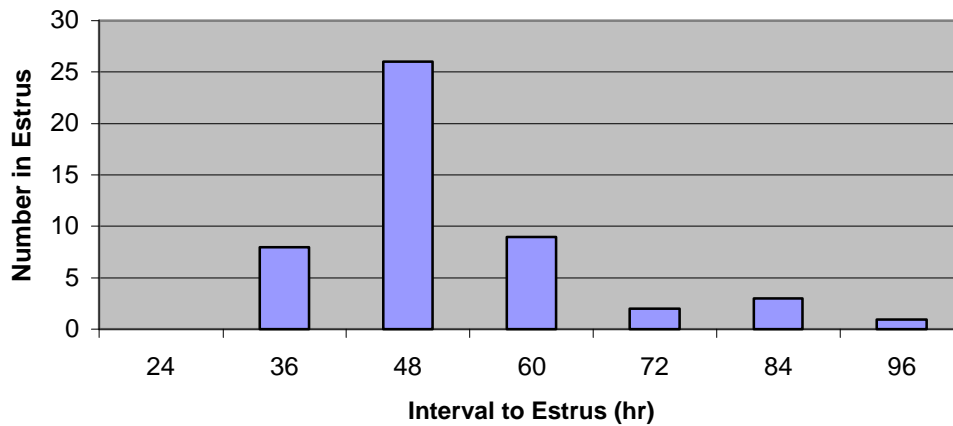


Table 3. The Rate of Embryo Transfer and Pregnancy in Recipients Following the Initial Synchronization or Resynchronization to Control the Timing of Estrus

Synchronization	Treatment	No.	Transfer Rate	Pregnancy Rate/ Transfer	Pregnancy Rate/ Recipient
Initial	Control	169	108/169 64%	67/108 62%	67/169 40%
Resynchronization	Implant	116	80/116 69%	53/80 66%	53/116 46%

Pregnancy rate per transfer and pregnancy rate per recipient following resynchronization of estrus and transfer of a second embryo were similar to those recorded in the Control group following the initial synchronization (Table 3). Hence, the resynchronization treatment used in this experiment enabled the immediate reutilization of non-pregnant recipients for the transfer of a second embryo. Furthermore, pregnancy rates following the second transfer were high, and not different from those following the initial synchronization and transfers in the Control group.

IMPLICATIONS

Ovulation control treatments combined with estrus synchronization enable the transfer of bovine embryos to recipients without the need for estrus detection. When coupled with the use of a progestin implant to resynchronize non-pregnant recipients in order to facilitate a second attempt at ET, these methods will enhance reproductive efficiency of recipients maintained for use in an embryo transfer program.