What’s new in timed artificial insemination and assisted reproduction in beef cattle?

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Abstract

Protocols designed to control follicular wave dynamics and ovulation in cattle have reduced, and even eliminated, the need for estrus detection. The addition of progestin-releasing devices and gonadotropin treatments, such as equine chorionic gonadotropin, have provided possibilities for increased pregnancy rates, especially in cows experiencing postpartum anestrus. In embryo transfer programs, these same treatment protocols have again eliminated the need for estrus detection, permitting fixed-time embryo transfer. In superovulation schemes, the control of follicular wave emergence and ovulation has also eliminated the need for estrus detection in donors, facilitating donor management. Alternative approaches to control follicular wave emergence have been developed recently by increasing the responsiveness of donors to gonadotropin releasing hormone. To further simplify superstimulatory treatments, follicle stimulating hormone has been diluted in a hyaluronan-based, slow-release formulation permitting the induction of superovulation with only two intramuscular injections. Collectively, new treatment protocols have facilitated the widespread application of artificial insemination and embryo transfer in beef cattle, primarily by eliminating the necessity of estrus detection.

Introduction

Estrus detection is time consuming and labor intensive, subject to environmental influences, and frequently inefficient and inaccurate. Therefore, reproductive performance is often unsatisfactory when artificial insemination (AI) is done following detection of estrus. Alternatively, treatments that synchronize both emergence of a new wave of ovarian follicles and ovulation, and employ fixed-time AI (FTAI; without estrus detection) can result in satisfactory reproductive performance because all animals are inseminated whether they show estrus or not. The same protocols are now being used for fixed-time embryo transfer (FTET) with improved pregnancy rates because of increased utilization of recipients. The occurrence of behavioral estrus is no longer relevant with FTAI and FTET protocols.

Although research efforts in recent years have resulted in little or no increase in the number of transferable embryos following superovulation, protocols that control emergence of the follicular wave and the timing of ovulation have allowed the treatment of groups of donors, regardless of the stage of the estrous cycle and permitted FTAI in donors, without the need to detect estrus. However, the most commonly used treatment for synchronization of follicular wave emergence for superovulation involves the use of estradiol-17β or one of its esters, which cannot be used in many countries because of concerns about the effects of steroid hormones in the food chain. The purpose of this paper...
is to review new developments in synchronization and superovulation of beef cattle using readily available pharmaceutical products.

Treatments that Synchronize Estrus and Ovulation

There are many options available for synchronization of estrus and ovulation in beef cattle, and these have been listed by the Beef Cattle Reproduction Leadership Team. For information, the reader is referred to http://beefrepro.unl.edu/resources.html. This is an excellent reference source put together by representatives of the AI and pharmaceutical industries, veterinarians, and reproductive physiologists from the Beef Reproduction Task Force. For another very good review of synchronization protocols, the reader is referred to the Purdue University website http://www.extension.purdue.edu/extmedia/AS/AS_575_ W.pdf.

Although prostaglandin F$_{2\alpha}$ (PGF) has been the most commonly used treatment for synchronization of estrus, poor estrus detection and variability in the interval from treatment to estrus and ovulation makes this treatment very inefficient. In order to avoid problems associated with estrus detection, treatments that synchronize dominant follicle emergence and time of ovulation have been utilized for FTAI. These treatments are generally divided into those that are estradiol-based and those that are gonadotropin releasing hormone (GnRH)-based.

Estradiol-based protocols

Estradiol treatments are commonly used to synchronize follicle wave emergence and ovulation in beef cattle in Canada and Mexico. The protocol consists of insertion of a progestin-releasing device and the administration of 2.5-5 mg estradiol-17β or 2 mg estradiol benzoate on day 0 (to synchronize follicular wave emergence), and PGF at the time of removal of the progestin device (to ensure luteolysis) on days 7 or 8. A lower dose of estradiol (usually 1 mg) is given 24 hours after progestin removal to synchronize a luteinizing hormone (LH) surge (approximately 16 to 18 hours after treatment) and ovulation (approximately 24-32 hours later). Fixed-time AI is typically done 30-34 hours after the second estradiol treatment. As mentioned earlier, estradiol is no longer available for use in food producing animals in many countries, including the US, so most of the remaining discussion will concentrate on GnRH-based protocols.

GnRH-based protocols

GnRH-based protocols have been used extensively for FTAI in dairy and beef cattle in the US. In cattle with a growing dominant follicle (>10 mm in diameter), treatment with GnRH induces LH release and ovulation, with emergence of a new follicular wave approximately two days later. Treatment with PGF six days or seven days after GnRH results in ovulation of the new dominant follicle, especially when a second GnRH injection is given 36-48 hours after PGF. The treatment protocol that utilizes GnRH and PGF for FTAI in dairy cattle has been called Ovsynch. Cosynch protocols are more commonly used in beef cattle because cows are FTAI at the time of the second GnRH. Most FTAI protocols utilized in the US today are variations on the Ovsynch protocol.

It has been shown that the emergence of a new follicular wave was synchronized only when GnRH treatment caused ovulation. However, recent studies have shown that the first GnRH results in ovulation in only 44 to 54% of dairy cows, 56% of beef heifers and 60% of beef cows. If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized, resulting in disappointing pregnancy rates following FTAI. In addition, ~20% of heifers show estrus before the injection of PGF, dramatically reducing fertility to FTAI. Prevention of the early ovulations by addition of a progestin-releasing device to a seven-day GnRH-based protocol has improved pregnancy rates after FTAI in heifers and cows. A GnRH-based protocol with the addition of a progestin-releasing device is illustrated in Figure 1.

Figure 1. Diagram of gonadotropin releasing hormone (GnRH)-based fixed-time AI (FTAI) protocol utilizing a progestin-releasing device. A first GnRH injection is given to induce ovulation (in 50-60% of animals) and synchronize follicular wave emergence. Prostaglandin (PGF) is given six or seven days later to induce regression of the corpus luteum, and a second GnRH is given in 48-60 hours to induce luteinizing hormone (LH) release and synchronize ovulation of the recruited dominant follicle. In beef cattle, a progestin-releasing device is usually inserted between the first GnRH and PGF to prevent early heats, and FTAI is normally done at the time of the second GnRH injection (Cosynch).
It has been shown that stage of the estrous cycle at the time that GnRH-based protocols are initiated affects pregnancy rates. Cattle in which GnRH was administered between days 1 and 4 or days 13 and 17 of the cycle had much lower pregnancy rates than at other times (20 vs 50%, respectively). When GnRH is administered during metestrus (days 1-4), the dominant follicle may not ovulate, beginning to undergo atresia at the approximate time that the PGF is injected. The dominant follicle of the second wave (days 13-17) may also not ovulate in response to the first GnRH treatment, and in the absence of ovulation, endogenous PGF may cause luteolysis and ovulation before FTAI, resulting in low pregnancy rates. Therefore, estrus detection and AI of cattle that come into estrus early, or the insertion of a progestin device between the first GnRH and PGF, are often done to improve pregnancy rates.

Pre-synchronization with either one dose or two doses of PGF 14 days apart, and administration of the first GnRH 12 or 14 days after the second dose of PGF, has improved pregnancy rates. The objective is to get cows between days 5 and 12 at the time of GnRH treatment. In a recent review, it was noted that pre-synchronization with PGF increased pregnancy rate by 11 to 13% in dairy cattle, and an interval of 11 days between the second PGF and the first GnRH resulted in higher pregnancy rates than the previously used 14-day interval. Wiltbank also reported that the application of a double Ovsynch protocol improved pregnancy rates, compared to Ovsynch by itself. Another approach is to palpate cattle before initiating the GnRH-based program; those with a functional corpus luteum (CL) are given PGF (and GnRH is administered 14 days later), and those with a large ovarian follicle or corpus hemorrhagicum are given GnRH (and the GnRH-based program is started eight days later).

We have investigated the effects of pre-synchronization with PGF prior to a Cosynch protocol on estrus synchrony, CL and preovulatory follicle diameters, and pregnancy rate following FTAI in beef heifers in Canada. Pre-synchronization reduced the proportion of heifers in estrus before FTAI, suggesting that this may be useful in the successful application of GnRH-based protocols in beef heifers. However, pregnancy rate to FTAI was not affected by pre-synchronization in this study. We also examined the effects of pre-synchronization with a progestin device on follicle size and ovulation rate to an initial injection of GnRH in lactating beef cows subjected to a Cosynch protocol in Canada. Pre-synchronization with a progestin device increased the proportion of cows responding to the first GnRH treatment, but pregnancy rate to FTAI was not affected. It would seem that there are several different pre-synchronization protocols that will increase the numbers of animals ovulating to the first injection of GnRH, and these may result in improved pregnancy rates in GnRH-based FTAI protocols. However, additional studies are needed with much larger numbers of animals to confirm these results in beef cattle.

The 5-day Cosynch Protocol

Bridges et al compared a seven-day Cosynch protocol plus progestin device with FTAI at 60 hours and a five-day Cosynch protocol with FTAI at 72 hours in postpartum beef cows. In that study, pregnancy rates were 11% higher with the five-day protocol. Santos et al reported similar findings in dairy cattle. The hypothesis proposed was that the five-day protocol provided for a longer proestrus with increasing estradiol concentrations due to continuous gonadotropin support for the dominant follicle. The ovulatory follicle of cows in the five-day program benefited from this extra time and additional gonadotropin support. However, due to a shorter interval between the first GnRH and induction of luteolysis in the five-day protocol, two injections of PGF six to eight hours apart were necessary to induce complete regression of the GnRH-induced CL.

More recently, Kasimanickam et al reported that heifers inseminated at 56 hours in a five-day Cosynch protocol had, on average, a 10.3% higher AI pregnancy rate than heifers inseminated at 72 hours. In addition, Colazo et al showed that pregnancy per FTAI did not differ between five-day and seven-day Cosynch protocols with a single administration of PGF in dairy heifers. In that study, the use of the first GnRH in the five-day Cosynch protocol also did not seem to be necessary, as pregnancy rates did not differ when it was not used. Conversely, Lima et al observed an increased pregnancy rate in dairy heifers receiving the final GnRH concurrent with AI at 72 hours after PGF, compared with 16 hours before AI, however, they also showed no benefit of a first GnRH. Additional research is needed to evaluate five-day Cosynch protocols in beef heifers.

Use of Equine Chorionic Gonadotrophin (eCG) to Improve Pregnancy Rates

Baruselli et al and Bó et al have reported that the use of 400 IU of eCG at the time of removal of a progestin device resulted in increased plasma progesterone concentrations and increased pregnancy rates in suckled beef cows treated during postpartum anestrus. Furthermore, in experiments designed to evaluate the effect of different treatments for the synchronization of ovulation on pregnancy rates following FTAI of lactating dairy cows in Argentina, pregnancy rates were significantly higher in cows treated with progestin devices, estradiol, and eCG (145/298; 48.7%) than in those treated with a GnRH-based protocol plus progestin devices without
eCG (117/298; 39.3%). Results also suggested that eCG increases pregnancy rates in cows with lower body condition score.\textsuperscript{19} In a Brazilian study,\textsuperscript{59} lactating \textit{Bos indicus} cows that received eCG had a larger dominant follicle at FTAI and a higher ovulation rate, resulting in greater plasma progesterone concentrations following the synchronized ovulation. These data suggested that the beneficial effect of eCG treatment was related to increased ovulation rates (follicle maturity) and the positive effect of increased concentrations of circulating progesterone on embryo development and pregnancy maintenance.

We have examined the effects of eCG at the time of PGF injection on preovulatory follicle size and fertility in lactating beef cows subjected to a Cosynch protocol in Canada.\textsuperscript{61} Treatment with eCG increased pregnancy rates in cows that were not pre-synchronized, especially the two-year old cows, confirming that eCG may be useful in Ovsynch or Cosynch protocols in lactating beef cattle, especially those that were early postpartum or under nutritional stress. It would seem that eCG increases progesterone concentration, and in turn, pregnancy rates. However, the lack of an effect in animals that were pre-synchronized suggests that the two different treatments may be different routes to same end; more research is needed.

**Fixed-time Embryo Transfer (FTET)**

The use of FTAI protocols for FTET has recently been reviewed in three different papers presented at the Annual Meetings of the International Embryo Transfer Society.\textsuperscript{9,10,22} Briefly, both estradiol- and GnRH-based protocols have been used successfully to synchronize ovulation in recipients that received \textit{in vivo}-\textsuperscript{1,22,23} or \textit{in vitro}-\textsuperscript{5,9,54} derived embryos. In two Brazilian studies, \textit{Bos indicus} x \textit{Bos taurus} crossbred heifers\textsuperscript{7,76} and cows\textsuperscript{76} were treated with a single dose of PGF and observed for estrus for five days or with a GnRH-based protocol without estrus detection. Seven days after estrus (PGF group) or after the second GnRH treatment (GnRH protocol), recipients with an ultrasonically detectable CL\textsuperscript{7} or a palpable CL\textsuperscript{75} were selected to receive frozen-thawed embryos by “Direct Transfer”. The overall pregnancy rate was higher in recipients treated with the GnRH-based protocol because it was not dependent on the detection of estrus. It is also noteworthy that in one of these studies,\textsuperscript{7} only 53.7% of the heifers treated with PGF were observed in estrus, a reflection of the difficulty of estrus detection in \textit{Bos indicus}-derived cattle.\textsuperscript{17}

In a US study,\textsuperscript{11,33} 499 lactating \textit{Bos taurus} crossbred cows (28 to 92 days postpartum) were assigned to one of three treatment groups: cows in the Control group were treated with GnRH on day 0, PGF on day 7, and detected for estrus using a Heat-Watch system (GnRH+PGF). Cows in the other two groups were treated with an Ovsynch protocol alone (Ovsynch), or plus a progestin device for seven days (Ovsynch+P) and were not observed for estrus. Six to eight days after estrus (GnRH+PGF group) or seven days after the second GnRH treatment (Ovsynch and Ovsynch+P groups), cows with a palpable CL received Grade 1 or 2 frozen-thawed embryos by “Direct Transfer”. Although the conception rate (recipients pregnant/recipient transferred) tended to be higher in the estrus detected (GnRH+PGF) group, overall pregnancy rates did not differ among groups, mainly because of the greater number of recipients selected for embryo transfer in the Ovsynch and Ovsynch+P groups. The same investigators did field trials involving 1637 recipients treated with a GnRH-based (Ovsynch) treatment protocol plus a progestin device without estrus detection; overall pregnancy rate was 59.9%. In summary, results of these studies indicate that acceptable pregnancy rates can be achieved when embryos are transferred to recipients that have received treatment protocols designed to synchronize ovulation, without the necessity of estrus detection.

**Advances in Superovulation**

Traditionally, gonadotropin treatments have been initiated during the mid-luteal phase, approximately nine to 11 days after estrus (reviewed in Bó et al\textsuperscript{15,16}), around the time of emergence of the second follicular wave.\textsuperscript{32} However, a greater superovulatory response occurred when treatments were initiated on the day of follicular wave emergence, as opposed to one day before, or one or two days after wave emergence.\textsuperscript{53} Therefore, conventional treatment protocols have two drawbacks: 1) the requirement to have trained personnel dedicated to the detection of estrus, and 2) the necessity to have all donors in estrus at the same time in order to initiate treatments in groups of animals. We have recently summarized current superovulation protocols for cattle.\textsuperscript{46}

**Synchronization of Follicle Wave Emergence for Superovulation**

In the 1990s, we reported on synchronization of follicular wave emergence, on average four days after treatment with progesterone and estradiol.\textsuperscript{15} This treatment has been used by practitioners around the world for superstimulation of cattle, but its use has now been restricted in several countries. This restriction leaves many embryo transfer practitioners with a serious dilemma, and created the need to develop treatments that do not involve the use of estradiol.

An alternative is to eliminate the suppressive effect of the dominant follicle by ultrasound-guided follicle aspiration and initiate superstimulatory treatments one or
two days later.\textsuperscript{13} The disadvantage of ultrasound-guided follicle aspiration is that it requires ultrasound equipment and trained personnel making it only appropriate when donors are held in an embryo production facility; it is very difficult to apply in the field.

Another alternative is to use GnRH to induce ovulation of the dominant follicle,\textsuperscript{44} which would be followed by wave emergence one to two days later.\textsuperscript{47} However, as indicated earlier, emergence of a new follicular wave was synchronized only when treatment caused ovulation\textsuperscript{47} and without pre-synchronization, the first GnRH results in ovulation in less than 60\% of animals.\textsuperscript{47,52} Not surprisingly, treatment with GnRH at random stages of the estrous cycle, prior to initiating superstimulatory treatments, resulted in lower superovulatory responses than treatments initiated after follicular aspiration or estradiol.\textsuperscript{28}

More recently, in a retrospective analysis of commercial data, Hinshaw (personal communication; AETA 2007) found no differences in the numbers of transferable embryos between donors superstimulated four days after treatment with estradiol and progesterone and those superstimulated two days after treatment with GnRH. In another study,\textsuperscript{49} progestin-treated dairy cows (n = 411) were superstimulated four days after receiving estradiol or two days after GnRH; there was no significant difference in the number of transferable embryos between groups. In yet another retrospective analysis of commercial data,\textsuperscript{62} dairy donors superstimulated 60 hours after the administration of GnRH (n = 245) produced a similar number of transferable embryos compared with those superstimulated four days after receiving estradiol (n = 691). Obviously, controlled studies with the use of GnRH must be conducted to validate these promising results.

**Superovulation During the First Follicle Wave Following GnRH-Induced Ovulation**

Another alternative is to induce ovulation and initiate superstimulatory treatments on the first follicular wave. Follicular wave emergence occurs consistently at the time of ovulation,\textsuperscript{39} and experiments performed in cattle\textsuperscript{55} and sheep\textsuperscript{61} have shown that it is possible induce superovulation of follicles in the first follicular wave. Adams et al\textsuperscript{5} also reported no difference in superovulatory response when follicle stimulating hormone (FSH) treatments were initiated at the time of emergence of the first or the second follicular wave. However, success relies upon determination of the time of ovulation or accurate estrus detection, with ovulation expected to occur one day later.

To avoid the need to detect estrus and ovulation in Nelore (Bos indicus) donors, Nasser et al\textsuperscript{55} induced synchronous ovulation with a protocol designed for FTAl. FSH treatments were initiated at the expected time of ovulation (and emergence of the first follicular wave). Superovulatory response did not differ from a contemporary group superstimulated four days after treatment with estradiol. However, the number of transferable embryos was reduced in cows superstimulated during the first follicular wave without an accompanying use of a progestin device.

We recently conducted a series of experiments with the overall objective of developing a protocol for superovulation during the first follicular wave after GnRH-induced ovulation, using progestin devices (reviewed in Bö et al\textsuperscript{20,21}). We considered previous reports indicating that ovulatory response to GnRH could be increased by the administration of PGF to regress the CL at the time of insertion of a progestin device that remained in place for seven to 10 days.\textsuperscript{61} In that study, ovulation and wave emergence occurred one to two days after the administration of GnRH, indicating that this approach could be used in groups of randomly cycling donors.

The recommended protocol consists of the administration of PGF at the time of insertion of a progestin device to induce luteal regression and cause a persistent follicle to develop. Seven days later (with the progestin device still in place), GnRH is administered to induce ovulation and follicle wave emergence, and FSH treatments are initiated 36 hours later. The protocol can be easily organized on a calendar, as illustrated in Figure 2. Although this protocol was designed for four days of FSH, a five-day superstimulation protocol can be accomplished by simply delaying the administration of the PGF and removal of the progestin device by one day. Overall in this series of experiments, more than 95\% of animals ovulated to the first GnRH administration (with expected follicle wave emergence) and superovulatory response and ova/embryo numbers and quality were similar to that obtained when the follicular wave was synchronized with estradiol.

**Subordinate Follicle Break-Through**

During a normal follicular wave, subordinate follicles regress because of decreasing concentrations of circulating FSH, caused by the secretions (estradiol and inhibin) of the cohort and especially of the dominant follicle.\textsuperscript{1,2} Small follicles require FSH to continue their growth, and evidence suggests that follicles as small as 1 mm in diameter will commence growth under the influence of FSH (reviewed by Adams et al\textsuperscript{3}). Perhaps all that is required to recruit follicles for superstimulation is to cause these follicles to grow to a diameter of 3 or 4 mm, at which time the regular four- or five-day superstimulatory treatment protocol could be initiated. Assuming a growth rate of 1 to 2 mm per day, this should take two to three days i.e., add two to three days to the
superstimulation treatment protocol. As the exogenous FSH replaces endogenous levels that are depressed by the secretory products of the dominant follicle, we hypothesized that the presence of a dominant follicle would not have any effect on superovulatory response.

We superstimulated donors successfully using this approach at random stages of the estrous cycle, without regard to the presence of a dominant follicle.\textsuperscript{20} Small doses of FSH were administered twice daily for two days, and then the regular FSH treatment protocol was begun with no increase in the total amount of FSH administered. As an alternative, the two days of FSH pretreatment could be replaced with a single injection of eCG. In another study, the administration of 500 IU of eCG two days before initiating FSH treatments tended to increase the superovulatory response in beef donors,\textsuperscript{24} and in beef donors that had previously produced unsatisfactory numbers of embryos, pretreatment with 500 IU of eCG resulted in increased embryo production over that achieved without eCG (cited in Bó et al\textsuperscript{20}). It was hypothesized that the eCG recruited additional follicles into the wave prior to initiating FSH treatments.

More recently, we have investigated the effect of lengthening the superstimulatory treatment protocol from the traditional four days to seven days in order to recruit more follicles into the wave.\textsuperscript{30} Lengthening the FSH treatment protocol to seven days, without increasing the total amount of FSH administered, increased the number of ovulations and the synchrony of ovulations, and tended to increase the mean numbers of total ova/embryos, fertilized ova, and transferable embryos. In other words, the lengthened superstimulatory treatment protocol resulted in more follicles reaching an ovulatory size and acquiring the capacity to ovulate with an increased number of ovulations, and with no decrease in oocyte/embryo competence. It was concluded that prolonged FSH treatment protocols may be an effective strategy to recruit small follicles into the follicular cohort available for superstimulation, while providing the additional time needed to reach an ovulatory size and acquire the capacity to ovulate. In addition, these results suggest that traditional four-day superstimulatory treatment protocols may not provide adequate time for all follicles within the cohort to acquire the capacity to ovulate. This requires further study.

**Reducing the Number of FSH Treatments in a Superstimulation Protocol**

Because the half-life of pituitary FSH has been shown to be five hours in the cow,\textsuperscript{39} traditional superstimulatory treatment protocols consist of twice-daily injections of pituitary FSH over four or five days.\textsuperscript{46} This requires frequent attention by farm personnel, and increases the possibility of failures due to non-compliance. In addition, twice-daily treatments may cause undue stress in donor cows with a subsequent decreased superovulatory response, and/or altered preovulatory LH surge.\textsuperscript{63} Thus, simplified protocols may be expected to reduce donor handling and improve response, particularly in less tractable animals.

More than 15 years ago, we reported that a single subcutaneous administration of FSH in beef cows in good body condition (>3 out of 5) resulted in a superovulatory

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**Figure 2.** Treatment schedule for superovulation of donor cows following gonadotropin releasing hormone (GnRH)-induced ovulation. Donors receive a progestin device along with prostaglandin (PGF) to induce the formation of a persistent follicle followed by GnRH seven days later (with the progestin device still in place). Thirty-six hours after GnRH superstimulation with follicle stimulating hormone (FSH) is initiated with twice daily doses over four days, and PGF is administered with the last two FSH injections; progestin devices are removed with the last FSH injection. Ovulation is induced with GnRH 24 hours after progestin device removal, donors are fixed-time inseminated (AI) 12 and 24 hours later, and ova/embryos are collected seven days after AI.
response equivalent to the traditional treatment protocol over four days. However, the results were not repeatable in Holstein cows, which had less adipose tissue. In a subsequent study in Holstein cows, the single injection was split into two, with 75% of the FSH dose administered subcutaneously on the first day of treatment and the remaining 25% administered 48 hours later, when PGF is normally administered. Although superovulatory response was improved, it was still numerically less than the twice-daily injection protocol.

An alternative to induce a consistent superovulatory response with a single injection of FSH would be to combine the pituitary extract with agents that cause the hormone to be released slowly over several days. These agents are commonly known as polymers, are biodegradable, and non-reactive in the tissue, facilitating use in animals. We have recently completed a series of experiments in which FSH diluted in a 2% hyaluronan solution was administered as a single intramuscular injection, to avoid the effects of body condition. Overall, the single injection protocol resulted in a number of ova/embryos similar to the traditional twice-daily FSH protocol. However, 2% hyaluronan was very viscous and difficult to mix with FSH, especially in the field. We speculated that although more dilute preparations of hyaluronan were less efficacious as a single injection, their use could be improved by splitting them into two injections 48 hours apart, as we had shown previously with subcutaneous injections of FSH. The split intramuscular treatment protocol consisted of diluting the FSH lyophilized powder with 10 mL of the hyaluronan solution and the administration of two-thirds of the total dosage of FSH on the first day, followed by a second injection with the remaining one-third of the total dosage of FSH 48 hours later, when PGF is normally administered.

An experiment was designed to compare the efficacy of the split intramuscular injection protocol of FSH in two different concentrations of hyaluronan, 1% and 0.5%, with the twice-daily intramuscular injection protocol over four days in beef cattle. Overall, the numbers of transferable embryos did not differ among treatment groups (Control: 4.0±0.8; 1% hyaluronan: 5.0±0.9; 0.5% hyaluronan: 6.1±1.3). Data were interpreted to suggest that splitting the FSH dose in either concentration of hyaluronan into two intramuscular injections 48 hours apart would result in a comparable superovulatory response to the traditional twice-daily intramuscular injection protocol in beef cattle. Furthermore, the less concentrated solutions of hyaluronan were not difficult to mix with FSH, even under field conditions.

Summary and Conclusions

The use of protocols that control follicular development and ovulation offers the advantage of applying assisted reproductive technologies without the need for detecting estrus. These treatments have been shown to be practical and easy to perform by the farm staff, and more importantly, they do not depend on accuracy in estrus detection. In estrus synchronization programs, treatments with GnRH and progestin-releasing devices have provided for FTAI and FTET in beef cattle, and the addition of progestin devices and eCG have been especially useful in increasing pregnancy rates in cows experiencing postpartum anestrus. In superovulation schemes, estradiol is very efficacious in synchronizing follicle wave emergence, but is not available in many countries. Although the administration of GnRH to synchronize follicular wave emergence yields variable results, pre-synchronization with a progestin-releasing device has been shown to improve the response to GnRH. This allows for superstimulation during the first follicular wave after ovulation, with results that did not differ from the use of estradiol. Recent studies with the use of a hyaluronan-based, slow-release formulation have shown that it is possible to induce a consistent superovulatory response following two intramuscular injections of FSH, without adversely affecting the number of transferable embryos.

Acknowledgements

Research was supported by the Instituto de Reproducción Animal Córdoba (IRAC), Bioniche Animal Health, Belleville, ON, and the University of Saskatchewan.

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