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Superstimulation of ovarian follicular development in beef cattle with a single intramuscular injection of Folltropin-V

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ABSTRACT

The need to inject FSH twice daily for superstimulation of ovarian follicular development in cattle necessitates frequent attention by farm-personnel and increases the possibility of failures due to mishandling and errors in administration of treatments. A series of three experiments were designed to evaluate the feasibility of superstimulation in beef cattle with a single intramuscular (IM) injection of Folltropin-V diluted in a hyaluronan-based slow-release formulation (SRF). In Experiment 1, cows were assigned to one of three treatment groups to compare two methods of injection as compared to the twice daily IM injection protocol. Superovulatory response of cows ($n = 6$) treated with twice daily IM injections over 4 days (Control) was greater than of cows treated with a single subcutaneous (SC) injection in SRF ($n = 6$), while superovulatory response of cows treated with a single IM injection in SRF ($n = 6$) was intermediate. Experiment 2 was designed to compare two concentrations of SRF (20 mg/mL hyaluronan, 100% compared to 10 mg/mL hyaluronan, 50%) in a single IM injection protocol. The mean number of corpora lutea (CL) were not significantly different ($P \geq 0.05$), but the numbers of total ova/embryos ($P < 0.05$), fertilized ova ($P < 0.01$) and transferable embryos ($P < 0.001$) were greater in cows treated with FSH in 100% SRF ($n = 20$) than cows treated with FSH in 50% SRF ($n = 20$). Experiment 3 was designed to compare superovulatory response in Red Angus donor cows treated with a single IM injection of Folltropin-V diluted in 100% solution of SRF with those treated with the traditional twice-daily IM injection protocol over 4 days. Mean (±SEM) numbers of CL (13.7 ± 1.2 compared to 13.8 ± 1.2), total ova/embryos (12.3 ± 1.5 compared to 13.7 ± 2.1), fertilized ova (7.2 ± 1.1 compared to 8.4 ± 1.4) and transferable embryos (4.9 ± 0.8 compared to 6.4 ± 1.3) were not significantly different between Control ($n = 29$) and Single injection ($n = 29$) groups, respectively.

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In summary, superstimulation of beef donor cows with a single IM injection of Folltropin-V diluted in 100% solution of SRF resulted in a comparable superovulatory response to the traditional twice-daily IM administration of Folltropin-V diluted in saline over 4 days.

1. Introduction

The main goal of superstimulatory treatments in cattle is to induce a greater number of ovulations that will result in a high number of transferable embryos and acceptable pregnancy rates after transfer (Bo et al., 2002). However, there is great variability in the response of donor cows to gonadotropin treatments which is an important cost factor for the embryo transfer industry. Recent progress in the knowledge of the estrous cycle of cattle and studies on follicular wave dynamics have shown that greater superovulatory responses are achieved when gonadotropin treatments are initiated at the time of follicular wave emergence, and methods of synchronizing the timing of follicular wave emergence have been devised (Bergfelt et al., 1997; Bo et al., 1995).

Superstimulation of follicular development in donor cattle has been accomplished traditionally by a single intramuscular (IM) injection of equine chorionic gonadotropin (eCG) or twice daily IM injections of a pituitary extract containing FSH (Mapletoft et al., 2002). Equine chorionic gonadotropin is a complex glycoprotein with a half-life of more than 40 h (Schams et al., 1977), and a single IM injection is required to elicit superstimulation in the cow. Conversely, the biological half-life of pituitary FSH in the cow has been reported to be less than 5 h (Laster, 1972; Demoustier et al., 1988) and frequent treatments are normally needed to induce superstimulation (Bellows et al., 1969; Monniaux et al., 1983). Twice daily IM treatments with a pituitary extract induce a greater superovulatory response than once daily treatments (Looney et al., 1981; Monniaux et al., 1983; Walsh et al., 1993). Therefore, the administration of pituitary-derived gonadotropins requires constant attention by farm-personnel, increasing the possibilities of failures due to mishandling and errors with administration. In addition, twice daily treatments can cause undue stress in donor cows with a subsequent decreased superovulatory response (Bo et al., 1994). Simplified protocols for superstimulation of follicular development in cattle may be expected to reduce donor handling costs and improve response, particularly in less tractable animals.

A single bolus injection of a crude pituitary extract (FSH-P), diluted in a gelatin-saline vehicle, induced a superovulatory response similar to that of a 5-days, twice-daily treatment protocol (Hill et al., 1985). However, the number of transferable embryos with the single injection treatment was very few. Others have used FSH in aluminum hydroxide gel (Kimura et al., 2007) or polyvinylpyrolidone (Yamamoto et al., 1994) with variable success. However, there is a concern that superstimulation with pituitary extracts combined with these types of substances may result in the development of antibodies against FSH (Sutherland, 1991).

The development of a single dose protocol of FSH would be a useful alternative to the traditional twice daily treatment protocols for superstimulation in the cow. A single dose protocol would likely improve compliance on farms that lack man-power or sufficiently trained personnel. In a study using a partially purified pituitary extract, Folltropin-V (Bioniche Animal Health Inc., Belleville, ON, Canada), a single subcutaneous dose of 400 mg NIH-FSH-P1 in beef cows in medium to high body condition (>3/5) resulted in a superovulatory response equivalent to the traditional twice daily, 4-days treatment protocol (Bo et al., 1994). However, results were not repeatable in Holstein cows that had less subcutaneous adipose tissue. In yet another study in Holstein cows, the single injection of Folltropin-V was split into two, with 75% administered subcutaneously on the first day of treatment, and the remaining 25% administered 48 h later, when prostaglandin is normally administered; superovulatory response did not differ from the traditional 4-days protocol (Lovie et al., 1994). As the use of a single subcutaneous injection of Folltropin-V was body condition- and user-dependent, the idea of a more efficient and “user friendly” method of superstimulation has gained great interest.

An alternative option to induce a consistent superovulatory response with a single injection of FSH would be to combine Folltropin-V with a biodegradable polymer that will result in sustained, slow release of the hormone over several days. There are several polymers of biological or synthetic origin with different properties used to facilitate sustained drug release. These compounds are biodegradable and not reactive in tissue, which facilitates use in animals (Sutherland, 1991). One of these compounds is a hyaluronan-based slow release formulation (SRF, Bioniche Animal Health Inc., Belleville, ON, Canada). In preliminary studies, a single injection of Folltropin-V diluted in the SRF provided promising results in the induction of superovulation. A series of experiments were designed to evaluate the superovulatory response in beef cows treated with a single IM injection of Folltropin-V diluted in SRF. Specific objectives were to determine the site of the single injection of Folltropin-V in SRF that would induce superovulation most consistently (Experiment 1); to compare the efficacy of two different concentrations of SRF given by IM injection (Experiment 2); and to confirm the efficacy of the single IM injection of Folltropin-V in SRF by comparing it with twice daily IM injections in saline in inducing superovulation in Angus cows.

2. Materials and methods

Experiment 1 was designed to compare two sites of injection of Folltropin-V diluted in SRF. Cross-bred beef cows ($n = 18$) were randomly assigned to one of three treatment groups. On Day 0 (beginning of treatment), all cows received 5 mg estradiol-17β (Estradiol-17β® Río de Janeiro, Laboratorios Allignani Hnos SRL, Argentina) plus 50 mg progesterone (Progesterona® Río de Janeiro) and an intravaginal device impregnated with 1.56 g progesterone (Cue-Mate, Bioniche Animal Health Inc., Belleville, ON Canada). On Day 4, follicular development of cows were superstimulated with one of following treatments: Group 1 (Control) received 400 mg NIH-FSH-P1 (Folltropin-V, Bioniche Animal Health) diluted in saline in twice-daily decreasing doses by IM injection in the neck over 4 days (80, 80, 60, 60, 40, 40, 20 and 20 mg); Group 2 received a single dose of 400 mg NIH-FSH-P1 diluted in 10 mL of SRF (20 mg/mL hyaluronan) by IM injection in the neck; and Group 3 received a single dose of 400 mg NIH-FSH-P1 diluted in 10 mL of SRF by subcutaneous (SC) injection at the base of the ear. For single injection, the Folltropin-V was dissolved in 1 mL of saline and then injected into a vial containing 9 mL of hyaluronan at a concentration of 22 mg/mL. After gentle shaking, the contents were aspirated into a 12 mL syringe and injected. In the AM and PM of Day 6 (48 h after initiating FSH treatment), all cows received 150 mg D-(+)-cloprostenol (PGF, Bioprost, Biotay, Argentina) and Cue-Mates were removed in the PM. In the AM of Day 8 cows received 12.5 mg pLH (Lutropin-V, Bioniche Animal Health) and were inseminated 12 and 24 h later. If cows were in standing estrus in the afternoon of Day 7, they were inseminated at the time of pLH and 12 h later. Embryos were collected non-surgically on Day 15 and evaluated following IETS recommendations (Wright, 1998). Embryos of IETS quality grades 1 and 2 were considered to be of transferable quality.

Experiment 2 was designed to compare two concentrations of SRF on superovulatory response following a single IM injection of Folltropin-V. The experiment was conducted in two phases; Phase 1 was with 15 Red Angus donor cows in which follicular development was superstimulated twice in a cross-over design, and Phase 2 was done with 10 Red Angus donor cows that were randomly allocated to one of the two treatment groups and follicular development was superstimulated once. Treatments were initiated in all cows on Day 4 of the single injection protocol described in Experiment 1 with 400 mg NIH-FSH-P1 (Folltropin-V) given IM in the neck. The material for the single injection was prepared by diluting the Folltropin-V lyophilized powder in 1 mL of saline and then mixing this in the 12 mL syringe (as opposed to the bottle) with 9 mL of SRF (22 mg/mL hyaluronan; 100% solution) or a 50% dilution of SRF (11 mg/mL hyaluronan; 50% solution) immediately before administration. Inseminations, ova/embryo collections and evaluations were conducted as in Experiment 1.

Experiment 3 was designed to confirm previous results and compare superovulatory response following a single IM injection of Folltropin-V diluted in 100% solution of SRF with the traditional twice-daily IM injection treatment protocol with Folltropin-V diluted in saline in Red Angus donor cows. The experiment was conducted in two phases; Phase 1 was done with 17 Red Angus donor cows in which follicular development was superstimulated twice in a cross over design, and Phase 2 was conducted with 24 Red Angus donor cows that were randomly allocated into one of the two treatment groups and follicular development superstimulated once. On Day 4 of the protocol described in Experiment 1, cows were superstimulated with 400 mg NIH-FSH-P1 (Folltropin-V) in twice-daily decreasing IM doses over 4 days or with a single IM injection in the neck. The single administration was prepared by diluting the Folltropin-V lyophilized powder in 1 mL of saline and mixed with 9 mL of the 100% solution of SRF (22 mg/mL hyaluronan) in the syringe, as described in Experiment 2, immediately before administration. Inseminations, ova/embryo collection and evaluations were done as in Experiment 1.

2.1. Ultrasonography

All cows were examined on Days 0 and 4 for the presence of a CL and follicle size and number. Cow ovaries were also evaluated ultrasonically daily during follicular superstimulatory treatments (Days 4–8) to determine follicle growth profiles and on Day 11, to determine the number of ovulations (Robertson et al., 1993). Superovulatory response was confirmed by counting the number of CL by rectal palpation on Day 15.

2.2. Statistical analysis

Data points (continuous variables with normal distribution and homogeneity of variances) in Experiment 1 were evaluated by ANOVA. Means were compared by the protected LSD test. Data in Experiments 2 and 3 were first transformed by square root and then analyzed by ANOVA, using the software Infostat® (Infostat, 2008). Means were compared by the protected LSD test. ANOVA for mixed models was used to detect the effect of day, treatment and the day-by-treatment interaction on the mean follicle diameter and the number of follicles ≥9 mm in diameter over treatment days (Infostat, 2008). For statistical comparison of the mean follicle diameters between treatment groups, the mean follicle diameter of each cow was first calculated by obtaining the mean diameter of all follicles >3 mm present in the ovaries on a given day of treatment.

3. Results

The results of Experiment 1 are summarized in Table 1. The numbers of total ova/embryos, fertilized ova and transferable embryos were greater ($P < 0.05$) in cows treated with 400 mg FSH given by twice daily IM injections over 4 days than those given a single injection of 400 mg FSH by SC injection at the base of the ear; the numbers of total ova/embryos, fertilized ova and transferable embryos in cows treated by the single IM injection were intermediate and not different from the other treatment groups. Fig. 1 depicts the mean number of follicles ≥9 mm in diameter during the superstimulation treatment. In all groups, the mean number of follicles ≥9 mm in diameter increased from Day 5 (i.e. 24 h after the first FSH treatment) to Day 8 and then decreased thereafter (day effect, $P < 0.001$). Furthermore, there was a day by treatment interaction ($P < 0.0001$) that was attributed to a greater number of follicles ≥9 mm in diameter on Days 6–8 in cows treated with twice-daily IM injections of Folltropin-V.

Table 1
Superovulatory response (mean ± SEM) of beef cows treated with 400 mg NIH-FSH-P1 Folltropin-V given by twice daily IM injections over 4 days or as a single injection diluted in slow-release formulation (SRF) and given by IM injection or SC injection at the base of the ear. Experiment 1.

Treatment	*n*	CL	Ova/embryos	Fertilized ova	Transferable embryos
Control (2× daily)	6	13.2 ± 3.1[a]	12.7 ± 4.0[a]	10.8 ± 3.8[a]	8.5 ± 2.7[a]
Single injection IM	6	11.0 ± 2.0[a]	8.2 ± 2.0[ab]	5.8 ± 1.7[ab]	4.7 ± 1.6[ab]
Single injection SC	6	4.2 ± 1.0[b]	2.0 ± 1.4[b]	1.3 ± 1.1[b]	1.3 ± 1.1[b]
P-Value		0.028	0.044	0.051	0.062

Means within a column with different superscripts are significantly different.

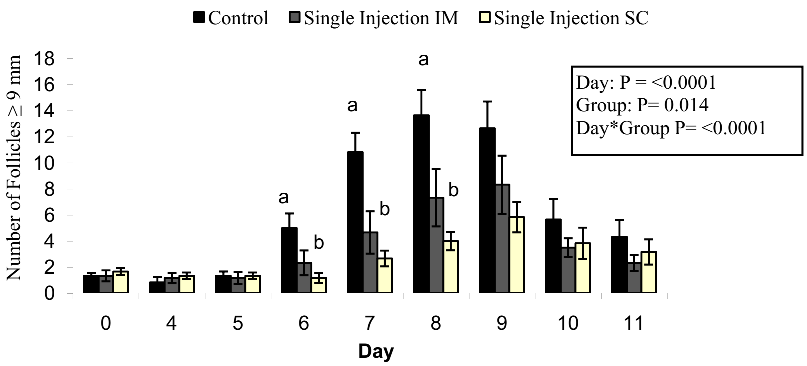

Fig. 1. Mean (±SEM) number of follicles ≥9 mm during superstimulation treatment in beef cows given 400 mg NIH-FSH-P1 Folltropin-V by twice daily IM injections over 4 days or diluted in a slow-release formulation (SRF) and given by a single IM injection or SC injection at the base of the ear. Experiment 1. [ab]Significant differences between control and single injection treatment groups ($P < 0.05$).

In Experiment 2, there were no differences between Phases 1 and 2, so data were combined. Although the mean (±SEM) number of CL were not significantly different, the numbers of ova/embryos ($P < 0.05$), fertilized ova ($P < 0.01$) and transferable embryos ($P < 0.001$) were greater in cows treated with the 100% rather than the 50% solution of SRF (Table 2). Two cows in the 50% group and one cow in the 100% group had ≤2 CL at the time of ova/embryo collection. Furthermore, 8 of 20 (40%) cows in the 50% group did not produce any transferable embryos, whereas, all the cows in the 100% group produced at least one transferable embryo.

Follicle characteristics over treatment days did not differ between groups. The number of follicles from 3 to 5 mm in diameter on the day of Folltropin-V injection (Day 4; 18.6 ± 1.5 compared to 16.4 ± 1.6), number of ovulations (13.2 ± 1.1 compared to 10.4 ± 1.4) and mean time of ovulation after pLH administration (30.8 ± 1.3 h compared to 30.1 ± 2.6 h) were not significantly different between cows treated with Folltropin-V diluted in 100% SRF and those treated with Folltropin-V diluted in 50% SRF. However, there was a tendency for a day by treatment interaction in the mean diameter profiles of all follicles >3 mm in diameter ($P = 0.0662$; Fig. 2) and ≥9 mm in diameter ($P = 0.08994$; Fig. 3) over treatment days, that was attributed to a greater mean follicle diameter (Fig. 2) and number of follicles ≥9 mm in diameter (Fig. 3) on Days 7 and 8, in cows treated with Folltropin-V diluted in 100% SRF than in those treated with Folltropin-V diluted 50% SRF.

In Experiment 3, there were no differences between Phases 1 and 2, so data were combined. The number of CL, total ova/embryos, fertilized ova and transferable embryos were not different between groups ($P > 0.05$; Table 3). One cow in each treatment group had ≤2 CL at the time of ova/embryo collection, and one cow in the Control group and two cows in the Single injection group did not produced any transferable embryos.

Follicle characteristics over treatment days did not differ between groups. There was a day effect ($P < 0.001$) on the mean diameter profile of all follicles ≥3 mm in diameter (Fig. 2) and the numbers of follicles 3–5 mm, 6–8 mm and ≥9 mm, but were not significantly different between groups and there were no interactions. Furthermore, the number of follicles 3–5 mm in diameter on the day of Folltropin-V injection (Day 4; 19.6 ± 1.5 compared to 17.6 ± 1.3), the number of ovulations (15.8 ± 1.3 compared to 14.5 ± 1.7) and the mean time of ovulation after pLH administration (31.3 ± 2.3 h compared to 35.1 ± 2.3 h)

Table 2
Superovulatory response (mean ± SEM) of beef cows treated with 400 mg NIH-FSH-P1 Folltropin-V diluted in a 100% or a 50% solution of slow-release formulation (SRF) given by a single IM injection in the neck. Experiment 2.

Treatment	*n*	CL	Ova/embryos	Fertilized ova	Transferable embryos	Cows with "0" transferable embryos
100% SRF	20	12.5 ± 1.1	11.5 ± 0.9	8.2 ± 0.7	5.8 ± 0.7	0
50% SRF	20	9.7 ± 1.3	7.1 ± 1.4	4.6 ± 1.1	2.4 ± 0.6	8
P-Value		0.099	0.015	0.010	0.001	0.001

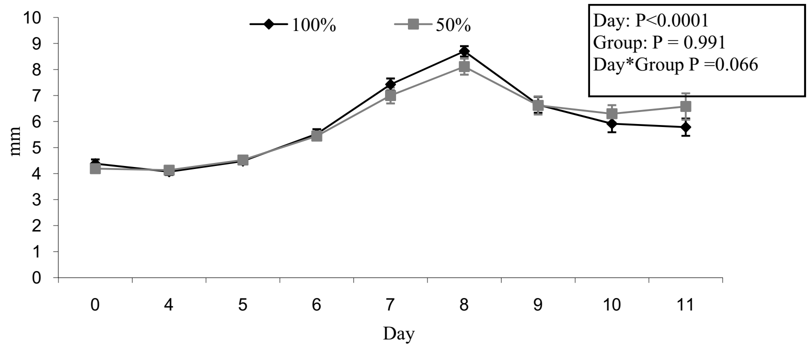

Fig. 2. Mean (±SEM) diameter profiles of all follicles >3 mm in diameter during the superstimulation of beef cows with 400 mg NIH-FSH-P1 Folltropin-V diluted in two concentrations of slow-release formulation (SRF; 100% or 50%) given by a single IM injection in the neck. Experiment 2.

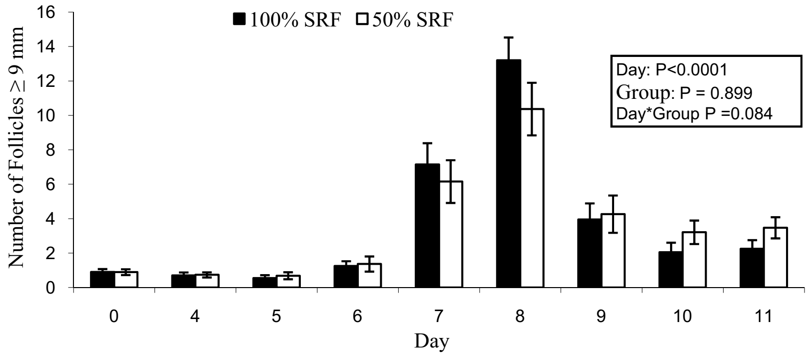

Fig. 3. Mean (±SEM) number of follicles ≥9 mm in diameter during the superstimulation of beef cows with 400 mg NIH-FSH-P1 Folltropin-V diluted in two concentrations of slow-release formulation (SRF; 100% or 50%) and given by a single IM injection in the neck. Experiment 2.

were not significantly different between cows in the Control group and those in the Single injection group.

4. Discussion

Overall results demonstrate that a single IM injection of Folltropin-V diluted in 20 mg/mL hyaluronan (100% solution of SRF) is highly efficacious in the superstimulation of Red Angus donors; results, both in terms of ova/embryo production and numbers of transferable embryos did not differ significantly from the traditional approach of superstimulation with twice-daily IM injections in saline. It is note-worthy that of the 49 cows in which follicular development was superstimulated with the single intramuscular injection of Folltropin-V in a 100% solution of SRF in Experiments 2 and 3, only four (8%) failed to produce any transferable embryos. This is much less than that reported by Looney (1986) for beef donors (24%), and by Hasler et al. (1983) for Holstein donors following superstimulation with twice-daily IM injections. In fact, of the 29 controls superstimulated with twice-daily IM injections in Experiment 3, only three (10%) produced no transferable embryos, obviously not different from those in which follicular development was superstimulated with the single IM injection.

Circulating concentrations of FSH following injection depends on absorption from the site of administration and metabolic clearance from the circulation. Based on metabolic clearance rates (Demoustier et al., 1988), it would be expected that FSH would be cleared from the circulation at the same rate, irrespective of its concentration and method of administration. Rapid absorption of the pituitary extract should result in rapid FSH clearance. Therefore, follicles exposed to greater concentrations of FSH for a very short period of time would appear to be unable to sustain growth until ovulation. Preliminary results collected as part of a study published previously (Bo et al., 1994) indicated that following a single IM or SC (in lean animals) injection of Folltropin-V in saline, blood concentrations of FSH reached similarly high concentrations

Table 3
Superovulatory response (mean ± SEM) of Red Angus cows treated with 400 mg NIH-FSH-P1 Folltropin-V diluted in saline and given by twice daily IM injections over 4 days (Control) or diluted in a 100% solution of slow-release formulation (SRF) and given by a single IM injection in the neck. Experiment 3.

Treatment	*n*	CL	Ova/embryos	Fertilized ova	Transferable embryos	Cows with “0” transferable embryos
Control (2× daily)	29	13.7 ± 1.2	12.3 ± 1.5	7.2 ± 1.1	4.9 ± 0.8	3
Single injection	29	13.8 ± 1.2	13.7 ± 2.1	8.4 ± 1.4	6.4 ± 1.3	4
P-Value		0.9634	0.935	0.648	0.372	0.585

and fell more rapidly than in cows in high body condition that received a SC single injection, presumably because of slower absorption in the cows with high body condition. The addition of FSH to a solution of polyvinylpyrrolidone resulted in slower absorption and more prolonged, elevated blood concentrations of FSH than when FSH was administered in a saline vehicle (Takedomi et al., 1993). We concluded that the mixing of Folltropin-V with a solution of hyaluronan also reduced the rate of absorption, resulting in sustained elevated levels of circulating FSH. Therefore, it would seem that characteristics affecting absorption rate rather than clearance of FSH would be the limiting factor in inducing superovulation with a single injection of Folltropin-V.

The results of Experiment 1 indicated that the administration of Folltropin-V mixed with hyaluronan as a single SC injection into the base of the ear was not efficacious, but the single IM injection resulted in a superovulatory response that did not differ significantly from that achieved with twice daily IM injections in saline (although numerically less than expected). However, the method of mixing the Folltropin-V and hyaluronan was of concern. A solution of 20 mg/mL hyaluronan (100% SRF) is quite viscous, and it was observed that following mixing with Folltropin-V, it was not possible to extract the full amount from the vial. Therefore, it was concluded that the numerically lower response following the single intramuscular injection may have been due to a lower amount of Folltropin-V actually being administered. This conclusion is supported by follicle growth profiles. Although numbers of recruitable follicles (3–5 mm) did not differ at the time of initiating gonadotropin treatment, the numbers of follicles reaching 9 mm or more in diameter was less in single injection groups (Fig. 1). It was decided that a more repeatable method of mixing Folltropin-V with 20 mg/mL hyaluronan had to be developed, and it was also for this reason that a lower concentration of hyaluronan (10 mg/mL hyaluronan; 50% SRF) was investigated in Experiment 2. The 50% solution of SRF was much less viscous and easier to extract from the vial.

The method of mixing Folltropin-V in saline and the solution of SRF in the syringe in Experiments 2 and 3 appeared to be much more efficacious in terms of inducing a superovulatory response. However, the 50% solution of SRF was less efficacious than the 100% solution in inducing superovulation. Follicle diameter profiles over the treatment period in Experiment 2 suggested that Folltropin-V diluted in a 50% solution of SRF induced a follicular superstimulatory response, but follicle growth seemed to slow down after 2 days (Day 6; Fig. 2) and it appeared that fewer follicles reached an ovulatory size than in the 100% SRF group (Fig. 3), presumably because of inadequate concentrations of circulating FSH in the second half of the treatment protocol, as was suggested following the subcutaneous administration of Folltropin-V in saline in Holstein cows (Lovie et al., 1994).

When compared to the traditional twice-daily IM injection protocol in saline over 4 days, the single IM injection of Folltropin-V in 20 mg/mL hyaluronan (100% SRF) proved to be very efficacious in Experiment 3. There were no significant differences in any of the measured end-points between groups, and the numbers of transferable embryos was high, especially when compared to results of twice daily treatment protocols published previously (Hasler et al., 1983; Looney, 1986). It is also important to note that the numbers of FSH-responsive follicles (3–5 mm diameter) at the time of initiating treatments did not differ significantly between groups, and did in fact, favor the Control group numerically. In addition, the numbers of ovulations and the interval from pLH treatment to ovulation did not differ between groups. The follicle growth profiles also did not differ between groups suggesting that the single IM injection of Folltropin-V provided sufficient FSH to support follicle growth through to ovulation.

Experiment 3 was considered a fairly rigorous test of the single IM injection of Folltropin-V in hyaluronan for the induction of superovulation in the cow. Not only did it support follicle growth profiles that were comparable to the traditional twice daily IM injection regimen, but there were no differences in any of the other variables that were measured, including ovulation rate, ova/embryo production and most importantly, numbers of transferable embryos. Although the optimal dose of Folltropin-V to be administered by a single IM injection was not determined, it would appear to be similar to that administered by twice-daily injections, and as has been shown previously with the single SC injection of Folltropin-V (Bo et al., 1994).

5. Conclusions

In summary, superstimulation of Angus donor cows with a single intramuscular injection of Folltropin-V diluted in 20 mg/mL hyaluronan resulted in a comparable superovulatory response to the traditional twice-daily intramuscular injection treatment protocol. The method described for the mixing Folltropin-V and hyaluronan at this concentration (20 mg/mL) would appear to be effective, but it is not clear whether the optimum dose of Folltropin-V for Angus cattle was used. A single intramuscular injection of Folltropin-V should reduce the labor and handling associated with follicular superstimulatory treatments and may be particularly useful in the superstimulation of follicular development in animals in which handling stress is an impediment to success.

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