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# THE EFFECT OF ESTRUS SYNCHRONIZATION SCHEME, INJECTION PROTOCOL AND LARGE OVARIAN FOLLICLE ON RESPONSE TO SUPEROVULATION IN BEEF HEIFERS

R.B. Staigmiller, M.D. MacNeil, R.A. Bellows, R.E. Short and D.A. Phelps

Fort Keogh Livestock and Range Research Laboratory, USDA-ARS, and Montana Agricultural Experiment Station
Route 1, Box 2021, Miles City, MT 59301, USA

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## ABSTRACT

Two trials were conducted to examine the effects of estrus synchronization scheme, gonadotropin injection protocol and presence of a large ovarian follicle on response to superstimulation of follicular development and the ensuing superovulation. Estrus was synchronized with either a progestin compound (MGA) or by the use of a luteolytic agent (PGF). Superstimulation was induced with 280 mg equivalents of pFSH administered either by a single subcutaneous injection or by a series of 8 intramuscular injections over 4 d. Follicular development was followed for 5 d with real-time ultrasound, and the heifers were retrospectively classified as to the presence or absence of a large follicle (≥8 mm; morphologically dominant follicle) at the start of superstimulation. The 2 trials differed by season of the year and genetic origin of the heifers. In Trial I (20 heifers), the ovulation rate was influenced by the 3-way interaction of the synchronization scheme, injection protocol and morphologically dominant follicle ($P=0.05$). The number of large follicles on Day 5 (Day 0 = day of start of superstimulation) and ovarian score (scale 1 to 5 based on extent of follicular development; 1=least, 5=most) on Day 5 were significantly correlated ($P<0.05$) with ovulation rate. In Trial II (20 heifers), the ovulation rate, number of embryos recovered, number of transferable embryos and ovarian weights were all greater ($P<0.05$ to $P<0.01$) with the 8-injection protocol than the 1-injection protocol. The number of medium follicles (5 to 7 mm) on Days 2 and 3, number of large follicles (≥8 mm) on Days 3, 4 and 5 and ovarian scores on Days 4 and 5 were all significantly correlated ($P<0.05$) with ovulation rate. In both trials, differences in follicle populations were not seen until Day 3 of the superstimulation procedure. Collectively, these trials do not provide strong support for a single injection of FSH, as used here, nor does it indicate a clear advantage for either MGA or PGF as a means of enhancing the ovulation rate or embryo quality.

Key words: Estrus synchronization, injection protocol, dominant follicle, superovulation, follicle development

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## INTRODUCTION

In the embryo transfer industry, the single most important measure of the success of superovulation economically is the number of live offspring born per donor (2). The number of offspring is a function of the number of embryos per donor that are transferred and the conception rate. The number of embryos available for transfer from each donor continues to show wide variation and represents a major problem to the industry. The ovulation rate and percentage of ovulated oocytes that develop to transferable embryos are the endpoints which determine the number of embryos available for transfer. A recent review of superovulation (2) has examined many of the factors that influence these 2 endpoints, including the presence of a dominant ovarian follicle at the start of superstimulation. Considerable attention has been given over the last several years to controlling follicular development so it can be appropriately synchronized with superstimulation protocol (1,3,9). Estrus synchronization is often used in conjunction with superstimulation. Two common methods of synchronization presently in use are 1) administration of a progestin for 12 to 14 d with superstimulation starting 14 d after the end of progestin treatment; and 2) an injection of PGF or a prostaglandin analog, with superstimulation starting 12 to 14 d later (see review, 18). It is not known to what extent either of these methods synchronize follicle development as well as estrus, hence possibly influencing availability of recruitable follicles and the presence or absence of a dominant follicle at mid cycle when superstimulation is initiated.

Over the past 2 decades, the most common protocols for superstimulation with FSH have involved multiple injections over a 3- to 5-d period (18). More recently, FSH preparations with greatly reduced concentrations of LH have been available, and there has been a renewed interest in developing superstimulation protocols requiring fewer injections and hence greatly reduced requirements of animal handling. Superstimulation with a single subcutaneous injection of FSH has been reported with encouraging results (4). The present study was conducted to determine if the ovulation rate and the number of transferable embryos are influenced by 1) the method of estrus synchronization, 2) a single versus multiple injections of FSH, 3) the presence or absence of a large follicle at the start of superstimulatory treatments, and 4) the interactions among these factors.

## MATERIALS AND METHODS

### Trial I

Trial I was conducted in October 1992. Twenty purebred Hereford heifers, averaging 17 mo of age and weighing 380 kg with a body condition score of 7.0 (scale 1 to 10 where 1=thin, 10=fat) were used in this trial. Breeding in the herd from which these heifers were selected has been closed since 1934 and selection has been based on measures of postweaning growth (14). Inbreeding coefficients for heifers used in this study ranged from 31 to 35%. In 1978 the herd was divided into 2 sublines, one in which the sires were selected for high yearling weight (Y) and one in which the sires were selected by independent culling levels for high yearling weight and below average birth weight (YB). Ten animals from each subline were used in the study. A genetic variable (subline) was included in the data analysis.

The experimental design was a 2X3 factorial with main effects for estrus synchronization scheme, FSH injection protocol, and genetic subline. Estrus was synchronized with either prostaglandin $F_{2\alpha}$[a] or the oral progestin, melengestrol

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[a]Lutalyse, Upjohn, Kalamazoo, MI 49001.

acetate.[b] One half of the heifers (PGF) received a single intramuscular injection of 25 mg of prostaglandin $F_{2\alpha}$, with the superstimulation protocol started 13 d later. The other half of the heifers (MGA) were fed 0.5 mg/hd/d of melengestrol acetate for 12 d with the superstimulation protocol started 14 d after the last feeding. Superstimulation treatments were started on the same day of the estrous cycle in both groups of heifers. Superstimulation was induced with Folltropin®-V[c], a preparation of pFSH with low LH content. All heifers received a total dosage of FSH equivalent to 280 mg of NIH-FSH-P1, which is 70% of the dose used for mature cows. One half of the heifers (MGA-8 and PGF-8) received the FSH in 8 equal intramuscular injections at 12-h intervals over 4 d. The other half of the heifers (MGA-1 and PGF-1) received the FSH in a single subcutaneous injection behind the upper part of the shoulder. Injections in both groups were begun on Days 10 to 12 of the synchronized estrous cycle, and were administered independently of the technician who would subsequently assess follicle development. The single injections of FSH were given commensurately with the second injection of the 8-injection protocol, and all heifers received an intramuscular injection of 25 mg of prostaglandin $F_{2\alpha}$ at 60 h after the first injection of the 8-injection protocol. The two injection protocols were selected on the basis of both having been successfully used and reported in the literature (4,23,25). Heifers from the 2 genetic sublines were assigned equally across the 4 treatment groups to complete the factorial design.

Follicular development was monitored throughout the period of superstimulation using real-time ultrasound with a 5 mhz probe. Observations were made on all heifers in the morning of the first day of FSH injections and were repeated at 24-h intervals for a total of 5 observations. The observations were made by the same technician throughout the entire study who relayed the data to an assistant for recording. Heifers in all treatment groups were penned together and the order through the chute for ovarian observations was random, thus the technician was unaware of either treatment assignment of the heifers or results of observations from previous days. Data recorded consisted of the number and diameter of all follicles greater than 4 mm. A follicle >8 mm in diameter and at least 3 mm larger than the next largest follicle at the start of superstimulation was classified as a morphologically dominant follicle. This follicle may simply have been the largest follicle and not expressing functional dominance. The term morphological dominant follicle is used to avoid confusion with the discussion of other follicles classified as large, and the term dominant follicle which implies functional dominance. The diameter was used to calculate both volume and surface area for each follicle to evaluate the increase in follicle size as well as the increase in follicle number. In addition, on the last 2 d of observations, each ovary was assigned a size index score based on the degree of follicular development. The scale for the scores was from 1 to 5 (1 = least development, 5 = most development).

When heifers displayed estrus following superstimulation they were mated with bulls of known high fertility. Heifers not showing estrus were bred by artificial insemination at 68 h and again at 80 h after the injection of prostaglandin. On Day 7 (day of mating = Day 0) embryos were recovered nonsurgically and the embryos were scored for stage of development and embryo quality as described by Elsden (8). Embryos scored as quality 1 and 2 were considered to be transferable. Laparotomy was performed on Day 8 and ovulation rate was determined by direct count of corpora lutea (CL).

Trial II

Trial II was conducted in February 1993. Twenty crossbred heifers (½

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[b]MGA, Upjohn, Kalamazoo, MI 49001.
[c]Vetrapharm, London, Ontario, Canada, N6E 2V6.

Simmental, ⅛ Angus, ⅛ Hereford, ⅛ Pinzgauer and ⅛ Red Poll) used in this trial averaged 23 mo of age, weighed 435 kg and had a body condition score of 7.3. Other aspects of the design and methodology of the trial are the same as Trial I with 2 exceptions. Heifers that did not show estrus were left penned with fertile bulls and were not artificially inseminated, and embryos were recovered by flushing the individual uterine horns following partial hysterectomy and ovariectomy on Day 7. The ovarian data recorded consisted of number of CL, ovarian weight, number of unovulated follicles >7 mm in diameter, number of luteinized follicles >7 mm, and weight of follicular fluid in both unovulated and luteinized follicles >7 mm. Follicles were considered luteinized if >50% of their visible surface was comprised of luteal tissue. Follicular fluid weight was determined by the difference in ovarian weight before and after the aspiration of the fluid.

Data Analysis

Analysis of variance was performed using the GLM procedure of SAS (21). In Trial I the model included genetic subline, synchronization scheme, injection protocol and all 2- and 3-way interactions. Genetic subgroups were not present in Trial II. All other aspects of the analyses were the same. Following the initial analysis, as described above, the heifers were further classified as to the presence or absence of a morphologically dominant follicle at the start of the superstimulation treatment. The model then contained main effects of morphologically dominant follicle, synchronization scheme, injection protocol, genetic subline (Trial I), and their interactions. The final analysis was accomplished with this model after eliminating the interactions that were not significant. For the analysis of follicular development, the follicles were classified as either medium (5 to 7 mm diameter) or large (≥8 mm diameter). Follicle data were analyzed using the GLM procedure for repeated measures with day as the repeated measure. Follicle number, volume and surface area were all tested for nonlinear patterns of change over the five days of follicle development during superstimulation. The number of medium and large follicles were also analyzed for each of the 5 days individually using the above described models.

## RESULTS

Trial I

At the time of embryo recovery 1 heifer was diagnosed as having a uterine infection and was eliminated from the study resulting in 19 animals remaining in the trial.

When analyzed with the initial model, without regard to the presence or absence of an morphologically dominant follicle, there were no significant differences in ovulation rates due to main effects or interactions of the main effects. However, when the presence or absence of a morphologically dominant follicle at the start of superstimulation was included in the model, that factor was significant (P=0.03). Further, the effect of the morphologically dominant follicle varied with synchronization scheme and injection protocol (interaction, P=0.05; Figure 1). More dramatic differences were seen due to synchronization scheme and injection protocol when a morphologically dominant follicle was present than when absent.

Ova and embryos recovered accounted for 41% of the CL and of those recovered 37% were transferable. The interaction of synchronization scheme-by-injection protocol approached significance (P=0.06) for the number of transferable embryos (Figure 2). Including a factor in the model for the morphologically dominant follicle had no effect on number of transferable embryos.

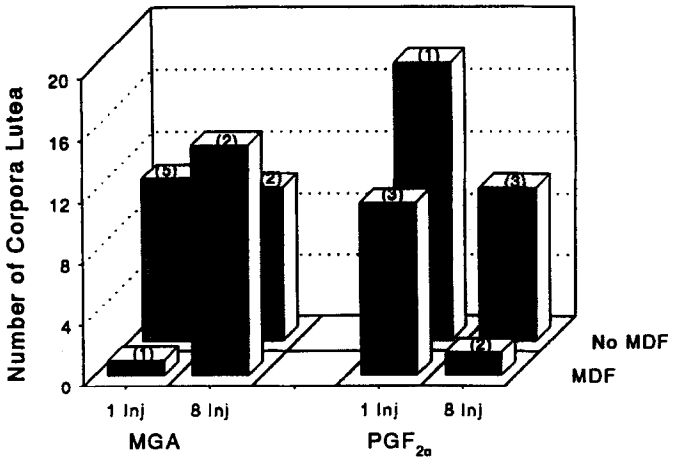


Figure 1. Mean number of corpora lutea following superstimulation of beef heifers synchronized with MGA or PGF, receiving FSH in 1 or 8 injections and with or without a MDF present at the start of injections in Trial I (MDF-by-synchronization scheme-by-injection protocol; P=0.05). MDF = morphologically dominant follicle. Number in parentheses = number of observations.

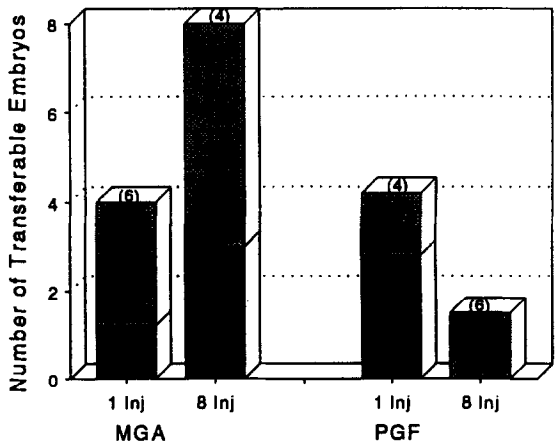


Figure 2. Mean number of total embryos (full columns) and transferable embryos (solid black) following superstimulation of beef heifers synchronized with MGA or PGF and receiving FSH in 1 or 8 injections in Trial I (synchronization scheme-by-injection protocol; P=0.06). Number in parentheses = number of observations.

At the start of superstimulation treatments there was no difference (P>0.40) between heifers synchronized with MGA vs PGF for number of medium (0.9±1.4 vs 1.7±1.8) or large follicles (0.5±0.8 vs 0.5±0.5). Analysis of follicular development with the initial model showed the change in number of medium follicles was influenced by the interaction of synchronization scheme-by-injection protocol-by-genetic subline (P=0.04). However, when the morphologically dominant follicle was included in the model the effect of genetic subline was removed and number of medium follicles was influenced by the 3-way interaction of morphologically dominant follicle, synchronization scheme and injection protocol (P=0.06; Figure 3). Development of medium follicles over the 5 d was also influenced by the interaction of morphologically dominant follicle and genetic subline (P=0.02). When a morphologically dominant follicle was present the two genetic sublines showed a similar pattern of medium follicle development, but in the absence of a morphologically dominant follicle more medium follicles developed in the YB line than in the Y line (data not shown). Analysis of number of follicles at each individual day indicated that no treatment differences existed until d 3 when the populations of medium follicles differed due to the interaction of synchronization scheme and injection protocol (P=0.03).

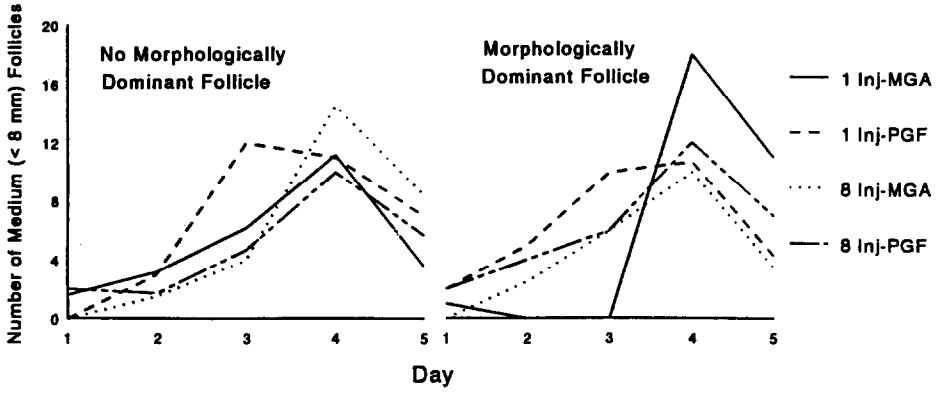


Figure 3. Development of medium sized follicles (4 to 7 mm) during superstimulation of beef heifers synchronized with MGA or PGF, receiving FSH in 1 or 8 injections and with or without a morphologically dominant follicle present at the start of injections in Trial I (MDF-by-synchronization scheme-by-injection protocol; P=0.06). See Figure 1 for the number of animals in each treatment group.

Development of large follicles showed many of the same trends over the 5 d as did medium follicles. The 3-way interaction (P=0.03) of synchronization scheme, injection protocol and genetic subline in the initial analysis gave way to an interaction of morphologically dominant follicle-by-synchronization scheme-by-injection protocol (P=0.06) when the morphologically dominant follicle was included in the model (Figure 4). The number of large follicles was influenced by the interaction of a morphologically dominant follicle and genetic subline (P=0.03). The greater number of large follicles was obtained in the YB heifers when a morphologically dominant follicle was present at the start of pFSH injections, but in the Y heifers in the absence of a morphologically dominant follicle (data not shown). For large follicles also, no treatment differences were seen until Day 3 of pFSH injections.

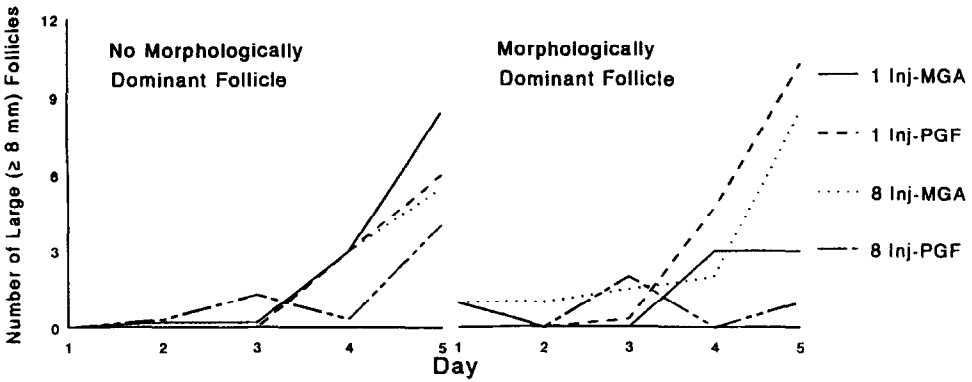


Figure 4. Development of large follicles (>8 mm) during superstimulation of beef heifers synchronized with MGA or PGF, receiving FSH in 1 or 8 injections and with or without a morphologically dominant follicle present at the start of injections in Trial I (MDF-by-synchronization scheme-by-injection protocol; P=0.06). See Figure 1 for the number of animals in each treatment group.

Trial II

One heifer was eliminated for reasons unrelated to the study resulting in 19 animals remaining in the trial.

The effects of treatment on ovarian characteristics and embryos are shown in Table 1. Unlike Trial I, analyzing the data with a factor in the model for the presence of a morphologically dominant follicle at the start of the pFSH injections had no effect on any of the endpoints analyzed. Hence, all analysis for this trial are reported on the initial model considering only the synchronization scheme and the injection protocol. Administering pFSH in a single injection resulted in fewer ovulations, recovered oocytes and transferable embryos, and in lower ovarian weight than the multiple injection protocol. There was a trend (P=0.10) toward a greater number of unfertilized oocytes with the single injection protocol than with the multiple injection protocol.

There was a trend (P=0.09) toward more large follicles at the start of superovulation in heifers synchronized with PGF (0.9±0.7) than with MGA (0.4±0.5), but there was no difference (P>0.40) for medium follicles (1.4±0.9 vs 1.5±1.3). The pattern of follicular development over the 5 d of superstimulation was influenced by an interaction between synchronization scheme and injection protocol (P=0.04) for medium follicles and by injection protocol (P=0.06) for large follicles (Figure 5). Number of large follicles did not differ until Day 3 of superstimulation then increased rapidly during the last 2 days. However, multiple injections resulted in a greater number of follicles than did the single injection.

In both trials, simple correlations were calculated across all treatment groups for the number of different size follicles on each of the 5 d, with both the number of CL and transferable embryos (Table 2). The results were not consistent between the trials except for a significant correlation between the number of large follicles on Day 5 and the number of CL.

Table 1. Embryo and ovarian characteristics in heifers synchronized with 2 different regimens and superovulated with 2 different injection protocols

| | MGA[a] | | PGF[a] | | |
|---|---|---|---|---|---|
| Characteristics | 1[b] | 8[b] | 1[b] | 8[b] | ems[c] |
| Corpora lutea (n)* | 7.3 | 20.0 | 15.2 | 22.4 | 59.3 |
| Embryo/oocyte recovered (n)* | 7.0 | 14.2 | 9.8 | 15.6 | 36.8 |
| Unfertilized oocytes (n)† | 1.0 | 0.8 | 4.6 | 0.2 | 7.6 |
| Degenerating embryos (n) | 0.7 | 0.8 | 2.0 | 1.4 | 2.3 |
| Quality 1-2 embryos (n)** | 5.3 | 11.6 | 2.2 | 10.8 | 26.2 |
| Quality 3-4 embryos (n) | 0.0 | 1.0 | 1.0 | 3.2 | 10.5 |
| Ovarian wt. (g)* | 26.7 | 65.3 | 46.8 | 79.6 | 1004.9 |
| Follicular fluid (g) | 4.6 | 4.2 | 7.8 | 2.6 | 23.9 |
| Unovulated follicles (n) | 7.0 | 5.6 | 5.2 | 4.6 | 9.1 |
| Luteinized follicles (n) | 0.3 | 1.2 | 1.2 | 0.6 | 1.4 |
| Maximum diameter of remaining follicle (mm) | 16.7 | 15.6 | 17.8 | 16.8 | 42.0 |

[a] Methods of estrus synchronization, see text for details.
[b] Number of injections to administer equivalent doses of pFSH.
[c] ems = error mean square.
† Effects of injection protocol differ, P < 0.10.
* Effects of injection protocol differ, P < 0.05.
** Effects of injection protocol differ, P < 0.01.

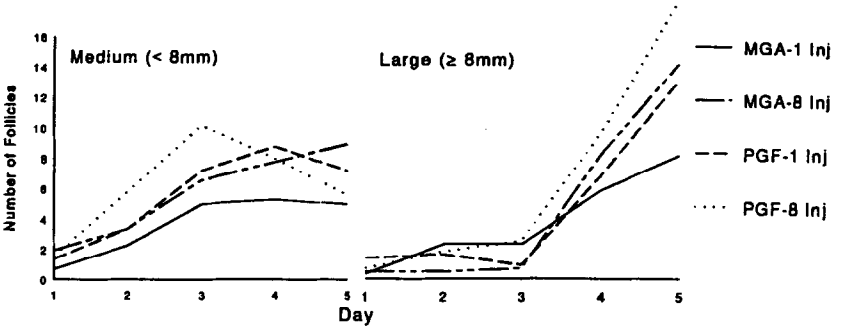


Figure 5. Development of medium (4 to 7 mm) and large (>8 mm) follicles during superovulation of beef heifers synchronized with MGA or PGF and receiving FSH in 1 or 8 injections in Trial II (medium follicles, synchronization scheme-by-injection protocol; P=0.04; large follicles, injection protocol; P=0.06). The number of animals for each treatment group is: 1 Inj-PGF, 6; 8 Inj-PGF, 5; 1 Inj-MGA, 3; 8 Inj-MGA, 5.

Complete analyses were conducted in both trials on volume and surface area of follicles as well as on the number of follicles (data not presented). While there were some differences in the analyses, they were not consistent between trials, nor were there any obvious trends in the differences that facilitated biological interpretation of the data.

Table 2. Correlation of the number of CL and the number of transferable embryos with the number of medium and large follicles during superovulation

| | Trial I | | Trial II | |
|---|---|---|---|---|
| Number of follicles[a] | CL no. | Transferable embryos[b] | CL no. | Transferable embryos |
| Medium - Day 1 | 0.064 | -0.368 | 0.114 | 0.119 |
| Large - Day 1 | -0.148 | 0.370 | 0.052 | -0.394 |
| Medium - Day 2 | 0.159 | 0.146 | 0.586** | -0.313 |
| Large - Day 2 | 0.065 | 0.066 | 0.096 | -0.478* |
| Medium - Day 3 | 0.174 | 0.248 | 0.795** | -0.049 |
| Large - Day 3 | 0.215 | 0.178 | 0.451* | -0.109 |
| Medium - Day 4 | -0.124 | -0.085 | 0.249 | -0.349 |
| Large - Day 4 | 0.306 | 0.340 | 0.900** | 0.078 |
| Medium - Day 5 | -0.220 | -0.144 | 0.676** | 0.276 |
| Large - Day 5 | 0.519* | 0.364 | 0.675** | 0.328 |
| Ovary Score - Day 4 | 0.374 | 0.194 | 0.739** | 0.480* |
| Ovary Score - Day 5 | 0.668** | 0.352 | 0.661** | 0.210 |

[a] Number of follicles by size classification on each of the five days of ovarian examination during superstimulation.
[b] Quality 1 and 2 embryos as classified by Elsden (8).
* $P < 0.05$.
** $P < 0.01$.

## DISCUSSION

The synchronization scheme appeared to have little effect on either the ovulation rate or the number of transferable embryos. It did, however, interact with the injection protocol and a morphologically dominant follicle to influence the ovulation rate in Trial I. There is no obvious explanation of this interaction, but it is likely that a large number of factors are highly interactive in controlling ovarian stimulation. There was no effect of synchronization scheme on the ovulation rate or the number of transferable embryos in Trial II. The lack of any differences in the distribution of medium and large follicles at the start of superovulation suggests that any differences that may have existed as a result of the synchronization scheme are lost or minimized by the middle of the following cycle. There is evidence that the occurrence of estrus synchronizes follicle development (19), and it is possible that the estrus occurring between synchronization and the start of superstimulation would remove most if not all of the effects of the synchronization scheme.

The injection protocol interacted with the synchronization scheme and a morphologically dominant follicle in Trial I but had a direct effect on both the ovulation rate and the number of transferable embryos in Trial II. However, the follicular development data did not show consistent patterns across all treatments, that multiple injections could either recruit or maintain more follicles for ovulation than the single injection. Nor did the structures remaining on the ovary following ovulation indicate that follicular development with incomplete ovulation had occurred. A more pronounced effect was seen in the single injection protocol on reducing the number of transferable embryos and increasing the number of unfertilized oocytes. This may be an indication that some follicles are recruited and carried on to ovulation that do not develop an internal endocrine environment which permits complete oocyte maturation, and results in a lower rate of fertilization and embryo development. Also, the large

instantaneous dose of FSH given with the single injection may have rescued, and carried through to ovulation, some follicles that were destined for atresia and that contained degenerating oocytes. Other authors (4,25) have reported similar ovulation rates between single and multiple injection protocols, but they did not report the number of transferable embryos obtained.

Previous reports have shown that the presence of a large follicle inhibits or restricts the development of smaller follicles (16,24), and further, that the presence of a dominant follicle limits the response to superovulation (11). Our objective was to determine if a single examination of the ovaries by ultrasound would detect a large follicle, which we have termed the morphologically dominant follicle, capable of influencing the response to superstimulation treatment. While the presence of a morphologically dominant follicle did interact with other treatment factors in Trial I, there was no compelling evidence across both trials that the morphologically dominant follicle did indeed alter the ovulation rate or the number of transferable embryos. Wilson et al. (26) reported similar results. Similarly, there was no effect on number of embryos collected when the criteria was the presence of a large follicle that had started to regress (10). In contrast, Guilbault et al. (11) used the criterion for a dominant follicle of growth or stability of the largest follicle for at least 4 d, and Huhtinen et al. (12) required 3 d beyond maximum growth. Both reports showed an inhibitory effect of a dominant follicle on response to superstimulation. From these combined studies it seems apparent that size alone is not a sufficient criterion for determining if a large follicle will limit response to super-stimulation. If indeed a dominant follicle does influence response to superstimulation, more specific criteria establishing the presence of a dominant follicle remain to be established if we are to eliminate the inhibitory influence, maximize response and reduce the high variability found in our preset superstimulation treatments. In a more recent study (5) the number of follicles 5 to 8 mm in diameter was used as the criterion for the presence of a dominant follicle, and when there were >10 follicles the response was greater to superovulation than when there were <10 follicles.

Analysis of the change in follicle populations during the superstimulation treatments did not show consistent effects of the treatment variables across both trials. In Trial I, however, the interaction involving genetic subline on the development of medium follicles may be an indication that there are subtle but important genetic influences on individual physiological traits such as follicular development. Some distinct patterns of follicular development were consistent across both trials. Follicles stimulated by the pFSH injections did not reach large size until after Days 3 and 4 of treatment. This is indicated by the slight increase in large follicles on Day 4 and by the dramatic decrease in medium follicles and increase in large follicles between Day 4 and Day 5 (Figures 3 and 4; data for Trial II not presented). Correlations were computed relating the populations of medium and large follicles on each of the 5 d with the ovulation rate and the number of transferable embryos in an attempt to find an indication early in the superstimulation process of the magnitude of respond for these two traits. Only the number of large follicles on Day 5 was correlated with the ovulation rate in both trials. Similar results have been reported previously (20). However, in Trial II, medium follicles on Days 2 and 3 and large follicles on Days 4 and 5 were all correlated with the ovulation rate. This could well be the same contingent of follicles on each of these days, and may simply reflect growth from medium to large follicles between Days 3 and 5. Follicle volume and surface area were computed hypothesizing that change in size may be a more sensitive measure of follicular dynamics than a change in number. However, these measures offered no advantage over follicle numbers in either understanding follicular growth during superstimulation or in estimating ovulatory response.

Between the 2 trials of this study there was lack of consistency in the effect of the variables tested on the 2 primary endpoints. Although the

experimental designs were similar for the 2 trials, the results were quite different. One possible explanation of the difference is the diverse genetic background of the animals used in the 2 trials. One group was an inbred line of heifers and thus would have been vulnerable to inbreeding depression. The other group was a highly fertile crossbred group and could thus be expected to display some degree of heterosis. Fertility traits have been shown to reflect the effects of both inbreeding (7,13) and heterosis (6,13). While there was no direct comparison of a genetic effect it must remain as a possible explanation for the observed differences.

A second difference between the 2 trials was the season of year in which the trials were conducted. One trial was conducted during moderate weather of the fall with decreasing daylength, and the other was conducted during the harsh weather of the winter with increasing daylength. Previous reports, however, have shown seasonal effects to be minor (23) or nondetectable (15,22). Both groups of animals were under good nutrition management and both showed similar body condition.

Along with differences between trials there are a number of significant 3-way interactions in this study that are difficult to interpret. While the number of animals in each trial was fully adequate, the number in each of the subgroups of the 3-way interactions may have been limited. This is particularly true for factors that are assigned on the basis of response, such as the presence of a morphologically dominant follicle. As shown in Figure 1, some subgroups had only a single observation. Hence, these interaction need to be interpreted with caution. However, the comparisons were all preplanned, and the fact that these interactions were significant justifies the comparisons.

In summary, the reduction in transferable embryos resulting from the single injection protocol in Trial II indicates more modifications are needed in the single injection protocol to make it acceptable. The effect of synchronization scheme does not indicate a clear advantage for either the single or multiple injection protocol of estrus synchronization. Likewise, the presence of a large follicle does not indicate functional dominance and it may or may not influence response to superstimulation. Any effect of a dominant follicle may well be influenced by its interaction with other factors yet undefined.

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