Effect of semen source and dose of FSH on superovulatory response and embryo production in Holstein heifers

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Abstract

This study evaluated fertilization rate and embryo quality in superstimulated Holstein heifers artificially inseminated with pooled semen from four high fertility bulls or commercial semen from one of four selected sires (not pooled). It also evaluated the superovulatory response to two different doses of FSH. Twenty heifers underwent two superstimulatory treatments in a crossover design. On Day 0, all heifers received an intravaginal progesterone device (CIDR). On Day 1, heifers were treated with estradiol benzoate (3 mg). Starting on Day 6, heifers were treated with one of two different amounts of FSH using progressively decreasing doses. Prostaglandin $F_{2\alpha}$ was given 12 h before CIDR removal, and GnRH (100 μg) was given 36 h after CIDR removal. Heifers received a single AI 12 h after the GnRH injection. Fertilization rate (83% vs. 77%, P = 0.35) and percentage of transferable embryos (52% vs. 45%, P = 0.55) recovered per flush for pooled high-fertility vs. commercial semen did not differ. The higher dose of FSH stimulated growth of more (P < 0.01) follicles > 8 mm in diameter per heifer (16.8 ± 1.6 vs. 12.9 ± 1.2). However, number (2.3 ± 0.5 vs. 3.3 ± 0.8, P = 0.21) and percentage (38.2 ± 8.9 vs. 59.8 ± 10.3, P = 0.28) of transferable embryos did not differ between the high and low FSH group. In addition, lowering the dose of FSH decreased (P = 0.04) the number of unfertilized ova (1.6 ± 0.5 vs 0.6 ± 0.2). In conclusion, decreasing FSH to 50% (200 mg) of the recommended dose (400 mg) did not reduce the number or percentage of transferable embryos. Moreover, number of unfertilized ova appeared to depend as much on FSH dose as semen quality, with increasing FSH dose decreasing fertilization efficiency and commercial semen from individual sires producing an acceptable fertilization rate in superstimulated heifers.

Keywords: dose of FSH; pooled semen; fertility.

Introduction

Embryo transfer is an extensively utilized and researched reproductive biotechnology; however, inconsistent results are commonly described. During superovulation, practitioners of embryo transfer seek an elevated and consistent yield of high quality embryos. Many factors may be associated with embryo quality during superstimulatory treatments. Some of these include semen fertility (Newcomb, 1980), variations in commercial FSH product (Lindsell et al., 1986), heat stress (Page et al., 1985), nutritional status (McEvoy et al., 1995), breed (Donaldson, 1984), season (Hasler et al., 1983), and age (Lerner et al., 1986). Consequently, embryo production from superovulation, as measured by number of transferable embryos per cow, has been found to be highly variable and unpredictable for an individual cow (Armstrong, 1993).

Fertilization rate and embryo quality can be impacted by semen quality, particularly since cows undergoing gonadotropin treatments have lower fertilization rates than single ovulating cows (Page et al., 1985; Hyttel et al., 1991; Saacke et al., 1998). Fertilization failures in superstimulated animals were found to range from 29 to 35% versus 10 to 15% in unstimulated cattle (Hasler et al., 1983). These results agree with a previous study in sheep (Hawk et al., 1987) that found detrimental effects on sperm transport mechanisms in superstimulated animals. Likewise, Hyttel et al. (1991) showed that superstimulation has an adverse effect on oocyte maturation as well as on sperm transport, both of which are crucial events in the context of adequate embryo production. The reason for the low fertilization rate in superstimulated females is not clear but may be due to suboptimal gamete quality or inadequate transport and storage of sperm in the oviduct (Page et al., 1985). Saacke et al. (1998) found much lower numbers of accessory sperm cells per ovum/embryo in superovulating (mean = 1.1) than in single ovulating (mean = 21.1) cattle, confirming that few sperm cells reach and/or penetrate the oocyte of superstimulated cattle.

Another variable that is well-known to alter superovulation is the dose of FSH used during superstimulation of follicle growth (Lerner et al., 1986; Gonzalez et al., 1990; Sugano and Watanabe, 1997;

Murphy *et al*., 1998). Comparisons of gonadotropin products with different amounts of residual LH showed conflicting results (Lerner *et al*., 1986; Mapletoft *et al*., 1988; Sugano and Watanabe, 1997; Takagi *et al*., 2001). Likewise, the ideal amount of FSH to be used in superstimulatory treatments is controversial. It has been shown that increasing doses of Folltropin® from 5 to 10 mg increased ovulation rate and transferable embryos; however, increasing the dose to 20, 30, or 40 mg did not increase embryo yield (Gonzalez *et al*., 1990). There were numerical indications that the 40 mg dose of Folltropin produced decreasing embryo quality. Interestingly, Donaldson and Darrell (1985) found a negative correlation between amount of purified FSH and number of transferable embryos. Thus, increasing FSH dose may improve superovulatory response but have a negative impact on embryo quality.

Thus, this experiment was designed to compare the superovulatory response of Holstein heifers to two different doses of FSH and to evaluate fertilization rate and embryo quality in superstimulated heifers artificially inseminated with either pooled semen from four high fertility bulls vs. commercial not pooled semen. We hypothesized that: 1) decreasing the dose of FSH to 50% of the recommended dose (from 400 mg to 200 mg of FSH) would decrease the number of superstimulated follicles as well as the number of ovulations and increase fertilization rate and the percentage of high quality embryos; and 2) superstimulated heifers inseminated with commercial conventional semen would yield a greater proportion of unfertilized ova (UFO) than heifers inseminated with pooled semen from four high fertility bulls.

Materials and Methods

Animals and management

The experiment was conducted during the summer of 2002. Twenty nulliparous cyclic Holstein heifers of 12 to 16 months of age were used. Body condition scores ranged from 3.0 to 3.5 (scale 1 to 5; Edmonson *et al*., 1989) with no detectable changes during the experimental period. Heifers were housed indoors at the Livestock Laboratory of the University of Wisconsin-Madison and fed a total mixed ration containing 70% alfalfa silage and 30% corn silage (16.8 % CP and 1.36 Mcal/kg) with free access to water. All animal procedures were approved by the College of Agriculture and Life Sciences Animal Care Committee of the University of Wisconsin-Madison.

Superovulation protocol

The heifers underwent two superstimulatory treatments at random stages of the estrous cycle using the protocol outlined in Fig. 1. Each animal received a total equivalent of either 200 or 400 mg of NIH-FSH-P1 (im, Folltropin-V, Bioniche Animal Health Inc., Belleville, ON, Canada) in a crossover design. Treatments and inseminations were assigned at random among animals. The superstimulation protocol began (Day 0) with the application of an intravaginal progesterone device (Eazi-Breed CIDR containing 1.9 g of progesterone, InterAg Co., Hamilton, New Zealand). On Day 1, heifers were treated with estradiol benzoate (EB, 3 mg i.m.; Sigma Chemical Co., St. Louis, MO) diluted in Sesame oil to synchronize emergence of a new follicular wave. Starting on Day 6 (5 d after EB) heifers were treated with one of two different amounts of FSH using progressively decreasing doses and i.m. injections (200 mg: 2.0, 2.0, 1.5, 1.5, 1.0, 1.0, 0.5, 0.5 ml or 400 mg: 4.0, 4.0, 3.0, 3.0, 2.0, 2.0, 1.0, 1.0 ml) over 4d (Day 6-9). Prostaglandin $F_{2\alpha}$ (25 mg, i.m., Prostamate, Phoenix Pharmaceutical Inc., St. Joseph, MO) treatment was given 12 h before CIDR removal (Day 9, PM), and GnRH (100 µg, i.m., Cystorelin, Merial Ltd, Iselin, NJ) was given 36 h after CIDR removal (Fig. 1).

All heifers received a single artificial insemination by the same technician into the body of the uterus 12 h after the GnRH injection using 0.5 ml frozen-thawed commercial semen from one of four selected sires (not pooled) or 0.5 ml of frozen-thawed semen containing equal number of sperm from each of four high fertility bulls selected for high non-return rates (post-thaw motility = 70%; ABS Global, De Forest, WI). This high fertility semen has produced 89 to 100% fertilization rates in single-ovulating heifers or cows in previous studies (Sartori *et al*., 2002). After non-surgical embryo collection (described below), heifers were treated with 2 $PGF_{2\alpha}$ injections 12 h apart in preparation for a second superovulatory treatment 10 d later.

Embryo collection and evaluation

Non-surgical embryo collection (Eldsen *et al*., 1976) was performed by one of two technicians on Day 18 of the protocol. The entire uterine horn was flushed, using a shallow technique as previously described (Sartori *et al*., 2003). Ova/embryos were collected, searched for at 10X magnification, and subsequently counted and examined under a stereoscopic dissecting microscope at 45X to 100X magnification for assessment of developmental stage and quality. Structures were classified according to the Manual of the International Embryo Transfer Society (1998). Unfertilized ova were designated when there was no sign of cleavage. The embryo searching and grading were done by a single technician.

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Ovarian ultrasonography

Transrectal ovarian ultrasonography was performed on Days 11, 13, and 18 (Fig. 1) with an ALOKA 500V equipped with a 7.5 MHz linear array transducer (Corometrics Medical Systems Inc., Wallingford, CT).

The number of follicles > 8 mm was estimated on the day of GnRH injection (Day 11). The ovulation rate was estimated by determining the number of preovulatory follicles that had disappeared on Day 13 (assumed ovulation) and confirmed by the number of corpora lutea (CL) counted on the day of embryo collection.
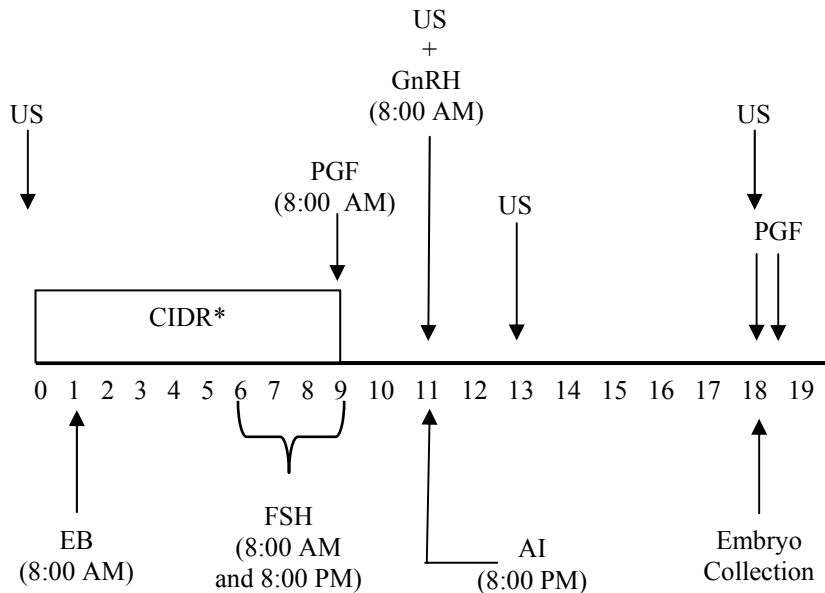

Figure 1. Superovulation protocol. AI = Artificial Insemination; CIDR = Controlled Internal Drug (Progesterone) Releasing Device; EB = Estradiol Benzoate; FSH = Follicle Stimulating Hormone; GnRH = Gonadotropin Releasing Hormone; PGF = Prostaglandin F2α; US = Ultrasound. * CIDR insertion occurred on Day 0 (08:00 AM) and was removed on Day 9 (08:00 PM).

Statistical analyses

Generalized linear mixed models (macro GLIMMIX of SAS; Littell *et al.*, 1996) were used for count variables that were assumed to follow a conditional Poisson distribution, such as number of ovulatory follicles, number of CL, number of embryos produced, number of transferable embryos, number of fertilized embryos, number of UFO, and number of degenerate embryos. Variables such as ovulation rate, percentage of fertilized ova, percentage of UFO, percentage of transferable embryos, and percentage of degenerated embryos were evaluated using a linear mixed effects model (proc MIXED of SAS; Littell *et al.*, 1996). In both models, heifer was treated as a random effect, and fixed explanatory variables such as technician, replication, treatment, and mating sire were used in the different models. Conclusions were based on a 5% level of significance with 10% being considered a tendency.

Results

Out of 40 superstimulations, 1 heifer was not flushed on the second replicate due to a uterine infection detected by ultrasound at the time of CIDR insertion. Thus, 39 superovulations and embryo flushes were performed and data were used for evaluating the 2 experimental hypotheses.

Semen effects

As expected, the type of semen used for AI did not alter number of ovulations or embryo recovery rate (Tab. 1). Surprisingly, there was also no difference (P = 0.35) in the percentage of fertilized ova (76.9 vs. 83.4%) although the number of UFO tended to be greater (P < 0.10) for the heifers bred with commercial not pooled semen (1.6 vs. 0.6 UFO/flush). There were also no differences in number or percentage of transferable embryos per flush related to whether commercial semen or pooled semen from four high fertility bulls was used for AI (Tab. 1). In addition, there was no difference between groups for percentage of degenerate embryos expressed either as a percentage of total structures or for only fertilized structures (Tab. 1).

Table 1. Results (mean ± SEM) from superovulated Holstein heifers inseminated with frozen-thawed commercial semen from one of four selected sires (not pooled) or pooled semen from four high fertility bulls.

Variables	Commercial (n = 19)	Pooled (n = 20)	P-value
Number of follicles > 8 mm	15.3 ± 1.6	14.6 ± 1.4	0.93
CL number [a]	11.4 ± 0.9	10.6 ± 1.3	0.77
Ovulation rate (%)	75.9 ± 4.6	72.6 ± 6.9	0.78
Total number of ova/embryos recovered [a]	6.3 ± 1.0	4.1 ± 0.7	0.12
% Ova/embryos recovered per CL	56.6 ± 5.9	42.2 ± 6.5	0.36
Number of fertilized ova [a]	4.7 ± 1.0	3.5 ± 0.7	0.51
% Fertilized ova	76.9 ± 5.0	83.4 ± 6.4	0.35
Number of UFO [a]	1.6 ± 0.4	0.6 ± 0.2	0.10
Number of transferable embryos [a]	3.2 ± 0.9	2.3 ± 0.6	0.48
% Transferable embryos	44.5 ± 10.1	52.4 ± 10.0	0.55
Number of degenerate embryos [a]	1.5 ± 0.4	0.9 ± 0.4	0.29
% Degenerate embryos	32.5 ± 8.9	25.6 ± 8.4	0.64
% Degenerate of fertilized ova	45.6 ± 10.8	31.9 ± 10.3	0.37

[a] per flush. UFO = Unfertilized ova.

Dose of FSH

There was substantial variation in the superovulatory response among animals with the number of ovulations ranging from 0 to 22 (CV = SD/Mean = 0.48) for the higher FSH (400 mg) and 0 to 18 (CV = 0.52) for the lower FSH (200 mg) group. Three heifers treated with the lower dose of FSH and 1 heifer treated with the higher FSH dose did not respond to the superovulatory treatments based on detection of ≤2 CL by ultrasonography. Nevertheless, their data were used in order to better compare the treatment effects.

There were greater (P < 0.01) numbers of follicles >8 mm in diameter at the time of GnRH injection (Day 11) in heifers treated with 400 mg of FSH (Tab. 2). The percentage of these follicles that ovulated was not different (P = 0.27) than heifers treated with 200 mg and 400 mg of FSH, and no difference in

number of CL (11.8 vs. 10.2) was detected. In addition, there were no treatment differences in total number of ova/embryos recovered or the percentage of ova/embryos recovered per CL (Tab. 2). However, there were more (P = 0.04) UFO in the heifers treated with 400 than 200 mg of FSH (1.6 vs. 0.6). In addition, treatment with the lower FSH dose resulted in numerically but not statistically (P = 0.21) greater numbers of transferable embryos (3.3 vs. 2.3) and numerically lower numbers of degenerate embryos (0.7 vs. 1.6; P = 0.25). A numerically higher percentage of transferable embryos (59.8% vs. 38.2%; P = 0.28) and lower percentage of degenerate embryos (20.8% vs. 36.5%; P = 0.14) was also found in heifers treated with the lower dose of FSH but again these were not statistically significant differences. There was also no difference in developmental stage between treatment groups (P = 0.42).

Table 2. Results (mean ± SEM) from superovulated Holstein heifers treated with a total of 400 mg of FSH or 200 mg of FSH.

Variables	400 mg FSH (n = 20)	200 mg FSH (n = 19)	P-value
Number of follicles > 8 mm	16.8 ± 1.6	12.9 ± 1.2	0.01
CL number [a]	11.8 ± 1.3	10.2 ± 1.2	0.35
Ovulation rate (%)	72.6 ± 5.8	77.0 ± 6.9	0.27
Total number of ova/embryos recovered [a]	5.4 ± 0.8	4.9 ± 1.0	0.79
% Ova/embryos recovered per CL	49.6 ± 6.6	49.9 ± 7.4	0.85
Number of fertilized ova [a]	3.9 ± 0.8	4.3 ± 1.0	0.80
% Fertilized ova	74.7 ± 7.9	86.0 ± 4.3	0.18
Number of UFO	1.6 ± 0.5	0.6 ± 0.2	0.04
Number of transferable embryos [a]	2.3 ± 0.5	3.3 ± 0.8	0.21
% Transferable embryos	38.2 ± 8.9	59.8 ± 10.3	0.28
Number of degenerate embryos [a]	1.6 ± 0.4	0.7 ± 0.3	0.25
% Degenerate embryos	36.5 ± 8.7	20.8 ± 7.4	0.14
% Degenerate of fertilized ova	49.6 ± 9.5	28.5 ± 10.0	0.27
Mean developmental stage [b]	5.4 ± 0.03	5.4 ± 0.02	0.42

[a] per flush. UFO = Unfertilized ova
[b] Developmental stage of transferable embryos according to International Embryo Transfer Society Manual – IETS, 1998 (4 = morula, 5 = early blastocyst, 6 = blastocyst, 7 = expanded blastocyst).

Discussion

This experiment is apparently the first study to compare production of embryos in superstimulated heifers using semen from individual commercial bulls with semen from a heterospermic preparation of sperm that may have had higher fertility due to the presence of sperm from multiple high-fertility bulls in the semen. We hypothesized that the pooled semen from 4 high fertility bulls would increase fertilization rates. In support of this hypothesis, there was a tendency for greater numbers of UFO when heifers were inseminated with commercial semen from single sires than with the pooled high fertility semen. Our result agrees with the study of Newcomb (1980), in which higher quality semen produced a greater proportion of fertilized ova than semen from bulls with lower semen quality. Likewise, other authors (Hawk *et al.*, 1988) reported a greater fertilization rate by using a pool of high fertility fresh ejaculates. However, in all of these studies, including our study, classification of a structure as a UFO could not be definitively separated from one-cell zygotes that had been fertilized but had not undergone cleavage. Nevertheless, percentage of ova deemed to be fertilized in our experiment (~80%) appeared to be acceptable for superovulation based on the results of Hasler (1992), and fertilization rate was similar between treatment groups. The number and percentage of transferable embryos or degenerate embryos were not altered by type of semen that was utilized for AI. These results are in agreement with a previous study (Garcia *et al.*, 1994) reporting that a single semen dose of 20×10^6 sperm/0.25 ml straw was able to produce suitable fertilization rates in superstimulated cows (~90%). Nevertheless, an earlier trial (Donaldson, 1985) found that superstimulated cows that received a single AI had a lower fertilization percentage than those receiving two AIs. Thus, our results as well as the results from some other studies, suggest that one dose of high quality semen in a well managed donor heifer can produce acceptable fertilization and embryo production results; however, these results do not rule out or minimize the improvements that can occur in certain situations by using multiple breedings or optimizing semen quality.

In the current trial, there was a greater number of superstimulated follicles (~4 follicles more) at the time of the GnRH injection in the 400 mg vs. the 200 mg FSH group, but no differences in CL number were detected between groups. Thus, a lower ovulation rate tended to occur in the high FSH group. A previous study has shown that the number of superstimulated follicles in response to an increasing dose of FSH follows a dose-dependent pattern (Kanitz *et al.*, 2002). Nevertheless, Greve *et al.* (1983) and Callensen *et al.* (1986, 1987) showed a correlation between greater doses of gonadotropin and lower ovulation rates. Similarly, very high doses of FSH were reported to decrease numbers of ovulations (Saumande and Chupin,

1986; Kanitz *et al.*, 2002). Some authors have suggested that decreases in percentage of follicles that ovulated in superstimulated cows may be due to a decrease in the proportion of animals with an LH surge and/or a decrease in the number of follicles that ovulate after the LH surge (Stock *et al.*, 1995; Kanitz *et al.*, 2002). In our study, it seems unlikely that lack of an LH surge reduced ovulation rates because only 2 of the superstimulation treatments resulted in no ovulations (1 in 200 mg group, 1 in 400 mg group). In addition, treatment with a final GnRH during our superstimulation protocol should assure an LH surge in all heifers. Therefore, the most plausible explanation for any reduction in ovulation rate is lack of ovulation of superstimulated follicles. The molecular mechanism for this problem will require further research but could involve lack of follicular gonadotropin receptors or deficiencies in other parts of the ovulation cascade in some superstimulated follicles (Kanitz *et al.*, 2002; Richards, 2005). Thus, we are left without a clear mechanistic explanation for the surprising observation that a doubling in the dose of FSH produced only a small change in number of large follicles and no increase in number of ovulations. It seems unlikely that we have reached the superstimulatory or ovulatory capacity for the heifers in light of the studies indicating that there is an average of 24 follicles in each follicular wave (Ginther *et al.*, 1996).

The higher dose of FSH also increased numbers of UFO as compared to the lower FSH dose. Thus, superstimulation of follicle growth with greater levels of FSH/LH appears to reduce fertilization success consistent with previous reports (Donaldson and Darrell, 1985; Donaldson and Ward, 1986). Although results from some studies are consistent with no detrimental effect of some LH in the FSH preparation on superovulation success (Lindsell *et al.*, 1986), other studies have shown reduced efficacy when any dose of contaminating LH was present in the FSH preparation (Donaldson *et al.*, 1985; Willmott *et al.*, 1990). Donaldson (1984) reported that fertilization rates are negatively related to the content of LH in the gonadotropin product. In addition, almost all measures of embryo quality (percentage degenerate, percentage transferable, number transferable) favored the group treated with the lower dose of FSH; although these differences did not reach statistical significance. Other studies have also indicated that increasing level of superstimulation can decrease embryo quality. These results could be due to excessive estradiol near ovulation (Langford *et al.*, 1980; Kanitz *et al.*, 2002) or reduced oocyte quality due to premature germinal vesicle breakdown (Revah and Butler, 1996) or excessive follicular stimulation (Alfuraiji *et al.*, 1993). Despite lack of a clear understanding of the cellular mechanism, it does appear that increasing the dose of FSH may negatively impact oocyte quality or reproductive tract environment, leading to reduced

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fertilization and perhaps suboptimal embryo development.

In conclusion, lower amounts of FSH/LH in the 200 mg FSH group can produce a comparable superovulatory response to the standard dose of FSH (400 mg), as evidenced by a similar percentage and number of transferable embryos recovered per heifer. Therefore, in order to reduce costs of superstimulatory treatments, a lower dose of FSH may be used in superstimulatory protocols for Holstein heifers. In addition, there may be some advantages of using high fertility semen in superstimulatory protocols but we were unable to obtain statistically superior results in fertilization success or embryo quality by using semen containing a mixture of 4 high fertility bulls.

References

Alfuraiji MM, Atkinson T, Broadbent PJ, Hutchinson JSM. 1993. Superovulation in cattle using PMSG followed by PMSG monoclonal antibodies. *Anim Reprod Sci*, 33:99-109.

Armstrong DT. 1993. Recent advances in superovulation of cattle. *Theriogenology*, 39:7-24.

Callensen H, Greve T, Hyttel P. 1986. Preovulatory endocrinology and oocyte maturation in superovulated cattle. *Theriogenology*, 25:71-86.

Callensen H, Greve T, Hyttel P. 1987. Premature ovulations in superovulated cattle. *Theriogenology* 28:155-166.

Donaldson LE. 1984. Dose of FSH-P as a source of variation in embryo production from superovulated cows. *Theriogenology*, 22:205-212.

Donaldson LE. 1985. Effect of insemination regimen on embryo production in superovulated cows. *Vet Rec*, 117:35-37.

Donaldson LE, Darrell NW. 1985. Superovulation in cattle: dose response to FSH-W with and without LH contamination. *Theriogenology*, 23:189. (abstract).

Donaldson LE, Ward DN. 1986. Effects of luteinizing hormone on embryo production in superovulated cows. *Vet Rec*, 119:625-626.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. 1989. A body condition scoring chart for Holstein dairy cows. *J Dairy Sci*, 72:68-78.

Eldsen RP, Hasler JF, Seidel GE Jr. 1976. Non-surgical recovery of bovine eggs. *Theriogenology*, 6:523-532.

Garcia A, Mapletoft RJ, Kennedy R. 1994. Effect of semen dose on fertilization and embryo quality in superovulated cows. *Theriogenology*, 41:202. (abstract).

Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. 1996. Selection of the dominant follicle in cattle. *Biol Reprod*, 55:1187-1194.

Gonzalez A, Lussier JG, Carruthers TD, Murphy BD, Mapletoft RJ. 1990. Superovulation of beef heifers with Folltropin: a new FSH preparation containing reduced LH activity. *Theriogenology*, 33:519-529.

Greve T, Callensen H, Hyttel P. 1983. Endocrine profile and egg quality in the superovulated cows. *Nord Vet Med*, 35: 408-421.

Hasler JF. 1992. Current status and potential of embryo transfer and reproductive technology in dairy cattle. *J Dairy Sci*, 75:2857-2879.

Hasler JF, McCauley AD, Schermerhorn EC, Foote H. 1983. Superovulatory responses in Holstein cows. *Theriogenology*, 19:83-99.

Hawk HW, Conley HH, Wall RJ, Whitaker RO. 1988. Fertilization rates in superovulated cows after deposition of semen on the infundibulum, near the uterotubal junction or after insemination with high numbers of sperm. *Theriogenology*, 29:1131-1142.

Hawk HW, Cooper BS, Conley HH. 1987. Inhibition of sperm transport and fertilization in superovulating ewes. *Theriogenology*, 28:139-153.

Hyttel P, Callesen H, Greve T, Schimidt M. 1991. Oocyte maturation and sperm transport in superovulated cattle. *Theriogenology*, 35:91-108.

Kanitz W, Becker F, Schneider F, Kanitz E, Leiding C, Nohner H, Pöhland R. 2002. Superovulation in cattle: practical aspects of gonadotropin treatment and insemination. *Reprod Nutr Dev*, 42:587-599.

Langford GA, Marcus GJ, Hackett AJ, Ainsworth L, Wolynetz MS. 1980. Influence of estradiol-17 beta on fertility in confined sheep inseminated with frozen semen. *J Anim Sci*, 51:911-916.

Lerner SP, Thayne WV, Baker RD, Hensche T, Meredith S, Inskeep EK, Dailey RA, Lewis PE, Butcher RL. 1986. Age dose of FSH and other factors affecting superovulation in Holstein cows. *J Anim Sci*, 63:176-183.

Lindsell CE, Rajkumar K, Manning AW, Emercy SK, Mapletoft RJ, Murphy BD. 1986. Variability in FSH-LH ratio among batches of commercially available gonadotropins. *Theriogenology*, 25:167. (abstract).

Littell RC, Milliken GA, Stroup WW, Wolfinger RD. 1996. *SAS system for mixed models.* Cary, NC: SAS Institute.

Manual of the International Embryo Transfer Society: a procedure guide and general information for the use of embryo transfer technology emphasizing sanitary procedures. 1998. 3rd. ed. Savoy, IL: IETS.

Mapletoft RJ, Gonzalez A, Lussier JG. 1988. Superovulation of beef heifers with Folltropin or FSH-P. *Theriogenology*, 29:284. (abstract).

McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Palmer RM, Roberston IS. 1995. Dietary-induced suppression of pre-ovulatory progesterone

concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova. *Anim Reprod Sci*, 39:89-107.

Murphy MG, Boland MP, Roche JF. 1998. The effects of dose and duration of administration of pFSH during the first follicular wave on the ovulation rate of beef heifers. *Theriogenology*, 49:557-569.

Newcomb R. 1980. Investigation of factors affecting superovulation and non-surgical embryo recovery from lactating British Friesian cows. *Vet Rec*, 106:48-52.

Page RD, Jordan JE, Johnson SK. 1985. Superovulation of Holstein heifers under heat stress with FSH-P or Folltropin, *Theriogenology*, 31:236. (abstract).

Revah I, Butler WR. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. *J Reprod Fertil*, 106:39-47.

Richards JS. 2005. Ovulation: new factors that prepare the oocyte for fertilization. *Mol Cell Endocrinol*, 234:75-79.

Saacke RG, DeJarnette JM, Bame JH, Karabinus DS, Whitman SS. 1998. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? *Theriogenology*, 50: 117-128.

Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci*, 85:2803-2812.

Sartori R, Suarez-Fernandez CA, Monson RL, Guenther JN, Rosa GJ, Wiltbank MC. 2003. Improvement in recovery of embryos/ova using a shallow uterine horn flushing technique in superovulated Holstein heifers. *Theriogenology*, 60:1319-1330.

Saumande J, Chupin D. 1986. Induction of superovulation in cyclic heifers: the inhibitory effect of large doses of PMSG. *Theriogenology*, 25:233-247.

Stock AE, Ellington JE, Fortune JE. 1995. A dominant follicle does not affect follicular recruitment by superovulatory doses of FSH in cattle but can inhibit ovulation. *Theriogenology*, 45:1091-1102.

Sugano M, Watanabe S. 1997. Use of highly purified porcine FSH preparation for superovulation in Japanese black cattle. *J Vet Med Sci*, 59:223-225.

Takagi M, Kim IH, Izadyar F, Hyttel P, Bevers MM, Dieleman SJ, Hendriksen PJ, Vos PLAM. 2001. Impaired final follicular maturation in heifers after superovulation with recombinant human FSH. *Reproduction*, 121:941-951.

Willmott N, Saunder J, Bo GA, Palasz A, Pierson RA, Mapletoft RJ. 1990. The effect of LH/FSH ratio in pituitary extracts on superovulatory response in the cow. *Theriogenology*, 33:347. (abstract).