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Timing of artificial insemination and embryo production in superovulated Holstein cattle

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Abstract

The objective was to evaluate the effect of the first artificial insemination (AI) on the number of viable and degenerated embryos, total embryos and unfertilized oocytes, and fertilization rate in Holstein donor cows. Cows (n=22) and heifers (n=50) were synchronized by the Crestar® (Intervet International B.V., Boxmeer, Holland) protocol where 3 mg of norgestomet are implanted subcutaneously in addition to an intramuscular injection of 3 mg of norgestomet and 5 mg of estradiol valerate on Day 1 (D1). On the sixth day, superovulation was initiated with 8 decreasing injections of FSH/LH (PLUSET®; I.F. Serono, Rome, Italy), twice daily at 12 hour intervals. On the eighth day, 0.5 mg of sodium cloprostenol (Sincrosin®, Vallée S.A., Montes Claros, Brasil) was injected, and the implant was withdrawn on the ninth day. Embryos were flushed seven days after the first AI and classified according to the developmental stage and quality. Animals were allocated ramdomly to one of two treatments where in AI was at 0 and 12 hours (T0; n=27) and at 12 and 24 hours (T12; n=45) after onset of estrus. Fertilization rates were similar between treatments (T0 versus T12) and parity (heifers versus cows): 87.1 ± 4.8% versus 85.9 ± 3.2% and 87.1 ± 2.9% versus 85.9 ± 4.9%, respectively. There was no difference between treatments (T0 versus T12) and parity (heifers versus cows) for average number of viable embryos (5.3 ± 1.0 versus 5.7 ± 0.7 and 5.7 ± 0.6 versus 5.3 ± 1.0), unfertilized structures (1.0 ± 0.4 versus 1.2 ± 0.3 and 1.3 ± 0.3 versus 0.9 ± 0.5), and degenerated structures (1.1 ± 0.4 versus 1.2 ± 0.3 and 1.5 ± 0.3 versus 0.9 ± 0.4), respectively. There was an interaction (P = 0.04) between parity and treatment. Heifers in T12 had more viable embryos (7.1 ± 0.8) than those in T0 (4.2 ± 0.9). It was concluded that superovulated Holstein cows, but not heifers, can be inseminated at the onset of estrus without any deleterious effect on embryo yield or quality.

Keywords: insemination; superovulation, embryo transfer; Holstein cattle.

Introduction

The ideal time to initiate artificial insemination (AI) in embryo transfer programs is somewhat controversial, according to the literature. Donaldson (1985) recommended inseminating superovulated cows 12 hours after the onset of estrus with a single dose of semen. However, Schiewe *et al*. (1987) recommended insemination at 24 hours with two doses. In some reports, fertilization rates of superovulated cows ranged from 65% to 70% with various insemination regimes (Donaldson, 1983; Hawk and Tanabe, 1986; Lerner *et al*., 1986; Saacke *et al*., 1998).

According to Moor (1975), there is great variation as to when estrus occurs and not all animals express estrus after superovulation. Yadav *et al*. (1986) observed the occurrence of ovulations 24 hours after the onset of estrus with the majority taking place within the first 12 hours, but Maxwell *et al*. (1978) observed more extended intervals. Thus, it is difficult to define the ideal time to first inseminate superovulated cows due to the lack of studies that precisely define the dynamics of ovulation. This is especially true for Holstein cows kept in tropical environmental conditions.

The objective of this study was to evaluate the effect of time of the AI on the number of viable and degenerated embryos, total embryos and unfertilized oocytes, and embryo fertilization rates of Holstein donor cows and heifers.

Materials and Methods

Synchronization and superovulation

Twenty-two lactating cows and fifty nuliparous heifers within a single farm were used in this study. Heifers and cows were allocated to treatments and the establishments of treatment groups were done in a contemporary fashion in 9 replicates. Animals were reproductively sound upon entering the experiment and cows were at least 60 days post partum with body condition scores of at least 3 (Wildman et al., 1982). Heifers were 21.3 ± 2.1 (mean ± SEM) months old, and, cows were 5.7 ± 3.0 years old with a mean parity of 3.1 ± 2.0.

After uterine/ovarian evaluation by rectal palpation, animals received a subcutaneous norgestomet (3 mg) ear implant (Crestar®, Intervet International B.V., Boxmeer, Holland) with an intramuscular injection of norgestomet (3 mg) and of estradiol valerate (5 mg) on the first day (Day 1). On Day 5, cows and heifers began to receive twice-daily injections of follicle stimulating hormone from swine pituitary extract (PLUSET®; I.F. Serono, Rome, Italy) at decreasing intramuscular dosages of 100, 75, 50, and 25 IU (cows) and 80, 60, 40, and 20 IU (heifers) for four days,

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respectively. Luteolysis was induced with 500 µg of DL Cloprostenol (SINCROSIN®, Vallee S.A., Montes Claros/MG, Brazil) 60 hours after the first FSH injection. All donors (n=72) used in this trial showed signs of estrus within 48 hours of the prostaglandin (PG) injection. At the seventh FSH injection, the implant was removed.

Treatments

Animals were randomly allocated to one of two treatments: 1) T0 (n=27; 10 cows and 17 heifers) AI at 0 and 12 hours after the onset of estrus and 2) T12 (n=45; 12 cows and 33 heifers) AI at 12 and 24 hours by a skilled technician and using semen from different bulls of proven fertility (Table 1). Observation of estrus was initiated 36 hours after (PG) injection and was maintained for 12 hours thereafter. Animals not showing estrus up to 48 hours after PG injection did not continue in the trial.

Table 1. Experimental treatment regime.

Treatments	AI (hours after first observed in estrus)		
	0	12	24
T0	First AI	Second AI	
T12		First AI	Second AI

Embryo recovery and evaluation

Embryo flushes were performed non-surgically, seven days after the first insemination using 1.0 L medium (Modified Dulbecco, Nutricell®, Campinas, SP, Brazil), and embryos collected in 100 µm filters (Wright, 1981). Embryo developmental stage and quality were assigned after agreement from two experienced veterinarians according to the procedures of Linder and Wright (1983).

Statistical analyses

Data were distributed according to a poisson curve after PROCUNIVARIATE analysis (SAS, 1995). Treatment and parity effects on mean numbers of structures, viable, and degenerated embryos were analyzed by the GENMOD procedure of SAS® (SAS, 1995). The effects of treatment and parity on fertilization rates were compared by chi-square analysis using the FREQ procedure of SAS® (SAS, 1995). Results are expressed as means and the standard errors of the means (SEM).

Results

Fertilization rates were not different between treatments or parities. The overall mean fertilization rate was 86.5 ± 4.2%. Neither treatment nor parity affected mean embryo yield. There were no effects of treatment or parity on the number of viable embryo produced; however, there was an interaction ($P < 0.04$) between parity and treatment. The mean number of viable embryos was greater ($P=0.04$) for heifers first inseminated at 12 hours as opposed to 0 hours (Table 2).

Table 2. Fertilization rate and number of viable embryos, degenerated embryos, and unfertilized structures in superovulated Holstein cattle first inseminated at 0 and 12 hours (T0) or 12 and 24 hours (T12) after the onset of estrus.

	Heifers		Cows					
	T0	T12	T0	T12	SEM[1]	Parity[2]	Treatment[3]	Parity versus Treatment[4]
						P*		
Fertilization rate (%)	86.74	87.49	87.50	84.26	20.64	0.83	0.83	0.73
Viable embryos	4.24	7.13	6.33	5.18	4.29	0.77	0.81	0.04
Degenerated embryos	1.42	1.51	0.83	0.87	1.78	0.18	0.88	0.96
Unfertilizated structures	1.05	1.62	1.00	0.81	1.90	0.42	0.80	0.51
Total embryos	5.77	8.53	7.51	5.62	4.75	0.65	0.67	0.12
Total structures	6.71	10.21	8.16	6.31	5.51	0.47	0.68	0.10

[1]Standard error of the mean.
[2]Nuliparous heifers versus cows (at least one lactation).
[3]Treatments - T0 (insemination at 0 and 12 hours after the onset of estrus; n=27) and T12 (insemination at 12 and 24 hours after the onset of estrus onset; n=45).
[4] Interaction between parity and treatment.
* Denotes the value of P for statistical analysis purpose.

Discussion

Fertilization rate was not different among treatments in the current experiment. Similar findings were reported by Donaldson (1985) with a single AI at 0, 12, or 24 hours after the onset of estrus in superovulated cows. However, Dalton *et al*. (2000) reported higher fertilization rates when donors were

inseminated 24 hours after the onset of estrus compared to inseminations at 0 or 12 hours. They argued that this difference may have occurred due to decreasing fertilization potential of sperm before ovulation occurred. Considering their results with an AI at 12 hours, we should have observed improved fertilization rates, which did not occur. A possible explanation for the similarity in fertilization rate between treatments is that, according to Kishida *et al*. (2004), all ovulations had occurred during the first 11 hours after the first ovulation (24 hours after the onset of estrus). According to Hunter (1980), the duration of spermatic viability in the female reproductive system is 24 hours, and approximately 4 to 6 hours are required for sperm capacitation. Therefore, in this trial, both AI times supplied an adequate amount of capacitated sperm within an optimal window of time for ova viability and maturation.

Dalton *et al.* (2000) reported that it is still unclear at which time AI, with conventional frozen-thawed semen, yields optimal embryo quality. In our trial, time of AI did not affect the number of degenerated embryos and structures or the total number of embryos and structures. Similar findings were observed by Donaldson (1985) where a single insemination at 12 hours after the onset of estrus did not alter the number of degenerated embryos compared to inseminations at 12 and 24 hours. However, Dalton *et al*. (2000) and Schiewe *et al*. (1987) reported an increase in the number of degenerated embryos with delayed fertilization. This may be related to ova that were ovulated early and underwent aging before the completion of sperm transport and fertilization. For cows in this trial, there was no effect of time of AI on embryo production because, in both treatments, there was probably adequate amounts of capacitated sperm present at the site of fertilization.

There was an interaction between parity and treatment for the number of viable embryos where heifers, but not cows, inseminated at 12 and 24 hours after the onset of estrus had a greater number of viable embryos. Similar findings were observed by Donaldson (1985) who reported a larger number of viable embryos when donors were inseminated 12 and 24 hours after estrus onset. The number of viable embryos in T0 may have been fewer in heifers than in cows due to a distinct uterine environment in heifers that may have resulted in an inadequate time for final sperm maturation and capacitation. According to Yadav *et al*. (1986) all ovulations in superovulated heifers occurred within 37 hours after the onset of estrus. Thus, ovulations occurring late may have increased the number of degenerated embryos and structures because of the interactions between semen and the uterus. Another possible explanation for the greater number of viable embryos seen in T12 heifers is that oocytes that were fertilized earlier, as in T0, probably spent more time in adverse uterine conditions. There maybe some important differences in how a cow reacts to the effects of superovulation as compared to heifers.

It was concluded that superovulated Holstein cows, but not heifers, can be inseminated at onset of estrus without any deleterious effect on fertilization and embryo yield and quality in embryo transfer programs. Furthermore, these results may imply that there are significant interactions between ovulation dynamics and semen capacitation within the reproductive tract of heifers and cows.

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