Factors Affecting Embryo Transfer Success in Recipient Heifers Under Field Conditions

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ABSTRACT

Recipient age at transfer (less than 525 d old or more than 525 d old), season (fall, winter, spring, summer), method of synchronization (natural or induced), and transfer technique (surgical or nonsurgical transfer) were associated with success of embryo transfer in a log-linear analysis. In a separate analysis, no significant association was found between success of transfer and transfer to the left or right uterine horn. Summer had the lowest proportion of successful transfers (58.9%). Pregnancy rates were 83% using the surgical transfer method and 68% with transcervical transfers. Proportion of pregnancies following a second transfer was not different from the proportion after first transfers. Success of embryo transfer was highest if recipients were >525 d old and if transfers were performed surgically in spring following synchronization of recipients with cloprostenol, an analog of prostaglandin $F_2\alpha$. Probability of success was lowest for transfers to young, prostaglandin-synchronized recipients, performed nonsurgically in summer.

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INTRODUCTION

Pregnancy rates from embryo transfer (ET) vary widely. Variation in donor response to superovulation, other donor factors, technical proficiency, recipient suitability, seasonal effects, and management factors are thought largely responsible for variation in observed success. Technique and donor effects on pregnancy rates have been studied most and reviewed recently (18), but there is considerably less literature on recipient factors affecting ET pregnancy rates. Although Shea et al. (20) reported decreased ET pregnancy rates in winter, seasonal effects on ET recipients remain largely undocumented. Variables affecting pregnancy rates in Holstein heifer recipients under field conditions in semiarid climates have not been reported.

Investigations of seasonal influence on female bovine reproduction have demonstrated effects on age at puberty and fertility. These effects are associated with photoperiod, nutrition, and thermal stress (9, 22, 23). Health and nutritional status of recipients are recognized as significant factors affecting recipient pregnancy rates (19). Effect of natural versus induced estrus on recipient pregnancy rates has not been established in dairy heifers, although no difference has been observed in beef recipients (26). Moreover, information about the conditional probability of ET pregnancy in heifers used as recipients following an unsuccessful transfer would aid in establishing eligibility criteria for recipient pools. Uterine horn of transfer as an influencing factor has been studied (14) but may be more variable under field conditions.

The hypothesis tested was that ET pregnancy rates on a large commercial dairy were affected by transfer method, uterine horn, recipient factors, and season.

MATERIALS AND METHODS

Holstein dairy heifers (n = 704) representing 823 ET on a large California dairy meeting the following inclusion and exclusion criteria were used. Heifers were included if they receved an ET between July 1, 1983 and July 31, 1984, if their record contained complete information on date of transfer, technique used, breeding history and pregnancy status, and estrus must have been either natural or induced with cloprostenol.[1] Heifers were excluded only if they had pneumonia of 3-wk duration or chronic foot problems.

All recipients were reared on a dairy in the southern San Joaquin Valley in central California. Housing was in shaded freestalls with access to a drylot. Southern San Joaquin Valley temperatures vary considerably between seasons ranging from an average daily minimum of 7°C in winter to an average daily maximum of 35°C in summer.

Estrus was detected by twice daily observation for mounting behavior and supplemented by tail-striping (24). Dates of estrus were recorded for all heifers. Heifers that were 8 to 15 d postestrus were selected for synchronization with donors. Donor cows were superovulated with follicle-simulating hormone[2] administered in intramuscular injections twice daily for 4 d.

Only heifers observed in estrus (natural or induced) in synchrony (± 12 h) with donors were designated as potential recipients. Potential recipients were removed from the freestall facility to a treatment area on the day of the ET. Rectal palpation of the reproductive tract was used to select heifers with a palpable corpus luteum and a normal reproductive tract. Pregnancy was diagnosed by rectal palpation at 40 to 50 d of gestation. Heifers found nonpregnant by return to estrus or palpation were eligible to be selected as recipients more than once.

Transcervical transfers were performed by ET specialists using an insemination gun and French straw as transfer instruments. Surgical transfers were performed by exposing the uterus through a surgical incision in the flank. All transfers were to the uterine horn ipsilateral to the corpus luteum. Embryos were transferred within 10 h of collection. For this study, winter began January 1, spring April 1, summer July 1, and fall October 1.

Using statistical techniques described by Fienberg (4), a log-linear analysis was used to test associations and interactions among the variables to find a model closely fitting the data. Variables examined were recipient age (less than 525 days, older than 525 d), season (fall, winter, spring, summer), transfer technique (surgical transfer, transcervical), uterine horn of transfer (left, right), method of synchronization (natural, induced), and number of times used as an ET recipient (one, two). The BMDP4F computer program (1) was used to select, in a stepwise manner, variables and interactions important in predicting tabulated observations. Then a logit model was derived to determine the conditional probability of embryo transfer pregnancy. The Chi-square statistic was used to evaluate the association of pregnancy and horn of transfer. The proportion of successful transfers on the first transfer was compared to the proportion of successful transfers on second attempts using a Z-test statistic (27).

RESULTS

Descriptive

Median age of recipients was 525 d. Slightly more than half (52%) of the transfers were performed surgically. Embryo transfers were made to the right uterine horn more frequently (57%) than to the left uterine horn (43%). Overall, 76% of the embryo transfers resulted in pregnancy. Pregnancy rates were 83% using the surgical transfer method and 68% with transcervical transfers. There were 111 transfers to heifers used once unsuccessfully as a recipient

[1] Estrumate, Haver Lockhart, Shawnee, KS.

[2] FSH-P, Burns Biotec, Omaha, NE.

and eight transfers to heifers used twice unsuccessfully. Mean interval between first and second transfers was 55 ± 3 d.

Preliminary Statistics

The proportion of successes on first transfers (P_1 = 535/704) was not significantly different (Z = .28, $P > .05$) from the proportion of successes for heifers given a second transfer after a failed transfer (P_2 = 83/111). The uterine horn to which a transfer was made did not affect embryo transfer success (χ^2 = 1.037, df = 1, P = .31). Because of these results, horn of transfer was not included in the log-linear analysis. Similarly, the number of times the heifer was used as a recipient was not used as a variable in the log-linear analysis.

Results of Log-Linear Analysis

The first goal of this analysis was to use the log-linear approach to obtain a parsimonious, yet well-fitting model for the logarithm of the expected cell frequencies derived from the multiway cross tabulation shown in Table 1. Because recipients were randomly selected, individual cell frequencies presented in Table 1 are assumed to be realizations of independent Poisson processes, each process yielding a count for the corresponding cell. Total number of observations was not fixed by design; therefore, Poisson rates are equal to the expected cell counts unconditionally.

The second goal of analysis was to consider pregnancy as a response variable and to assess the effect of the other variables on it. Because we were interested in the effect of the variables on pregnancy, we examined, using logits, the ratio of ET pregnancies to ET pregnancy failures for each combination of age, synchrony, technique, and season.

The BMDP4F computer program was used to select appropriate models for the data and to estimate the parameters of the models. From the several models selected by the computer program, we used a backward procedure (where terms are removed from the model if they do not significantly improve the fit) to find the most parsimonious one. The likelihood ratio (G^2) was used to test the significance of fit of each model tested. We selected the model EST, ESM, EA, STM, SMA, TA where the first letter of each variable is used to show its inclusion in the model: ET outcome (E), season (S), transfer technique (T), method of synchronization (M), and age (A). Groups of initials separated by commas represent variable interactions. This notation implicitly includes all combinations of first and second order effects that can be formed out of the third order interactions of the model. This model was selected because it is parsimonious and because it fit the data well (G^2 = 21.58, df = 20, P = .364). The log-linear parameter estimates of the interactions included in the model that involve pregnancy are given in Table 2, from which the magnitude of effect of each interaction on pregnancy can be evaluated. Parameter estimates near zero are less important. Effects having the largest impact on probability of pregnancy were, in order, surgical transfers, induced transfers in spring, summer transfers, and nonsurgical transfers in spring.

Results of Logit Analysis

After deriving the log-linear model, we treated pregnancy as the response variable and conditioned on the remaining four explanatory variables to get a logit model. From this model we estimated the conditional probabilities of being pregnant, which are presented in Table 1. The relative importance of synchrony, season, and method of synchronization on ET success are shown in Table 2. Highest probability of ET success (rank = 1) was in older recipients with induced heats and surgical transfers in spring. Lowest probability of ET success (rank = 29) was in young recipients with induced heats and nonsurgical transfers in summer. Confidence intervals about the estimated conditional probabilities of pregnancy also are presented in Table 1.

Figure 1 shows the generally improved success of ET with older recipients, surgical transfer methods, and the interactions between synchronization regimen and season.

DISCUSSION

The most remarkable finding of this study was the influence of season on pregnancy rates of embryo transfers. Decreased pregnancy rates in summer may be, in part, a result of thermal stress. Decreased duration of estrus and intensity (5, 25), prolonged cycle length, altered

TABLE 1. Percent of heifers pregnant according to age, method of synchronization (Sync method), transfer technique, and season (the row totals); and the ranking of probability of embryo transfer (ET) success.

Age	Sync method	Transfer technique	Season	Embryo transfer data: Percent pregnant	Embryo transfer data: Total ET	Probability $\bar{X}$	Probability CI	Probability rank
Young	Natural	Surgical	Winter	86.0	50	.892	.059[1]	7
			Spring	70.5	88	.671	.087	22
			Summer	76.2	21	.764	.153	14
			Fall	86.4	22	.880	.075	8
			Total	77.3	181			
		Nonsurgical	Winter	73.2	41	.724	.100	18
			Spring	62.5	48	.635	.115	23
			Summer	47.2	53	.532	.108	26
			Fall	70.0	10	.757	.116	15
			Total	60.5	152			
	Induced	Surgical	Winter	86.4	22	.835	.094	10
			Spring	94.4	18	.902	.093	5
			Summer	100.0	1	.677	.262	21
			Fall	100.0	1	.375	*	
			Total	90.5	42			
		Nonsurgical	Winter	77.8	18	.615	.152	24
			Spring	87.5	8	.899	.102	6
			Summer	36.4	22	.424	.194	28
			Fall	...[2]		.213	...[3]	
			Total	60.4	48			
Old	Natural	Surgical	Winter	93.5	46	.919	.047	3
			Spring	60.7	27	.737	.094	17
			Summer	80.0	10	.817	.132	11
			Fall	90.9	77	.905	.057	4
			Total	85.7	161			
		Nonsurgical	Winter	80.0	40	.783	.088	13
			Spring	76.2	21	.705	.116	19
			Summer	69.0	42	.610	.109	25
			Fall	81.7	71	.811	.084	12
			Total	77.6	174			
	Induced	Surgical	Winter	88.0	25	.874	.076	9
			Spring	94.4	18	.935	.065	1
			Summer	...		.743	.241	16
			Fall	...		.453	...[3]	
			Total	90.7	43			
		Nonsurgical	Winter	42.9	14	.688	.140	20
			Spring	83.3	6	.924	.079	2
			Summer	100.0	2	.503	.216	27
			Fall	...		.272	...[3]	
			Total	59.1	22			

[1] Confidence interval.

[2] Missing value.

[3] Confidence interval infinitely large due to missing values.

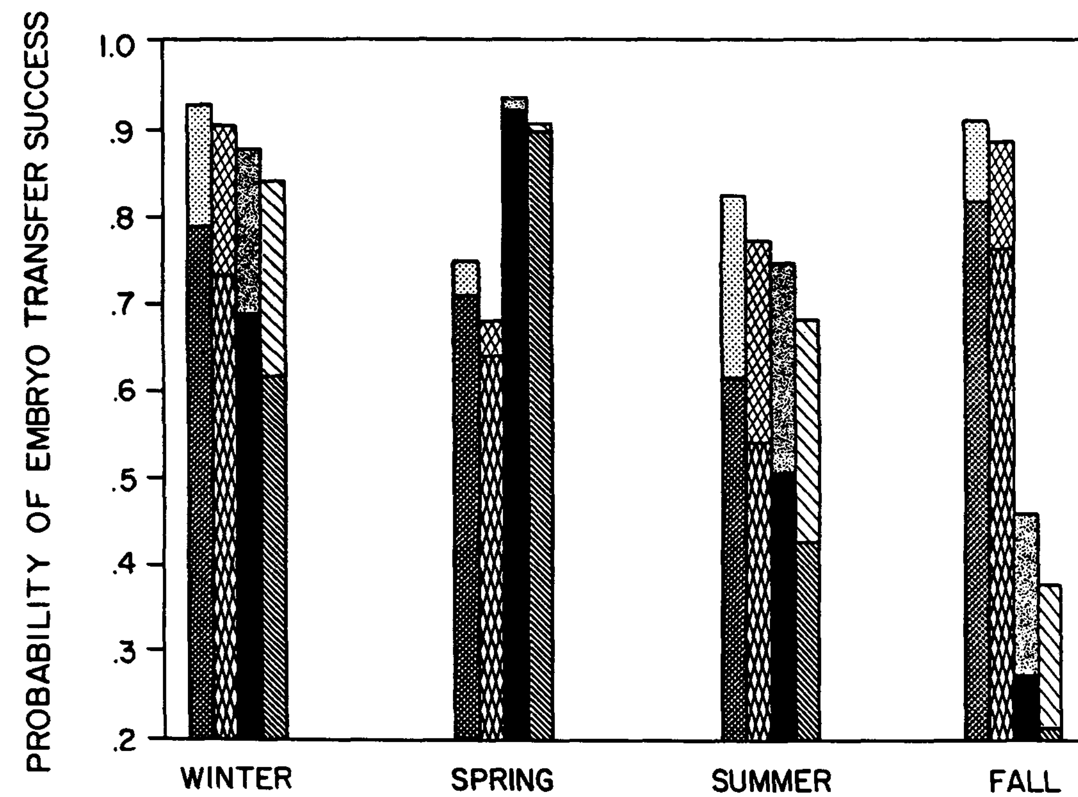

Figure 1. Probability of embryo transfer success in Holstein recipients for combinations of the variables of season, age, synchronization method, and transfer technique (▒ = heifers>525 d old, natural estrus; ▒ = heifers≤525 d old, natural estrus; ■ = heifers>525 d old, induced estrus; ▧ = heifers<525 d old, induced estrus). Lighter shaded, upper portion of each bar depicts the increased probability of embryo transfer success for transfers performed surgically rather than nonsurgically.

hormonal profiles (22), increased uterine temperatures (6), and decreased conception rates (7, 12, 22, 23) have been associated with high environmental temperatures. Observed low pregnancy rates in summer by nonsurgical transfer are not only a result of technique and season but also an interaction between the two. This interaction may be a result of direct effects of environment on the embryo before transfer. Increased embryonic mortality has been associated with prolonged incubation at 37°C before transfers (15). Cervical passage during nonsurgical transfer often takes longer than surgical placement of the embryo. It is possible that embryos transferred nonsurgically had a longer exposure to high ambient temperature and other environmental effects than did embryos transferred surgically.

That an ET following an ET had an equal probability of success was another important finding. Although Seidel (19) reported decreased pregnancy rates on successive transfers, this study agrees with a more recent report (2) that shows recipients can be used at least twice without decreasing pregnancy rate. This field study confirmed the superior efficacy of surgical transfer and the unimportance of horn of transfer (14). Age of heifer was shown to influence pregnancy rates. Ease and speed of cervical passage has been shown by others to affect pregnancy rates (3, 21, 26). Increased age may have facilitated cervical passage or surgical exteriorization of the reproductive tract, thereby improving ET success.

Improved pregnancy rates in recipients with prostaglandin $F_2\alpha$-induced estrus in spring and generally decreased pregnancy rates with induced estrus overall are difficult to explain. It is possible that pregnancy rates were different because accuracy of detection of estrus be-

TABLE 2. Estimates of the log-linear parameters for effects and interactions associated with embryo transfer success.

Show effect or interaction	Estimated parameter
Mean	.516*
Spring, induced	.517*
Fall, natural	.502
Spring	.288
Winter	.210
Surgery	.197*
Spring, nonsurgery	.157*
Natural	.109
Winter, surgery	.090
Old	.080
Summer, surgery	.065
Winter, natural	.015
Fall, surgery	.002
Summer, natural	.000
Summer, induced	.000
Spring, natural	–.517*
Fall, induced	–.502
Fall	–.279
Summer	–.219*
Nonsurgery	–.197*
Spring, surgery	–.157*
Induced	–.109
Winter, nonsurgery	–.090
Young	–.080
Summer, nonsurgery	–.065
Winter, induced	–.015
Fall, nonsurgery	–.002

*Parameter estimate differs from zero ($P<.05$).

tween groups or seasons was different. Several investigators (10, 11, 17) have noted improved conception and pregnancy rates with prostaglandin $F_2\alpha$ induction and insemination at observed estrus. Those studies did not examine seasonal influences. The sometimes improved fertility with cloprostenol induction may have been the result of seasonal/drug interaction rather than induction alone. Seasonal effects on corpus luteum development and structure are documented (16), but these changes are not reflected in plasma progesterone nor are progesterone concentrations at time of transfer useful for recipient selection (8, 13, 14). Although cloprostenol likely causes luteolysis in all seasons, fertility following induction may vary as a result of seasonal influences on corpus luteum function.

Improved accuracy and precision of detection of estrus also may occur following induction of estrus and result in higher pregnancy rates. The lack of information about accuracy of detection of estrus and the inconsistent effect of induction of estrus in this study suggest further research is needed to explain the season and drug interaction.

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