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REPRODUCTIVE TECHNOLOGIES: TECHNOLOGY STACKING

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Introduction

There have been several reproductive technologies developed over the past several years. Although these technologies have great potential to improve productivity of beef and dairy operations, they have only been minimally used. The reproductive technologies that may offer great value include estrus synchronization, embryo transfer, *in vitro* fertilization (IVF), sex-sorted semen, and embryo splitting. Synchronization of estrus protocols have been developed that consistently synchronize fertile ovulations with high fertility–fertility equal to breeding at estrus–and can be as valuable in synchronizing recipient cows as it is for AI cows. These procedures are often viewed as individual technologies. I have a more comprehensive viewpoint and propose combined utilization of the technologies. Within this succinct report, I will provide approaches to increase the reproductive potential of cattle.

Twinning

There are three basic approaches to twinning: transfer of two embryos, embryo splitting, and transfer of one embryo after an AI. With embryo splitting, the embryo is split using an embryo splitter and the demi-embryos are transferred into the cow. Since these are identical twins there is no concern with freemartinism. The following are data (Table 1) published by Dahlen et al. (2002). In that study, there was an increase of 84 pregnancies (calves) per 100 cows.

Table 1. Pregnancy rate and pregnancies with demi-embryos.

Item	**One Whole Embryo**	**Twin Demi-Embryos**
Pregnancy Rate (# pregnant / # cows treated)	15/37 (40%)	30/37 (81%)
Twinning (d 27)	0/15 (0%)	16/30 (53%)
Pregnancies (# fetal offspring / # cows treated)	15/37 (40%)	46/37 (124%)
Number of Calves / 100 Treated	40	124

There obviously will be cost associated with using this technology; however, the question is "Does the value of the additional calves justify the cost?"

Similarly, if one transfers two embryos per cow, calf production can be increased. However, one can also transfer an embryo into a cow seven days after an AI. A potential problem with these two concepts is freemartinism (assuming that one wishes to use the females for reproductive purposes). However, with the development of sex-sorted semen one can create embryos using sex-sorted semen and about 90% of the embryos will be of the desired sex. This greatly reduces the incidence of freemartinism. If one artificially inseminates with sex-sorted semen and follows with an embryo created with sex-sorted semen, then freemartinism will again be greatly reduced. This concept is by no means new; however, when it was first developed, sex-sorted semen was not on the horizon. Because of the high cost of sex-sorted semen, use for AI may be of questionable value. However, when used with embryo transfer, again one must assess if the value of the additional calves justifies the cost. Both Anderson et al. (1979) and Holy et al. (1981) demonstrated the efficacy of these procedures (summarized in Tables 2 and 3).

Table 2. Pregnancy rate and pregnancies in cows transferred one or two embryos (Anderson et al., 1979).

Item	**ET Two Embryos**	**ET One Embryo + AI**
Pregnancy Rate	15/21 (71%)	12/17 (71%)
Aborted	1/15 (7%)	1/12 (8%)
Produced Twins	10/14 (71%)	9/11 (82%)
Number of Calves / 100 Treated	114	117

Table 3. Twinning rate of cows transferred one embryo after AI (Holy et al., 1981).

Item	**Single**	**Twin**
Frequency	35/64 (55%)	29/64 (45%)
Single from AI	13/35 (37%)	—
Single from ET	22/35 (63%)	—

The pregnancy rate in the Holy et al., study was 62%. Clearly, both procedures increased the production of calves. Combined, 114 to 124 calves were produced using these three procedures. The number of animals used in these studies was limited; however, these data demonstrate the feasibility of these procedures.

Technology Stacking

I conducted another study wherein we used multiple reproductive technologies: estrus synchronization, sex-sorted semen, *in vitro* fertilization, and embryo transfer. In this study 486 beef cows were used. These cows were synchronized using CO-Synch with or without the CIDR. The day of the second injection of GnRH was considered day 0. On day 7 cows received either one or two grade 1 balastocysts. The embryos were created *in vitro*. Oocytes were collected from slaughterhouse ovaries and fertilized *in vitro* with frozen-thawed semen that had been sex-sorted to produce female offspring. The presence of corpora lutea were determined *per rectum* without grading quality. If a corpus luteum was present, transfers were done. Luteal tissue was verified via ultrasonography and transfers were done in every cow with luteal tissue. Single embryos were placed in the uterine horn ipsilateral to the ovary with the corpus luteum. In cows that received two embryos, they were transferred bilaterally (one in each uterine horn). Pregnancy was diagnosed 40-55 days post-transfer via ultrasonography.

Luteal tissue was detected in 463 cows (95%) at the time of transfer (Table 4). In thirteen cows, ultrasound revealed the presence of luteal tissue. Although, embryos were transferred into these cows, only 1 (8%) became pregnant. No luteal tissue was detected in 10 cows and transfers were not done in these cows. Based on this information, the corpora lutea should be detected only *per rectum*. However, clearly the CO-Synch estrus synchronization protocol (with or without the inclusion of the CIDR) was an effective protocol to prepare cows for embryo transfer. We have previously observed this using frozen-thawed embryos (Table 5).

Table 4. Pregnancy rate of cows based on classification of luteal tissue at transfer.

Item	**Number**	**Pregnancy Rate**
Cows:	486	—
with no luteal tissue	10	no transfers
detected only via ultrasound	13[b]	1/13 (8%)
detected *via per rectum* examination	463 (95%)[a]	200/463 (43%)

[a]Ninety-five percent (95%) of the cows received one or two embryos.
[b]These 13 cows are not included in Table 5.

Table 5. Pregnancy rates in synchronized cows transferred embryos.

Item	**Transferred**	**Pregnancies/Treated**
CO-Synch (expt. 1)	94%	50%
CO-Synch + CIDR (expt. 1)	94%	59%
CO-Synch (expt. 2)	92%	62%
CO-Synch + CIDR (expt. 2)	92%	65%

Pregnancy rates (# pregnant / # transferred) were not affected (P>.10) by inclusion of the CIDR; however, pregnancy rates was higher (P<.01) in cows receiving two embryos (51%) vs. one embryo (38%).

Table 6. Pregnancy rate and calving rate of cows synchronized with CO-Synch with and without the CIDR receiving one or two embryos at transfer.

Item	**CO-Synch**	**CO-Synch + CIDR**	**CO-Synch +/- CIDR**
Pregnancy Rate (# pregnant/# transferred):			
single transfer	60/145 (41%)	49/140 (35%)	109/285 (38%)[a]
twin transfer	43/ 88 (49%)	48/ 90 (53%)	91/178 (51%)[b]
Calving Rate (# calves/# recipients):			
single transfer	45/144 (31/100)	39/136 (29/100)	84/280 (30/100)[c]
twin transfer	38/ 85 (45/100)	41/ 87 (47/100)	79/172 (46/100)[d]

[a,b]Values with different superscripts differ [P<.01] (CIDR, P=.80; single/twin X CIDR interaction P=.27).
[c,d]Values with different superscripts differ [P<.01] (CIDR, P=.88; single/twin X CIDR interaction P=.62).

Sex of calf was assessed at calving and 90% (158/176) of the calves were heifers. Twenty-two percent of the cows with twin transfers calved with twins. There tended (P=.06) to be a higher pregnancy loss (# calving / # diagnosed pregnant) in cows with twin transfers than in cows with single transfers. Inclusion of CIDR in the synchronization did not affect (P>.10) pregnancy loss. Calving rate (# calves born / # recipients) was not affected by inclusion of the CIDR; however, calving rate was higher (P<.01) in cows receiving twins (46/100) vs. one embryo (30/100). Eight of the cows were diagnosed with hydramnios/hydrallantois and was not affected by CIDR inclusion nor number of embryos transferred. Calving difficulty was no greater than in previous years even though the Holstein calves were larger than beef calves and even though a large number of cows gave birth to twins. No excessive large calves were observed.

In summary, synchronization of ovulation with the CO-Synch protocol, with or without the CIDR, effectively prepared cows for transfer and twinning, regardless of transferring unilaterally or bilaterally, improved pregnancy rates. Furthermore, these data demonstrate the utility of the combined use of estrous synchronization, sex-sorted semen, in vitro fertilization, and embryo transfer technologies.

Conclusions

One novelty not mentioned is that the oocytes and semen were all of Holstein genetics. The idea here was to develop a source of income for the research unit. Holstein heifers are of high value. This is only one way to generate profit and I encourage you to explore other possibilities that can be obtained by stacking the technology. Combing these technologies give producers options for increasing calf crop and profit as well. As the technologies mature, utilization will increase and cost will decrease. Consider the possibilities.

References

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