The revolution and future frontiers of reproductive management of dairy cattle

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Abstract

The 21-d pregnancy rate is determined by an interaction between the AI service rate and pregnancies per AI (P/AI) and is a key performance indicator for the reproductive efficiency of dairy farms. Over the past 2 decades, the reproductive performance of lactating dairy cows increased. A greater understanding of endocrinology and the physiology of lactating dairy cows generated fertility programs (the Presynch-Ovsynch and Double Ovsynch protocols for TAI) that increase the service rate and P/AI compared with detection of estrus. Previously, synchronization of ovulation in heifers was associated with poor reproductive performance compared with detection of estrus. Several modifications determined through randomized-controlled studies gave rise to the 5-d CIDR-Synch protocol that has similar and more P/AI when heifers are inseminated with conventional and sexed semen, respectively, compared with detection of estrus. A hierarchy of reproductive needs exists for dairy farms that require fulfillment before a new need can emerge. Dairy farms must have a high 21-d pregnancy rate and good heifer management before adopting other advanced reproductive technologies such as genomic selection, sexed and beef semen, and in vitro-produced embryos. Adopting these advanced reproductive technologies benefits dairy farms, however, challenges still exist that need further investigation through randomized-controlled experiments to maximize the benefits.

Key words: synchronized ovulation, dairy cattle, pregnancies per AI

Introduction

The proportion of cows producing milk in a herd and their stage of lactation determines the total milk production of a dairy herd and is affected by the rate at which cows become pregnant. The rate at which lactating dairy cows or heifers become pregnant (21-d pregnancy rate) is determined by an interaction between the AI service rate (the rate at which eligible cows are inseminated) and pregnancies per AI (P/AI; the proportion of inseminated cows that become pregnant) and is a key performance indicator for reproductive efficiency and profitability of dairy farms.[3] Poor reproductive performance in lactating dairy cows associated with increased genetic selection for milk production and other management factors[70] initiated a pursuit to further understand dairy cow reproductive physiology and the implications of management on reproductive efficiency. Over the past 2 decades, the U.S. dairy industry underwent a reproductive revolution with increased reproductive performance of lactating dairy cows primarily driven by improvements in periparturient management and the adoption of fertility programs.[32] This advancement in the reproductive performance of lactating dairy cows is solely possible because of the randomized-controlled research studies conducted by several laboratories to understand the endocrinology and physiology of lactating dairy cows, now allowing dairy farms to adopt other advanced reproductive technologies. More randomized-controlled studies are now being published exploring the adoption of advanced reproductive technologies on dairy farms and other key knowledge gaps in the reproductive management of dairy cattle. Thus, the objective of this review is to briefly summarize the scientific literature over the past 29 years that initiated a reproductive revolution increasing reproductive performance in lactating dairy cows and the opportunities increased reproductive performance provides to dairy farms. Further, we will forecast the future frontiers of reproductive management in dairy cattle to be investigated to fill current knowledge gaps.

Lactating dairy cow reproductive management

Detection of estrus

Pregnancy establishment in lactating dairy cows depends on the accuracy of detection of behavioral estrus for the correct timing of insemination relative to ovulation. From the first mounting event[124] or the onset of activity,[115] the mean time to ovulation is approximately 28 h. While the adoption of hormonal synchronization protocols has increased, detection of estrus remains an important part of the reproductive management of a dairy farm. Previously, the rate of detection of estrus occurred primarily via visually observation and was poor (< 50%) for most dairy farms.[104] Challenges with accurate and efficient detection of estrus occur because of decreased duration of expression of estrus associated with increased milk production,[69] lack of cyclicity,[130] facilities,[87] and silent ovulations.[87,115] The development of automated activity monitoring (AAM) technologies such as pedometry,[55,71,99] radiotelemetry,[124, 28] and accelerometers[48,51] that use secondary characteristics of estrus[97] improved the efficiency of monitoring the expression of behavioral estrus.

For lactating Holstein cows fitted with an accelerometer and synchronized to express estrus, 71% of cows were detected in estrus with 95% ovulating within 7 d after induction of luteolysis.[115] Interestingly, of the 29% of cows not detected in estrus by the accelerometer system, 35% of those cows ovulated within 7 d after induction of luteolysis.[115] Santos et al. (2017) reported that 78% of lactating Holstein cows submitted to a protocol to synchronize estrus were detected in estrus via visual observation and AI.[102] In a recent study from our laboratory, detection of estrus occurred twice daily via visual observation of rubbed tail paint, with 75% of lactating Jersey cows detected in estrus after submission to a protocol to synchronize estrus.[59] Fewer primiparous cows tended to be detected in estrus and AI than multiparous cows (69.5 vs. 77.1%, respectively),[59] likely because primiparous cows have a greater prevalence of anovulation.[4,80] Sitko et al. (2023) reported that 71 to 74% of primiparous lactating Holstein cows were detected in estrus via visual observation or AAM technology after $PGF_{2\alpha}$ treatment regardless of genetic merit for fertility.[106] Despite recent improvements in the rate of detection of estrus and method, insemination rates based on the detection of estrus only approach about 70 to 80%[115, 102,106,59] because of failure of cows to express estrus, failure in detection of estrus, and the inability of cows to return to cyclicity. Thus, insemination rates based on the detection of estrus cannot achieve a 100% insemination rate and can be improved through the submission of undetected cows to a synchronized ovulation protocol for TAI.

Ovsynch

The original Ovsynch protocol was the first hormonal synchronization protocol to use 3 sequential hormonal treatments to manipulate ovarian function to synchronize ovulation.[89] The first GnRH treatment of the Ovsynch protocol induces an LH surge and ovulation of a dominant follicle to initiate a new synchronized follicular wave. Ovulation of a dominant follicle also creates an accessory corpus luteum (CL) that increases circulating progesterone (P4) during the growth of the preovulatory follicle. Luteolysis is induced 7 d later with $PGF_{2\alpha}$ as the second treatment of the Ovsynch protocol to decrease P4 concentrations to synchronize the initiation of proestrus. The final GnRH treatment of the Ovsynch protocol induces an LH surge to synchronize ovulation for the timing of AI to occur at an optimal time relative to ovulation for fertilization and initiation of pregnancy. The initial field studies with the Ovsynch protocol randomly assigned lactating dairy cows to be reproductively managed based on detection of estrus and AI or an Ovsynch protocol for TAI through their lactation.[88] Cows submitted to the Ovsynch protocol had similar P/AI (37.0 vs. 39.0%[88]; 37.8 vs. 38.9%[91]) but fewer median days to the first AI (54 vs. 83 d) and days open (99 vs. 118 d) than cows detected in estrus and AI, respectively.[88] Thus, the Ovsynch protocol increased the 21-d pregnancy rate not by increasing P/AI, but by overcoming the physiologic and management limitations with detection of estrus by increasing the service rate.

From there, the Ovsynch protocol was refined through several randomized-controlled experiments to determine the effect of cyclicity, timing of insemination, and day of the estrous cycle at the beginning of the protocol on fertility. Ovular cows had more P/AI (32 vs. 35%) than anovular cows (9 vs. 11%), but there was no difference in P/AI for cows submitted to an Ovsynch protocol for TAI than cows AI after a detected estrus, respectively.[41] Pursley et al. (1998) reported that inseminating cows at either 8, 16, or 24 h after the second GnRH treatment had the most P/AI, suggesting a timing of AI of 16 h after the second GnRH treatment.[90] Brusveen et al. (2008) submitted lactating dairy cows to an Ovsynch protocol and randomized cows to receive GnRH treatment concurrent with TAI at 48 or 72 h (Co-synch 48 or 72) or GnRH treatment 56 h after $PGF_{2\alpha}$ treatment and 16 h before TAI (Ovsynch-56).[13] Cows submitted to an Ovsynch-56 protocol had more P/AI than cows submitted to a Co-synch 48 or 72 protocol (39 vs. 29 vs. 25%, respectively).[13] Thus, the current optimal timing of insemination relative to ovulation is approximately 16 h after the last GnRH treatment of an Ovsynch protocol. Vasconcelos et al. (1999) reported that ovulation to the first GnRH treatment depends on the day of the estrous cycle, with cows beginning the Ovsynch protocol on d 5 to 9 having the greatest ovulatory response.[121] An optimal stage of the estrous cycle to begin an Ovsynch protocol is on d 6 or 7 of the estrous cycle[121] because the dominant follicle has ovulatory capacity and circulating P4 concentrations are moderate with less attenuation of the GnRH-induced LH surge for ovulation.[38] This data would be the foundation for randomized-controlled studies to develop presynchronization strategies to manipulate ovarian function for more cows to begin an Ovsynch protocol on d 6 or 7 of the estrous cycle.

Presynch-Ovsynch

The first strategy for presynchronization before an Ovsynch protocol tested in a randomized-controlled experiment was with 2 $PGF_{2\alpha}$ treatments given 14 d apart with the second $PGF_{2\alpha}$ treatment given 12 d before beginning an Ovsynch protocol (Presynch-Ovsynch).[82] When comparing only ovular cows, cows submitted to a Presynch-Ovsynch protocol had a 68% relative increase in P/AI than cows submitted to an Ovsynch protocol at a random stage of the estrous cycle (25 vs. 43%, respectively).[82] The initial intention behind the Presynch-Ovsynch protocol was to presynchronize cows for more cows to be at an optimal stage of the estrous cycle when beginning the Ovsynch protocol with 100% TAI.[82]

Dairy herds interested in prioritizing detection of estrus began inseminating cows detected in estrus after the second $PGF_{2\alpha}$ treatment ("cherry-picking") with undetected cows being submitted to the Ovsynch protocol.[24] Approximately 50 to 70% of cows are detected in estrus after the second $PGF_{2\alpha}$ treatment of a Presynch-Ovsynch protocol.[24,42,32] Borchardt et al. (2016) conducted a meta-analysis with 3 randomized-controlled studies[24,42,32] with 1,689 lactating dairy cows to compare the P/AI of cows submitted to a Presynch-Ovsynch protocol with 100% TAI or insemination after a detected estrus with undetected cows receiving TAI.[9] The combination of cows being inseminated after a detected estrus after the second $PGF_{2\alpha}$ treatment and undetected cows receiving TAI decreased the odds of pregnancy by 35% than when all cows were required to complete the protocol for scheduled TAI.[9] This combination negates the presynchronization effect because cycling cows that are presynchronized to begin the Ovsynch protocol at an optimal stage of the estrous cycle are removed from the protocol thereby decreasing P/AI.

Further, modifications to the Presynch-Ovsynch protocol have occurred such as the time interval from the second $PGF_{2\alpha}$ treatment to the first GnRH treatment of the Ovsynch protocol. The original Presynch-Ovsynch protocol used a 12 d interval from the second $PGF_{2\alpha}$ treatment to the first GnRH treatment of the Ovsynch protocol to increase the proportion of cows between d 5 and 12 of the estrous cycle at the beginning of the Ovsynch protocol.[82] Galvão et al. (2007) tested the effect of shortening the interval from the second $PGF_{2\alpha}$ treatment to the first GnRH treatment of the Ovsynch protocol from 14 to 11 d on ovulation to the first GnRH treatment and P/AI.[35] Cows with an 11 d interval had a greater ovulatory response (61 vs. 45%, respectively) and tended to have more P/AI at 66 d after AI (36 vs. 30%, respectively) than cows with a 14 d interval from the second $PGF_{2\alpha}$ treatment to the first GnRH treatment of the Ovsynch protocol.[35] Most cycling cows are detected in estrus within 3 to 4 d after the second $PGF_{2\alpha}$ treatment, thus using a 10 or 11 d interval increases the proportion of cows on d 6 to 8 of the estrous cycle to increase the ovulatory response to the first GnRH treatment of the Ovsynch protocol and P/AI.

Strickland et al. (2010) compared the P/AI of 1,371 lactating Holstein cows randomized for insemination after a detected estrus or a 14/11 Presynch-Ovsynch protocol with 100% TAI.[111] Cows submitted to the 14/11 Presynch-Ovsynch protocol had more P/AI than cows inseminated after a detected estrus (44.0 vs. 31.0%, respectively) despite cows submitted to the Presynch-Ovsynch protocol having greater DIM at first AI.[111] The

Presynch-Ovsynch protocol is effective at presynchronizing cows and increasing P/AI relative to detection of estrus and the Ovsynch protocol, but 2 limitations exist. First, presynchronizing cows solely with 2 $PGF_{2\alpha}$ treatments is ineffective for anovular cows to induce cyclicity before beginning the Ovsynch protocol. Second, 2 treatments with $PGF_{2\alpha}$ do not tightly synchronize follicular growth.

Double-Ovsynch

The combination of GnRH and $PGF_{2\alpha}$ treatments for presynchronization overcomes the limitations of solely using 2 $PGF_{2\alpha}$ treatments to presynchronize cows and is the basis for newer presynchronization strategies such as G6G[6] and the Double-Ovsynch[107,46,21] protocols. Treatment with GnRH to presynchronize cows resolves the anovular condition and more tightly regulates follicular development to have a greater proportion of cows begin the Ovsynch protocol on d 6 or 7 of the estrous cycle to optimize the response to subsequent treatments.[17] Cows submitted to a Double-Ovsynch protocol had more P/AI than cows submitted to a Presynch-Ovsynch protocol for TAI (49.7 vs.41.7%[107]; 46.3 vs. 38.2%[46]) but this increase in P/AI was primarily for primiparous cows. This difference between primiparous and multiparous cows we now know is partially due to incomplete luteolysis for multiparous cows[129] because cows with a young CL (d 6) are not as responsive to 1 $PGF_{2\alpha}$ treatment.[101,40] This was rectified by increasing the dose[39,5] and including a second $PGF_{2\alpha}$ treatment 24 h later within the Breeding-Ovsynch portion of the Double-Ovsynch protocol to induce complete luteolysis for low P4 at TAI.[129,17,112] Borchardt et al. (2018) conducted a meta-analysis of 7 experiments to determine the effect of an additional $PGF_{2\alpha}$ treatment 24 h after the first treatment during the Ovsynch protocol on luteolysis and P/AI[10]. Treatment with an additional $PGF_{2\alpha}$ treatment during the Ovsynch protocol increased luteolysis and P/AI by 11.6 and 4.6 percentage points, respectively.[10] Thus, fertility programs should include a second $PGF_{2\alpha}$ treatment 24 h after the first treatment during the Ovsynch protocol.

Three randomized-controlled experiments directly compared P/AI of cows AI after a detected estrus compared with cows submitted to a Double-Ovsynch protocol for TAI.[102,106,59] Of these 3 experiments, 2 were conducted in our laboratory using a protocol to synchronize estrus (GnRH treatment with 2 $PGF_{2\alpha}$ treatments 7 and 21 d later, respectively) to have cows submitted to a Double-Ovsynch protocol for TAI and cows inseminated at a detected estrus to be at a similar DIM. In the first experiment,[102] 578 lactating Holstein cows were randomized to receive TAI after a Double-Ovsynch protocol[107,46,18] or AI after a synchronized estrus. Cows submitted to a Double-Ovsynch protocol had more P/AI 63 d after AI than cows inseminated after a synchronized estrus (44.6 vs. 36.4%, respectively).[102] Further, 85.3% of the cows synchronized to the hormonal synchronization protocols and did not differ between treatments.[102] Synchronized cows submitted to a Double-Ovsynch protocol had 10 percentage points more P/AI at 33 d after AI than cows inseminated after a synchronized estrus (54.7 vs. 44.5%, respectively).[102]

In the second experiment,[59] 1,272 lactating Jersey cows were allocated by odd vs. even ear tag number, which was randomly allocated within the herd, within parity and semen type (conventional beef vs. sexed Jersey semen) to receive TAI after a Double-Ovsynch protocol or AI after a synchronized estrus. For cows inseminated with sexed Jersey or conventional beef semen, cows submitted to a Double-Ovsynch protocol for TAI tended to have and had more P/AI than cows inseminated after a synchronized estrus (sexed, 49.2 vs. 43.6 %; beef, 64.2 vs. 56.3 %, respectively).[59] Overall, 29.1% of cows submitted to a Double-Ovsynch protocol expressed estrus with 5.0 and 24.2% of cows detected in estrus ≥ 24 h before and at TAI, respectively, and there was no difference in P/AI 61 ± 4 d after AI based on expression of estrus at TAI.[59] The synchronization rate was greater for cows submitted to a Double-Ovsynch protocol for TAI than cows inseminated after a synchronized estrus (92.1 vs. 79.2%, respectively); however, synchronized cows submitted to a Double-Ovsynch protocol for TAI had more P/AI 61 d after AI than cows inseminated after a synchronized estrus (55.0 vs. 49.2%, respectively).[59]

In the third experiment,[106] primiparous Holstein cows from 6 commercial dairy farms were randomized within genetic fertility groups based on a Reproductive Index (CLARIFIDE®; Zoetis; Low, Medium, and High) and farm to either prioritize insemination after a detected estrus with undetected cows submitted to TAI or be submitted to a Double-Ovsynch protocol for TAI. Overall, cows submitted to a Double-Ovsynch protocol for TAI had approximately 10 percentage points more P/AI than cows inseminated prioritizing insemination after a detected estrus (58.7 vs. 48.7%, respectively).[106] For first insemination, cows in the High fertility group had more P/AI than the Low fertility group (59.8 vs 47.7%, respectively), but did not differ from the Medium fertility group (58.7 vs. 53.6 %, respectively).[106] Cows submitted to a Double-Ovsynch protocol for TAI for first insemination had more P/AI at pregnancy reconfirmation than cows inseminated prioritizing detection of estrus in the High (61.5 vs. 53.8%, respectively), Medium (55.8 vs. 45.4%, respectively), and Low (52.0 vs. 43.4%, respectively) fertility groups.[106] Differences in P/AI between reproductive management strategies had been largely attributed to differences in synchrony.[40,67,16] We concluded from these experiments that regardless of semen type and genetic merit, the major contributor to differences in P/AI between cows submitted to a Double-Ovsynch protocol for TAI versus cows inseminated after a detected estrus is the physiological mechanisms the Double-Ovsynch protocol embodies to optimize oocyte and embryo quality.

Table 1 summarizes randomized-controlled studies that directly compared cows inseminated after a detected estrus versus TAI after submission to a synchronized ovulation protocol. Lactating cows submitted to a Presynch-Ovsynch or a Double-Ovsynch protocol for TAI had more P/AI than cows inseminated after a detected estrus (Table 1). Thus, the Presynch-Ovsynch and Double-Ovsynch protocols are deemed fertility programs because they not only increase the service rate but also P/AI to increase the 21-d pregnancy rate.

Physiology of fertility programs

The steady-state P4 concentration in the circulation of lactating dairy cows is a balance between P4 produced by the corpus luteum and catabolized by the liver.[131] Milk production is highly correlated ($R^2 = 0.88$) with DMI[44] and DMI is highly correlated with increased hepatic blood flow ($R^2 = 0.93$).[30] With the increased feed intake of a high-producing dairy cow, there is increased hepatic catabolism of steroid hormones such as P4 thereby decreasing circulating P4 concentrations in high-producing dairy cows.[100] Around the time of deviation, decreased P4 concentrations likely cause a delay in the FSH nadir and increase LH pulses, allowing for co-dominant follicles increasing the risk for twins.[131] Follicles that undergo prolonged periods of dominance and then ovulate have fewer P/AI[78,79] likely due to increased LH pulses in a low P4 environment decreasing oocyte

Table 1: Effect of submitting lactating Holstein or Jersey cows for first insemination to various synchronized ovulation protocols for timed artificial insemination (TAI) versus insemination after a detected estrus on pregnancies per AI (P/AI; adapted from Fricke and Wiltbank, 2022).[33]

Experiment	TAI protocol	P/AI (%)		*P*-value
		Estrus	TAI	
Pursley et al., 1997[a]	Ovsynch[1]	39.0	37.0	NS
Pursley et al., 1997[b]	Ovsynch[1]	38.9	37.8	NS
Chebel and Santos, 2010	Presynch-Ovsynch[2]	25.3	31.1	0.20
Strickland et al., 2010	Presynch-Ovsynch[3]	30.5	44.3	< 0.01
Gümen et al., 2012	Presynch-Ovsynch[2]	33.3	49.2	< 0.05
Fricke et al., 2014	Presynch-Ovsynch[2]	29.0	38.0	< 0.01
Santos et al., 2017	Double-Ovsynch[4]	36.4	44.6	0.05
Rial et al., 2022	Double-Ovsynch[5]	37.3	44.6	0.02
Sitko et al., 2023	Double-Ovsynch[6]	46.6	55.4	< 0.01
Lauber and Fricke, 2024	Double-Ovsynch[7]	49.1	58.0	0.04

1 Lactating Holstein cows; Estrus: all cows inseminated after detected estrus; TAI: all cows submitted to an Ovsynch protocol for TAI.

2 Lactating Holsteins; Estrus: all cows detected in estrus after the second $PGF_{2\alpha}$ treatment of presynchronization inseminated with undetected cows submitted to Ovsynch protocol for TAI; TAI: all cows submitted to Presynch-Ovsynch protocol receive scheduled TAI.

3 Lactating Holstein cows; Estrus: all cows detected in estrus after first or second $PGF_{2\alpha}$ treatment of presynchronization inseminated with undetected cows submitted to Ovsynch protocol for TAI; TAI: all cows submitted to Presynch-Ovsynch protocol receive scheduled TAI.

4 Lactating Holstein cows; Estrus: all cows inseminated after a synchronized estrus; TAI: all cows submitted to Double-Ovsynch protocol for TAI.

5 Lactating Holstein cows; Estrus: cows inseminated after detection of estrus (74.6%) and undetected cows submitted to an Ovsynch protocol; TAI: all cows submitted to Double-Ovsynch protocol for TAI.

6 Primiparous Holstein cows; Estrus: cows inseminated after detection of estrus (~70%) and undetected cows submitted to an Ovsynch protocol; TAI: all cows submitted to Double-Ovsynch protocol for TAI.

7 Lactating Jersey cows inseminated with conventional beef semen; Estrus: all cows inseminated after a synchronized estrus; TAI: all cows submitted to Double-Ovsynch protocol for TAI.

quality potentially from premature meiotic resumption.[56,94] A high P4 environment during the development of the preovulatory follicle decreases follicle size compared with a low P4 environment[73] and double ovulations.[73,117] Cows that ovulate a medium-sized preovulatory follicle (15-19 mm) had more P/AI than cows with smaller or larger follicles.[108]

The preovulatory follicle during a Double-Ovsynch protocol is exposed to a high P4 environment when cows ovulate to the first GnRH treatment of the Breeding-Ovsynch portion of the protocol[39,18,77] and is associated with more P/AI.[39,11] At the last GnRH treatment of the Double-Ovsynch protocol, the follicle induced to ovulate is similar in size (~ 15 to 16 mm) to an ovulatory follicle in a nonlactating heifer.[33] Treatment with exogenous GnRH to induce ovulation mitigates overexposure of an oocyte to LH pulses preventing premature meiotic resumption[56,94] and timing of insemination relative to ovulation is more precisely controlled compared with AI after a detected estrus.[115] Thus, submission of cows to a fertility program increases circulating P4 concentrations during the growth of the preovulatory follicle, decreases exposure to LH pulses to induce ovulation of an optimal-sized follicle with better oocyte quality, and more precisely controls timing of insemination relative to ovulation than detection of estrus.

Nulliparous heifer reproductive management

Detection of estrus

In the United States, approximately 69% of nulliparous heifers are submitted for first insemination based on spontaneous or induced estrus.[83] The primary method for detection of estrus is once-daily observation of rubbed tail chalk, rump-mounted patches, or visual observation of primary or secondary estrous behaviors.[97] In addition, some herds are adopting AAM technologies into their heifer reproductive management. Aggressive reproductive management of heifers based on the detection of estrus requires a high estrous detection rate and consistent PGF_{2a} treatments to induce estrus.[110,68]

Heifers are considered the most fertile females on a farm with an observed conception rate of 57% for U.S. heifers inseminated based on detection of estrus with conventional semen.[57] Inseminations with conventional dairy semen, however, are decreasing as more dairy herds are adopting sexed semen, particularly for heifers, to create genetically elite replacements.[62] Further, heifer rearing costs from birth until calving are approximately $2,500, with feed accounting for 46 to 54% of total rearing costs.[2,54] Many dairy farms are inseminating heifers with sexed semen using once-daily detection of estrus as they have previously done with conventional semen, but recent randomized-controlled studies suggest this may be costly.[101,75,58] Thus, 2 limitations exist for reproductively managing heifers for first insemination based on once-daily detection of estrus. First, are the increased days to first insemination and pregnancy thereby increasing days on feed. Second, there are fewer P/AI with sexed semen likely because timing of insemination relative to ovulation is not optimized.

5-d CIDR synch

Only 2% of heifers in the U.S. for first insemination are submitted to a synchronized ovulation protocol for TAI.[83] Previously, synchronized ovulation protocols were associated with decreased fertility compared to a detected estrus in heifers. Nulliparous heifers submitted to an Ovsynch protocol for TAI had fewer P/AI than heifers inseminated after a detected estrus (35.1 vs 74.4%).[91] The challenge of submitting heifers to an Ovsynch protocol is that 17.7% of heifers express estrus before the first $PGF_{2\alpha}$ treatment[95] necessitating the inclusion of a P4 insert to mitigate premature expression of estrus.[96] Another modification that improved fertility was decreasing the interval between GnRH and PGF_{2a} treatment from 7 to 5 d while extending the period of proestrus.[12,92,93] The final modification was the addition of a GnRH treatment when inserting the P4 insert followed by 2 PGF_{2a} treatments 5 and 6 d later to increase follicle turnover and complete luteolysis[64,65] to yield similar P/AI to heifers inseminated based on detection of estrus with conventional semen.[65,105] Together, these modifications gave rise to the current gold standard for synchronizing ovulation in dairy heifers, the 5-d controlled internal drug release (CIDR)-Synch protocol.[65] One limitation of the 5-d CIDR-Synch protocol is that 27 to 33% of heifers are detected in estrus ≥ 24 h before scheduled TAI.[105,74,58]

Three randomized-controlled experiments across 4 publications[105,74,75,58] have compared the insemination dynamics, P/AI, and economics of submitting heifers for first insemination to a 5-d CIDR-Synch protocol versus once-daily detection of estrus and AI. In the first experiment,[105] 611 nulliparous Holstein heifers from 3 commercial dairy farms were randomized within farm for first insemination to either a 5-d CIDR-Synch protocol for TAI or treatment with $PGF_{2\alpha}$ and once-daily detection of estrus. Most heifers were inseminated with conventional dairy semen, but some heifers were inseminated with sexed semen.[105] Nonpregnant heifers were re-inseminated based on detection of estrus over the 84-d breeding period.[105] Heifers submitted to a 5-d CIDR-Synch protocol had 8 fewer days to first AI and 12 fewer days to pregnancy than heifers inseminated after a detected estrus.[105] Overall, P/AI did not differ between heifers submitted to a 5-d CIDR-Synch protocol or detected in estrus and AI (62.8 vs. 58.3%, respectively).[105] Interestingly, for the few heifers inseminated with sexed semen, heifers submitted to a 5-d CIDR-synch protocol had more P/AI 60 d after AI than heifers inseminated after a detected estrus (54.8 vs. 31.6%).[105] Silva et al. (2015) conducted a partial budget using actual farm costs for hormonal treatments, detection of estrus, semen and AI, pregnancy diagnosis, and feed to determine the cost per pregnancy.[105] As expected, submitting heifers to a 5-d CIDR-Synch protocol increased hormonal treatment costs, but these costs were offset by fewer days on feed and decreased feed costs.[105] The cost per pregnancy was $17.00 less for heifers submitted to a 5-d CIDR-Synch protocol than heifers inseminated after a detected estrus.[105]

In the second experiment,[74] 966 nulliparous Holstein heifers were randomized to either prioritize detection of estrus, TAI after a 5-d CIDR-Synch protocol, or a combination of both for first insemination with sexed semen. Heifers were followed for a 100-d breeding period after first insemination and nonpregnant heifers were re-inseminated with conventional semen after a detected estrus or TAI after a 5-d CIDR-Synch protocol.[74] At first service, P/AI did not differ for heifers inseminated after detection of estrus after PGF_{2a} treatment and heifers submitted to a 5-d CIDR-Synch protocol for TAI (42.0 vs. 43.8%, respectively).[73] The lack of treatment difference in this experiment is likely due to heifers submitted to the 5-d CIDR-Synch protocol receiving GnRH treatment at CIDR insertion but only 1 PGF_{2a} treatment at CIDR removal.[74] Fertility of heifers was not improved with GnRH treatment at CIDR insertion and only 1 PGF_{2a} treatment at CIDR removal[64] likely due to lack of complete luteolysis.[65] The hazard of pregnancy was greater for heifers inseminated with sexed semen after synchronization with a 5-d CIDR-Synch protocol than prioritizing detection of estrus.[74] In a follow-up economic analysis,[75] reproductive management strategies that used TAI or detection of estrus followed by TAI for first insemination with sexed semen were more economically favorable than solely prioritizing detection of estrus.

We conducted the final experiment[58] to evaluate reproductive management programs for submission of Holstein heifers for first insemination with sexed semen. One limitation of the 5-d CIDR-Synch protocol is that 27 to 33% of heifers are detected in estrus ≥ 24 h before scheduled TAI.[105,74,58] Heifers detected in estrus before scheduled TAI must be inseminated for proper timing of insemination relative to ovulation, particularly for sexed semen, resulting in a synchronization protocol that does not allow for 100% TAI. Most heifers in estrus after the first PGF_{2a} treatment of a 5-d CIDR-Synch protocol are likely in proestrus (18 to 24 d after previous estrus) at the initiation of the protocol.[20] In this experiment, we had a treatment group in which CIDR removal was delayed by 24 h to prevent early expression of estrus before TAI while maintaining P/AI based on a previous experiment from our laboratory with heifers inseminated with conventional semen.[58]

Nulliparous Holstein heifers (n = 736) from 3 commercial dairy farms were randomized within farm to 1 of 3 treatments for first insemination with sexed semen: 1) a 5-d CIDR-Synch protocol (CIDR5), 2) a 5-d CIDR-Synch protocol with CIDR removal delayed by 24 h (CIDR6), or 3) $PGF_{2\alpha}$ treatment followed by once-daily detection of estrus and AI (EDAI)[58]. All heifers were followed for an 84-d breeding period with most nonpregnant heifers being re-inseminated based on detection of estrus with sexed or conventional semen.[58] Delaying CIDR removal by 24 h in a 5-d CIDR-Synch protocol suppressed early expression of estrus before TAI than removal at the first $PGF_{2\alpha}$ treatment (0.004 vs. 27.8%, respectively).[58] With conventional semen, P/AI did not differ if CIDR removal occurred at the first $PGF_{2\alpha}$ treatment or was delayed by 24 h (54.8 vs. 54.3%).[58] By contrast, CIDR6 heifers tended to have 7 percentage points fewer P/AI than CIDR5 heifers (44.8 vs. 51.8%, respectively).[58] Fewer P/AI with sexed semen from delaying CIDR removal in a 5-d CIDR-Synch protocol likely occurred because heifers were inseminated too early relative ovulation and sexed semen has a shorter lifespan in vivo than conventional semen.[103]

Days to first AI (1.7 vs. 10.7 d, respectively) and pregnancy (18.3 vs. 27.4 d, respectively) were fewer for CIDR5 than EDAI heifers.[58] Overall, CIDR5 heifers tended to have more P/AI 64 d after AI than EDAI heifers (51.8 vs. 44.9%, respectively).[58] Table 2 contains a partial budget using actual farm costs for hormonal treatments, detection of estrus, semen and AI, pregnancy diagnosis, and feed to calculate the cost per pregnancy (U.S.$) based on the reproductive management strategy.[58] As expected,

Table 2: Partial budget for the cost per pregnancy (US$) based on the reproductive management strategy of nulliparous Holstein heifers for first insemination with sexed semen (adapted from Lauber et al., 2021).[58]

Actual Farm Costs[2] (US$)	Treatment[1]			*P*-value
	EDAI	CIDR5	CIDR6	
No. of heifers	181	225	218	
Hormonal treatment	4.05 ± 0.38^a	22.29 ± 0.36^b	21.85 ± 0.36^b	< 0.01
Detection of estrus	3.04 ± 0.19^a	2.03 ± 0.18^b	2.18 ± 0.17^b	< 0.01
Semen and AI	70.50 ± 2.47	69.78 ± 2.37	72.02 ± 2.28	0.39
Pregnancy diagnosis	9.55 ± 0.24	9.50 ± 0.14	9.42 ± 0.13	0.42
Feed	82.79 ± 3.01^a	50.10 ± 2.73^b	56.84 ± 2.56^b	< 0.01
Total per pregnancy	169.92 ± 5.55^a	153.26 ± 5.36^b	162.75 ± 5.03^{ab}	0.04

a-c Within a row, costs with different superscript lowercase letters differ ($P < 0.05$).

1 Nulliparous Holstein heifers were submitted for first insemination with sexed semen to either $PGF_{2\alpha}$ treatment with once-daily detection of estrus and AI (EDAI) or a CIDR-Synch protocol with CIDR exposure for 5 (CIDR5) or 6 d (CIDR6).

2 Actual farm costs for each input. Costs were calculated per individual pregnant heifer with additional feed cost per nonpregnant heifer during the 84-d breeding period divided among pregnant heifers.

submitting heifers to a 5-d CIDR-Synch protocol had increased hormonal treatment costs, but hormonal treatment costs were offset by decreased feed costs from fewer days on feed (Table 2).[58] The total cost per pregnancy was $16.66 less for heifers submitted to a 5-d CIDR-Synch protocol for TAI than for heifers inseminated after a detected estrus with sexed semen.[58]

Feed accounts for 46 to 54% of the total rearing costs of a heifer from birth until calving.[2,54] We conducted a sensitivity analysis to determine the effect of varying feed costs on the total costs per pregnancy to reflect geographical and market condition differences.[58] Five scenarios with feed costs of $1.25, $1.50, $1.75, $2.00 and $2.25 per heifer/d were created with all other costs held constant.[58] Feed costs for nonpregnant heifers, heifers labeled as "do not breed", or heifers that were moved to a bull pen during the 84-d breeding period were calculated and allocated to the feed costs for heifers that became pregnant during the 84-d breeding period.[58] Table 3 summarizes the cost per pregnancy under these 5 feed cost scenarios for the reproductive management strategies for first insemination with sexed semen.[58] When feed costs were ≥ $1.50 per heifer/d it tended or was more economical to submit heifers to a 5-d CIDR-Synch protocol for TAI than inseminate heifers after a detected estrus.[58] When feed costs were ≥ $1.50 per heifer/d, CIDR5 heifers tended and had a decreased cost per pregnancy of $12.81, $17.62, $22.43 and $27.24 compared with EDAI heifers.[58] Thus, submitting heifers to a 5-d CIDR-Synch protocol for TAI with sexed semen is an efficient and economical reproductive management strategy for heifers despite the limitation of early expression of estrus before TAI.

Hierarchy of reproductive needs and future frontiers

High 21-d pregnancy risk

Dr. Abraham Maslow proposed a theory of human motivation that a hierarchy (Maslow's Hierarchy of Needs) exists in which specific needs, such as physiological and safety, require fulfillment before a new need can emerge, such as belonging and self-esteem, for a person to self-actualize to reach their potential.[76] The point of the hierarchy is that you begin at the bottom and must achieve each stage before you progress to the next as you progress to the top; there is no skipping stages on your way to self-actualization. We believe that the reproductive management of dairy farms functions similarly to Maslow's Hierarchy of Needs. Figure 1 depicts our hierarchy of reproductive needs that require fulfillment for a dairy farm to reach its full potential. A high 21-d pregnancy rate of lactating dairy cows and heifers serves as the foundation for dairy farm profitability and is the first need a dairy farm is required to fulfill before new needs such as heifer management, genomic selection, sexed and beef semen, and in vitro produced (IVP) embryos can emerge and be fulfilled. We will discuss the benefits and challenges associated with these emerging needs for dairy farms and current knowledge gaps that could prevent dairy farms from self-actualization.

Heifer management: insemination eligibility and mature body size

From 2010 to 2021, age at first calving (AFC) decreased by 1.0 mo in U.S. Holsteins[19] and depicts the current trend in the dairy industry to achieve an earlier age at conception to decrease rearing costs to generate income from milk production sooner. Further, the U.S. heifer inventory decreased by 15% from 2016 to 2022[114] likely because more dairy farms are using a sexed and beef semen insemination strategy[62] to regulate herd inventory and are creating more genetically elite but fewer replacement heifers. This strategy decreases rearing costs but may not consider growth benchmarks for mature body size (MBS) of heifers to achieve their genetic potential for future milk production. Mature body size is defined as the mature body weight (MBW) and mature height (MH) of 3rd+ lactation cows in a herd measured at a consistent DIM. Current benchmarks for heifer growth are 55% MBW and 90% MH at first insemination, 94% MBW and 95% MH immediately pre-calving, and 85% MBW and 95% MH post-calving.[120,118,45] Only 36% of U.S. dairy heifer growers record body weight (BW) and average daily gain of heifers[84] despite the existence of MBS benchmarks and measurement tools. Increasing reproductive efficiency decreased AFC from 25 to 21 mo and decreased rearing costs by 18% under

Table 3: Sensitivity analysis of varying feed costs per heifer/d on the total cost per pregnancy (US$) of nulliparous Holstein heifers based on reproductive management strategy for first insemination with sexed semen (adapted from Lauber et al., 2021).[58]

Feed cost[2] (US$ per heifer/d)	Treatment[1]			*P*-value
	EDAI	CIDR5	CIDR6	
$1.25	147.97 ± 4.81	139.99 ± 4.65	147.70 ± 4.37	0.17
$1.50	160.17 ± 5.21[A]	147.36 ± 5.05[B]	156.07 ± 4.74[A]	0.08
$1.75	172.35 ± 5.63[a]	154.73 ± 5.44[b]	164.43 ± 5.11[a]	0.03
$2.00	184.53 ± 6.06[a]	162.10 ± 5.84[b]	172.78 ± 5.47[c]	0.01
$2.25	196.70 ± 6.48[a]	169.46 ± 6.23[b]	181.14 ± 5.84[c]	0.004

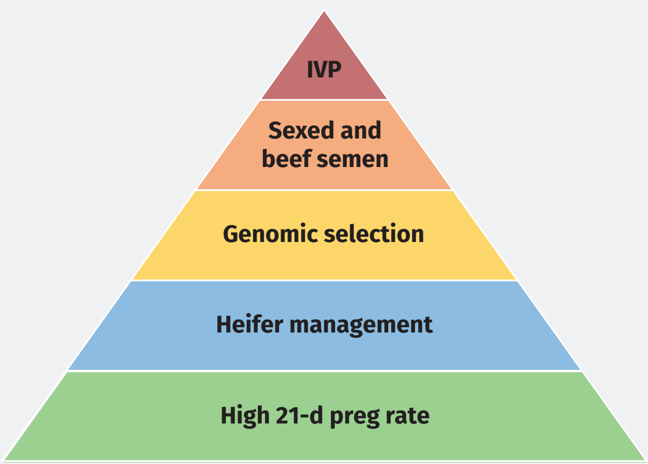
[a-c] Within a row, costs with different superscript lowercase letters differ ($P < 0.05$).

[A-B] Within a row, costs with different superscript capital letters differ ($P < 0.10$).

[1] Nulliparous Holstein heifers were submitted for first insemination with sexed semen to either $PGF_{2\alpha}$ treatment with once-daily detection of estrus and AI (EDAI) or a CIDR-Synch protocol with CIDR exposure for 5 (CIDR5) or 6 d (CIDR6).

[2] Varying feed costs (US$ per heifer/d) were evaluated with all other input costs from the partial budget held constant to determine the effect on the cost per pregnancy. For each scenario, feed costs were calculated per individual pregnant heifer with the additional feed cost per nonpregnant heifer during the 84-d breeding period divided among pregnant heife

Figure 1: Hierarchy of reproductive needs dairy farms must fulfill before a new need can emerge. A high 21 d pregnancy rate is the foundational need required to be fulfilled before other needs can be addressed.



the assumption of adequate growth relative to MBS.[113] Few U.S. dairy farmers measure heifer growth, and likely, the adoption of an earlier age at conception was often done without regard to MBS benchmarks and could limit heifers from achieving their genetic potential for milk production.

In a retrospective cohort study from our laboratory,[60] we evaluated the association between insemination eligibility and reproductive performance of heifers BW at 30 DIM and milk production during wk 4, 8 and 12 of lactation of primiparous cows in a commercial dairy farm. Nulliparous heifers (n = 1,849) were eligible for first insemination solely based on age (380 d) and were inseminated based on detection of estrus with sexed semen.[60] We extracted the incidence of bovine respiratory disease (BRD), parent average predicted transmitting abilities (PTA) values, P/AI, BW at 30 DIM, and primiparous milk production (wk 4, 8, 12) from the herd management software for each heifer.[60] Only heifers with a normal gestation length (250 to 300 d) were included. The mean MBW of the herd was 1,510 lb and was estimated by weighing a random subset of 3rd and 4th lactation cows (n = 75) at 30 to 40 DIM.[60] We created quartiles based on the BW at 30 DIM and %MBW as primiparous cows as follows: Q1 (lightest; n = 462), Q2 (light-moderate; n = 456), Q3 (moderate; n = 472), and Q4 (heaviest; n = 459).[60]

Table 4 describes the BW at 30 DIM, % MBW, AFC, and PTA values of each quartile.[60] By design, mean BW at 30 DIM and %MBW increased by quartile, but only Q3 and Q4 cows achieved the industry benchmark of ≥ 85% MBW post-calving.[120] Overall, Q4 cows had greater genetic potential for milk production than Q1 and Q3 cows, but not Q2 cows.[60] The PTA values for fertility traits (DPR and HCR) and productive life differed among quartiles, with Q1 cows having greater genetic potential for fertility and productive life than Q4 cows.[60] Body weight quartile was associated with P/AI at first service as nulliparous heifers with Q1 cows having approximately 9 to 26 percentage points more P/AI at first insemination as heifers than Q2, Q3, and Q4 cows (Figure 2).[60] By conceiving and calving approximately 20 d earlier than Q4 cows, Q1 cows had fewer days on feed and thus a shorter growth period as heifers before beginning the first lactation to achieve ≥ 85% MBW post-calving.[60] Thus, the separation of heifers into these BW quartiles in this analysis was primarily because of differences in genetic merit for fertility traits among heifers.

We found no association of BRD incidence with milk production at wk 4, 8 and 12 of lactation for primiparous cows that were healthy or ≥ 1 incidence of BRD as heifers.[60] Incidence of BRD likely affects primiparous milk production indirectly through decreased growth during the rearing period and could be underestimated because of survivorship bias.[109,1,49] We found a positive association between BW quartile and primiparous milk production at wk 4, 8 and 12 of lactation with Q4 cows yielding approximately 5 kg per cow/d more milk than Q1 cows (Figure 3).[60] Based on a shortened growth period to achieve 85% MBW, Q1 cows were unable to achieve their genetic potential for milk production because energy was likely partitioned to growth rather than to lactation. We concluded that the insemination eligibility of heifers should be defined by age and 55% MBW to maximize genetic potential for future milk production. Thus, once a heifer is of appropriate age and 55% MBW, she can be submitted to an aggressive reproductive management strategy such as a 5-d CIDR-Synch protocol to decrease days on feed and the cost per pregnancy without sacrificing future milk production. In the future, automatic monitoring through computer vision systems could be a more efficient method to measure BW, hip height, and body condition score of heifers[125,31] to create individualized growth benchmarks. Randomized-controlled studies are needed to further understand the effects of insemination eligibility and reproductive performance, calfhood disease incidence, and genetic potential on heifer growth, survivability, and subsequent milk production.

Heifer management: hormonal synchronization strategies

The protocols for presynchronization and synchronization of nulliparous heifers for TAI are limited compared with lactating dairy cows. For many years, research primarily focused on the reproductive physiology of lactating dairy cows because their reproductive performance was poor compared with heifers. Now, this has shifted as a greater proportion of heifers are inseminated with sexed semen, and dairy farmers are realizing the costs of more days on feed. The fertility of sexed semen is 70% relative to conventional semen in heifers[20] and is less than the 85% relative to fertility in lactating dairy cows.[24] In studies from our laboratory,[58,59] the P/AI of nulliparous heifers inseminated with sexed semen after a detected estrus (44.9%) or a 5-d CIDR-Synch protocol (51.8%) are similar to lactating cows inseminated with sexed semen after a detected estrus (43.8%) or a Double-Ovsynch protocol (49.2%). The 5-d CIDR-Synch protocol does not function as a high-fertility program as a Double-Ovsynch protocol functions in lactating dairy cows because only the service rate is increased, but not P/AI with conventional semen. Thus, more randomized controlled experiments are needed to further understand the endocrinology and physiology of heifers to develop more effective synchronized ovulation protocols.

Recently, more randomized-controlled studies have focused on implementing presynchronization strategies and modifying the 5-d CIDR-Synch protocol to optimize synchronization of ovulation in heifers. Presynchronization of heifers with a $PGF_{2\alpha}$ treatment 2 d before a 5-d CIDR-Synch protocol decreased the proportion of heifers ovulating before TAI (0 vs. 23.8%, respectively), increased the ovulatory response to the first GnRH treatment (86.3 vs. 19.0%, respectively) and P/AI (67.4 vs. 54.7%, respectively) with conventional semen than heifers not presynchronized.[53] Leão et al. (2022) presynchronized Holstein heifers with a $PGF_{2\alpha}$ treatment 2 d before GnRH treatment and randomized heifers to a 5-d or 6-d protocol without a CIDR.[63] Interestingly, the proportion of heifers with a functional corpus luteum at the first $PGF_{2\alpha}$ treatment, complete luteolysis, and ovulatory response after $PGF_{2\alpha}$ treatment did not differ, but heifers without a CIDR had a greater ovulatory follicle size than heifers with a CIDR.[63]

Table 4: Mean (± SEM) body weight (BW; lb) at 30 DIM, mature body weight (MBW; %), age at first calving (AFC; d), and parent average predicted transmitting ability (PTA) values of primiparous Holstein cows based on weight quartiles (Q1-Q4) at 30 DIM (adapted from Lauber and Fricke, 2023).[60]

	BW quartile			
	Q1	Q2	Q3	Q4
Item	n = 462	n = 456	n = 472	n = 459
BW at 30 DIM (lb)/(kg)	(1,127.3^a ± 1.78) (512.4^a ± 0.81)	(1,215.7^b ± 1.80) (552.6^b ± 0.82)	(1,283.3^c ± 1.76) (583.3^c ± 0.80)	(1,387.5^d ± 1.78) (630.7^d ± 0.81)
MBW[1] (%)	74.7^a ± 0.001	80.5^b ± 0.001	85.0^c ± 0.001	91.9^d ± 0.001
AFC (d)	674.6^a ± 1.25	681.8^b ± 1.25	688.2^c ± 1.24	694.6^d ± 1.25
PTA[2]				
Milk (kg)	173.1^b ± 9.75	188.6^{ab} ± 9.83	179.2^b ± 9.67	215.0^a ± 9.79
Fat (kg)	12.8^b ± 0.27	13.3^b ± 0.27	13.1^b ± 0.26	14.4^a ± 0.27
Protein (kg)	7.7^b ± 0.24	7.9^b ± 0.24	7.9^b ± 0.24	9.1^a ± 0.24
Stature	-0.56^c ± 0.03	-0.52^{bc} ± 0.03	-0.46^b ± 0.03	-0.29^a ± 0.03
Feed Saved (kg)	31.9^a ± 2.0	24.6^b ± 2.0	13.4^c ± 2.0	5.7^d ± 2.0
Net Merit $ (NM$)	274.7^A ± 3.2	272.7^{AB} ± 3.2	263.4^B ± 3.1	270.4^{AB} ± 3.2
Productive Life (PL)	2.4^a ± 0.04	2.2^{bA} ± 0.04	2.1^{bcB} ± 0.04	1.9^d ± 0.04
Daughter Pregnancy Rate (DPR)	0.37^a ± 0.05	0.27^{abA} ± 0.05	0.26^{ab} ± 0.05	0.11^{bB} ± 0.05
Heifer Conception Rate (HCR)	0.03^a ± 0.04	0.0^a ± 0.04	-0.08^{ab} ± 0.04	-0.16^b ± 0.04

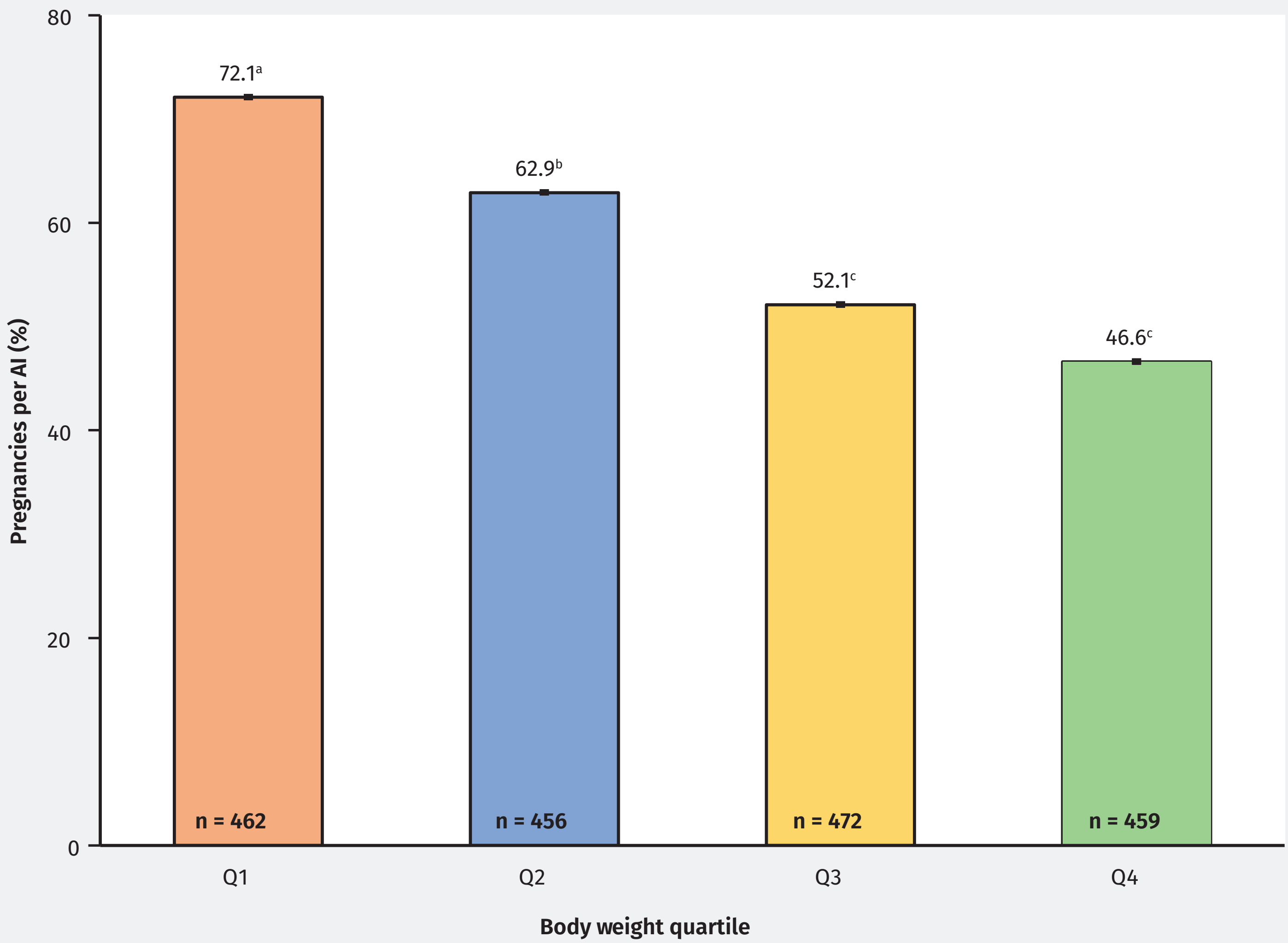
[a-d] Within a row, means with different lowercase superscripts differ ($P \leq 0.05$).

[A-B] Within a row, means with different uppercase superscripts tended to differ ($0.05 < P \leq 0.10$).

1 Percent mature body weight (%MBW) was calculated as the recorded weight of primiparous cows at 30 DIM divided by the MBW of the herd of 1,510 lb determined by the mean weight of a random sample of 3rd and 4th lactation cows (n = 75) at 30 to 40 DIM.

2 Parent average predicted transmitting abilities (PTA) estimated from DairyComp 305 software.

Figure 2: Pregnancies per AI (P/AI; %) at first insemination as heifers after detection of estrus and AI with sexed semen by BW quartile (Q1-Q4) at 30 DIM as primiparous cows. Percentages with different lowercase superscripts differed ($P \le 0.05$; adapted from Lauber and Fricke, 2023).[60]



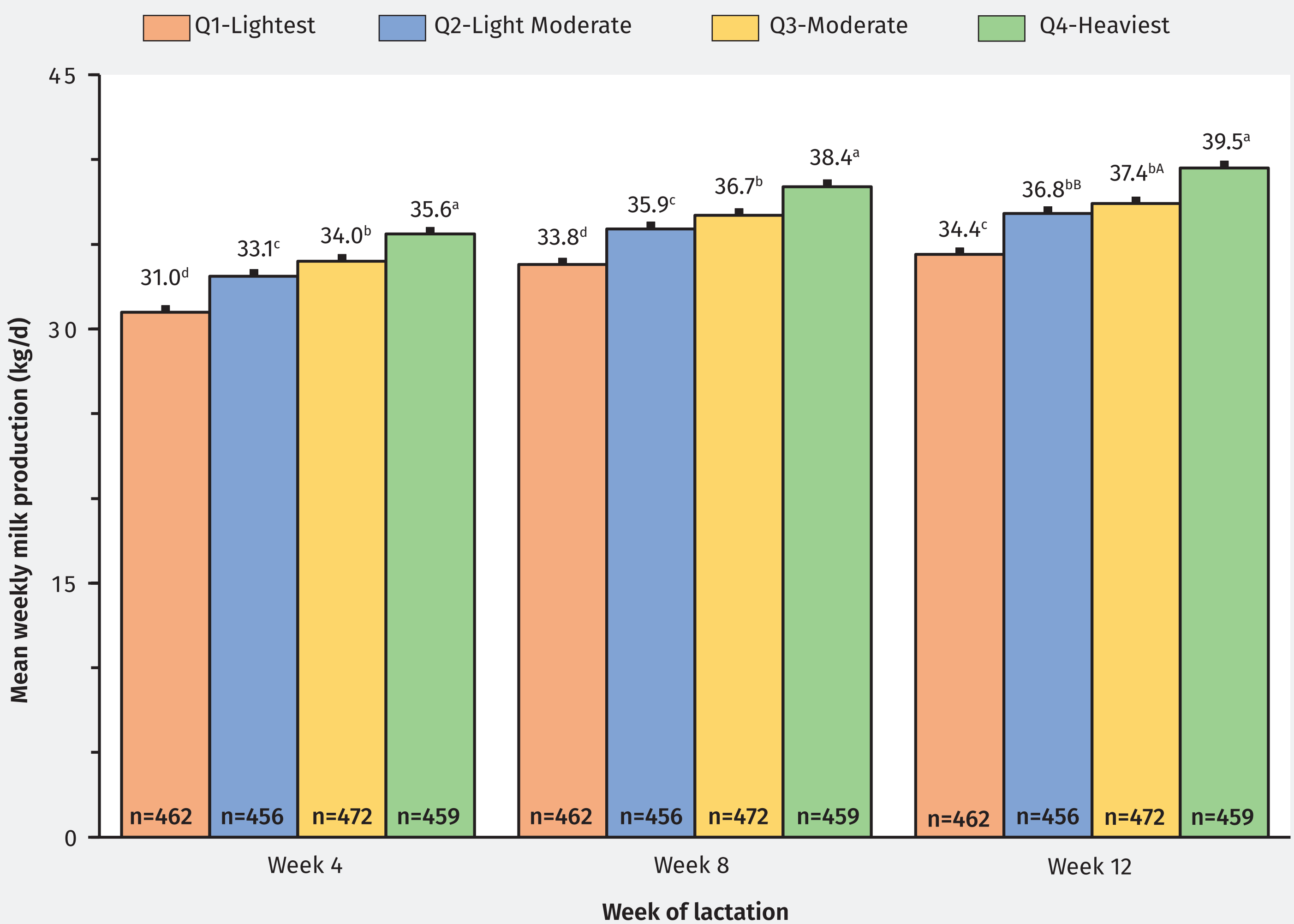
As previously described, our laboratory explored using a 6-d CIDR-Synch protocol to prevent early expression of estrus while maintaining P/AI. By contrast, we observed that heifers submitted to a 6-d CIDR-Synch protocol had fewer P/AI with sexed semen likely because heifers were inseminated too early relative to ovulation.[58] Moore et al. (2023) evaluated the effect of delaying TAI by 8 h with sexed semen in a 6-d progesterone-releasing intravaginal device (PRID)-Synch protocol to optimize TAI relative to ovulation.[81] Nulliparous heifers (n = 823) across 7 Irish dairy farms were submitted to a 6-d PRID-Synch protocol and randomized for TAI with sexed semen concurrent with the last GnRH treatment or 8 h later.[81] Delaying insemination with sexed semen by 8 h, approximately 20 h before expected ovulation, had 9 percentage points more P/AI than standard TAI (59.1 vs. 50.4%, respectively).[81] Thus, advancements in the reproductive management of heifers will rely on more randomized-controlled studies in collaboration with dairy farms, bovine practitioners, and industry.

Genomic selection

A high 21-d pregnancy rate and successful heifer management strategy allow dairy farms to produce enough replacements to implement genomic selection. Selection of heifers after weaning based on parent average PTA values and disease incidence can lessen the loss compared with removing heifers later in life when it would be more beneficial to have a phenotype of milk production to make selection decisions.[86] Despite the upfront costs of genotyping, the genetic gains in lifetime net merit in most cases from genomically testing are greater than parent averages.[126] The reliability of traits is greater using genomic testing compared with parent averages, even with low heritable traits such as daughter pregnancy rate (DPR) with a 17-percentage point gain in reliability than parent average.[127] Genomic selection from its adoption has decreased the generation interval and increased the annual genetic gain for low heritable traits such as DPR, somatic cell count, and productive life by 3 to 4 fold.[37]

Several studies have demonstrated the positive effects of greater genetic merit for fertility traits. Parent-average[57] and genomic merit[122] for DPR were positively associated with P/AI at first insemination in nulliparous heifers. Further, genomic merit for heifer conception rate (HCR) and DPR tends to be positively associated with the hazard of both pregnancy and estrus, respectively, in nulliparous heifers.[122] Physiologically, heifers genomically selected for greater DPR have larger follicles and greater steroidogenesis, whereas heifers selected for greater HCR had smaller follicles but greater concentrations

Figure 3: Mean (± SEM) weekly milk production (kg/d) at wk 4, 8 and 12 of lactation of primiparous Holstein cows by BW quartile at 30 DIM (Q1-Q4). Means with different lowercase letters differed ($P \leq 0.05$). Means with different uppercase letters tended to differ ($0.05 < P \leq 0.10$; adapted from Lauber and Fricke, 2023).[60]



of insulin-like growth factor 1.[123] In lactating Holstein cows, a positive association exists between genomic DPR and P/AI at first service, number of services per conception, days to first service, and days open.[66] Interestingly, there is a negative correlation between genomic DPR and HCR with genomic milk yield in Holstein heifers.[122] Lima et al. (2020) reported only an interaction between genomic DPR and milk production services per conception in primiparous cows, with high-producing primiparous Holstein cows having fewer services per conception as genomic DPR increased, but there was no relationship for low-producing cows.[66] A challenge that may exist to further propel genetic gain for fertility traits is this antagonistic relationship with milk production and that many dairy farmers remove cows from the herd primarily on milk production.

Genomic selection, when paired with a sexed and beef semen insemination strategy to increase dam selection intensity, could increase genetic gain and dairy farm profitability.[119] Genomic traits follow a normal distribution, and as parity increases, the normal distribution generally shifts to the left, thereby indicating lesser genetic merit on average. If a dairy farm solely selects heifers and cows for specific semen types based on parity, some genetically inferior heifers would be inseminated with sexed semen while some genetically superior cows would be inseminated with conventional or beef semen. Thus, to maximize genetic gain and profit, dairy farms should genomically test their herd to accurately identify herdmates that should be retained and produce the next generation of replacements.

Sexed and beef semen

Dairy farms that fulfill their needs for a high 21-d pregnancy rate, excellent heifer management, and genomic selection can address their next need of adopting a sexed and beef semen insemination strategy to regulate herd inventory. Implementing a sexed and beef semen insemination strategy allows a dairy farm to precisely manage herd inventory to create essential, genetically elite replacements with sexed semen and increased market value for non-replacements as beef x dairy crossbred calves. Poor reproductive performance (~15% 21-d pregnancy rate) dairy farms seldom use sexed and beef semen insemination strategies because insufficient replacements are produced with a positive income from calves over semen costs (ICOSC).[15] By contrast, herds with average or excellent reproductive performance (~20 to >30% 21-d pregnancy rates) can implement sexed and beef semen insemination strategies because sufficient production of replacements and positive ICOSC.[15] The use of genomic reliabilities in economic modeling found the optimal insemination strategy using a combination of sexed, conventional and beef semen.[23] Only insemination with conventional semen reduced profit by $63/cow per year.[23]

We characterized semen type prevalence and allocation to inseminate U.S. Holstein females by year, parity, service number and herd size.[62] The final data set included 8,244,653 total inseminations of 4,880,752 Holstein females across 9,155 herds from October 2019 to July 2021.[62] Semen types were categorized based on the National Association of Animal Breeder uniform code as beef, conventional Holstein, sexed Holstein or other dairy.[62] The top 4 beef breeds used to produce beef x Holstein crossbred calves were Angus (55.1%), Limousin (13.9%), Simmental (11.7%), and Crossbreed Beef (11.3%).[62] From 2019 to 2021, the use of sexed semen to inseminate Holstein females increased from 11.0 to 17.7%, and the use of beef semen to inseminate Holstein females increased from 18.2 to 26.1%.[62]

Figure 4 depicts the relative frequency of beef, conventional Holstein, sexed Holstein and other dairy semen inseminations of U.S. Holstein females (nulliparous to multiparous) by service number (1 to 3 +) from 2019 to 2021.[62] The use of beef semen to inseminate Holstein females increased with increasing parity and service number, whereas the use of sexed semen decreased with increasing parity and service number supporting that farmers used sexed semen more aggressively in higher fertility and younger females with greater genetic merit.[62] Figure 5 illustrates the relative frequency of beef, conventional Holstein, sexed Holstein, and other dairy semen inseminations of U.S. Holstein females (nulliparous to multiparous) by herd size (< 100 cows to ≥ 1,000 cows) from 2019 to 2021.[62] Interestingly, the increase in sexed and beef semen inseminations was driven primarily by larger herds.[62] The greater use of sexed and beef semen by larger dairy herds may be due to increased reproductive performance and economies of scale.[62] The average 21-d pregnancy rate in Holstein herds increases with increasing herd size (< 100 cows, 13.7%; 100 to 250 cows, 18.2%; 250 to 500 cows, 22.7%; 500 to 1,000 cows, 24.8%; and > 1,000 cows, 28.0%).[116]

Despite increased adoption and selective use of sexed semen, insemination of heifers and lactating dairy cows with sexed semen only yields approximately 70% and 80% relative fertility compared with conventional dairy semen, respectively.[20,27] Several studies have investigated strategies to increase fertility with sexed semen such as increasing sperm concentration per straw and delaying timing of insemination. Increasing sperm concentration per sexed semen straw does not compensate for decreased in vivo fertility.[24,25,26] Delaying timing of insemination with sexed semen for lactating dairy cows[27] and nulliparous heifers[20] did not increase P/AI compared with standard timing of insemination. Earlier induction of ovulation to inseminate primiparous cows closer to presumed ovulation in a Double-Ovsynch protocol decreased P/AI by 7-percentage points than standard induction ovulation.[61] Currently, the best reproductive management strategies to optimize fertility with sexed semen in lactating dairy cows and heifers is a Double Ovsynch[59] and 5-d CIDR-Synch protocol,[58] respectively. Sexed semen inseminations in vitro decreased the cumulative embryonic cleavage[7] and blastocyst rates[7,128,72] indicating altered embryo developmental kinetics and fewer transferable embryos compared with conventional semen. Thus, more randomized controlled studies are needed to understand the effect of semen processing on in vivo and in vitro fertility to maximize the benefits of a sexed and beef semen insemination strategy.

IVP embryos

At the top of the hierarchy of reproductive needs is the transfer of IVP embryos. From 2020 to 2021, the transfer IVP embryos increased by 32.8%.[50] The transfer of IVP embryos traditionally occurred to further genetic gains by creating genetically elite replacements from an elite donor. In addition, abattoir-derived ovaries of beef cattle are being used to harvest oocytes and produce purebred beef IVP embryos for transfer into lactating dairy cows. Particularly for lactating Jersey cows in which the beef x Jersey crossbred calves have less market acceptance because of more days on feed, poorer primal cut yields, and more yellow-colored fat than beef x Holstein crossbred calves.[8] Purebred beef calves from IVP embryos had more efficient feedlot and carcass performance than Angus x Jersey crossbred calves calved by Jersey cows.[34]

Crowe et al. (2023) randomized lactating dairy cows for submission to an 8-d PRID-Synch protocol for TAI with dairy or beef semen or transfer of a fresh or frozen, dairy or beef IVP embryos.[22] Pregnancies were similar for cows inseminated with dairy or beef semen and recipient cows of a dairy or beef IVP embryo.[22] The transfer of fresh dairy or beef IVP embryos had more pregnancies at d 32 than frozen embryos (56.1 vs. 41.6%, respectively).[22] By contrast, the pregnancy loss between d 32 and 62 after insemination was greater for IVP dairy and beef embryos than TAI with dairy or beef semen (15.1 vs. 4.7%, respectively), with the greatest losses observed for frozen beef, fresh beef and frozen dairy compared with fresh dairy IVP embryos (18.5 vs. 17.3 vs. 19.2 vs 6.0%, respectively).[22]

Increased pregnancy losses of IVP embryos compared with AI[22]and *in vivo*-derived embryos,[43] in addition to cost,[52] is one of the greatest challenges for the adoption of IVP embryos by dairy farms. One management strategy proposed is to increase P4 concentration with treatment with GnRH or human chorionic gonadotropin (hCG) to increase conceptus elongation, thereby pregnancy establishment and decreasing pregnancy loss. Holstein and Holstein-crossbred heifer recipients of a fresh, IVP expanded blastocysts treated with GnRH 5 d after ovulation had similar pregnancies per ET (P/ET; 35.7 vs. 33.5%, respectively), but decreased pregnancy loss compared with untreated controls (15.2 vs. 27.1%, respectively).[36] Niles et al. (2019) randomized Holstein heifers (n = 291) to receive no treatment or 2,000 IU of hCG at the time of embryo transfer (ET) of a fresh IVP embryo after submission to a 5-d CIDR-Synch protocol.[85] The P/ET did not differ based on treatment with or without hCG, but decreased pregnancy loss for heifers treated with hCG (10% vs. 22.0%; respectively).[85] By contrast, El Azzi et al. (2023) reported inconsistent effects of hCG or GnRH treatment of heifers and lactating dairy cows at ET on P/ET.[29]

In a preliminary experiment from our laboratory,[47] we investigated the effect of recipient synchronization type and the effect of treatment with hCG at ET on pregnancy outcomes. Multiparous Jersey cows (n = 349) were randomly assigned to be submitted to a protocol to synchronize estrus or a Double-Ovsynch protocol[102,59] with or without treatment with 2,500 IU of hCG at the time ET. All cows with a CL ≥ 13 mm received a frozen, grade 1, stage 7 IVP embryo produced from abattoir-derived ovaries from commercial black Angus-like dams and 3 Angus sires selected for calving ease.[47] Embryo transfers only occurred for synchronized estrus cows detected in estrus within 3 to 4 d after the last $PGF_{2\alpha}$ because of the time availability of the bovine practitioner to transfer the embryos.[47] With only approximately 75% of cows detected in estrus and because transfers occurred only 2 days of

Figure 4: Relative frequency of beef, conventional Holstein, sexed Holstein, and other dairy semen inseminations of U.S. Holstein females (nulliparous to multiparous) by service number (1 to 3+) from 2019 to 2021 (adapted from Lauber et al., 2023).[62]

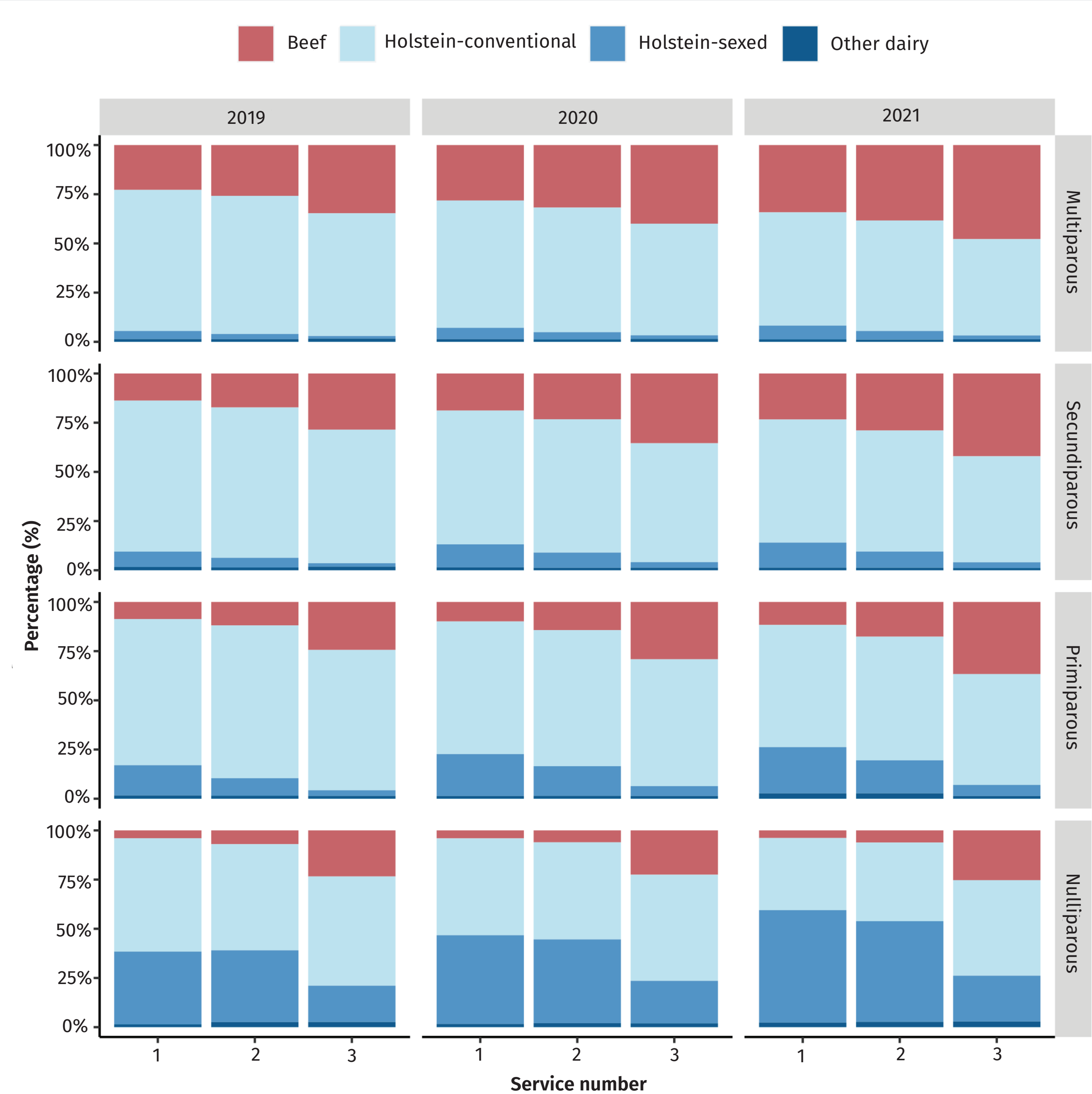
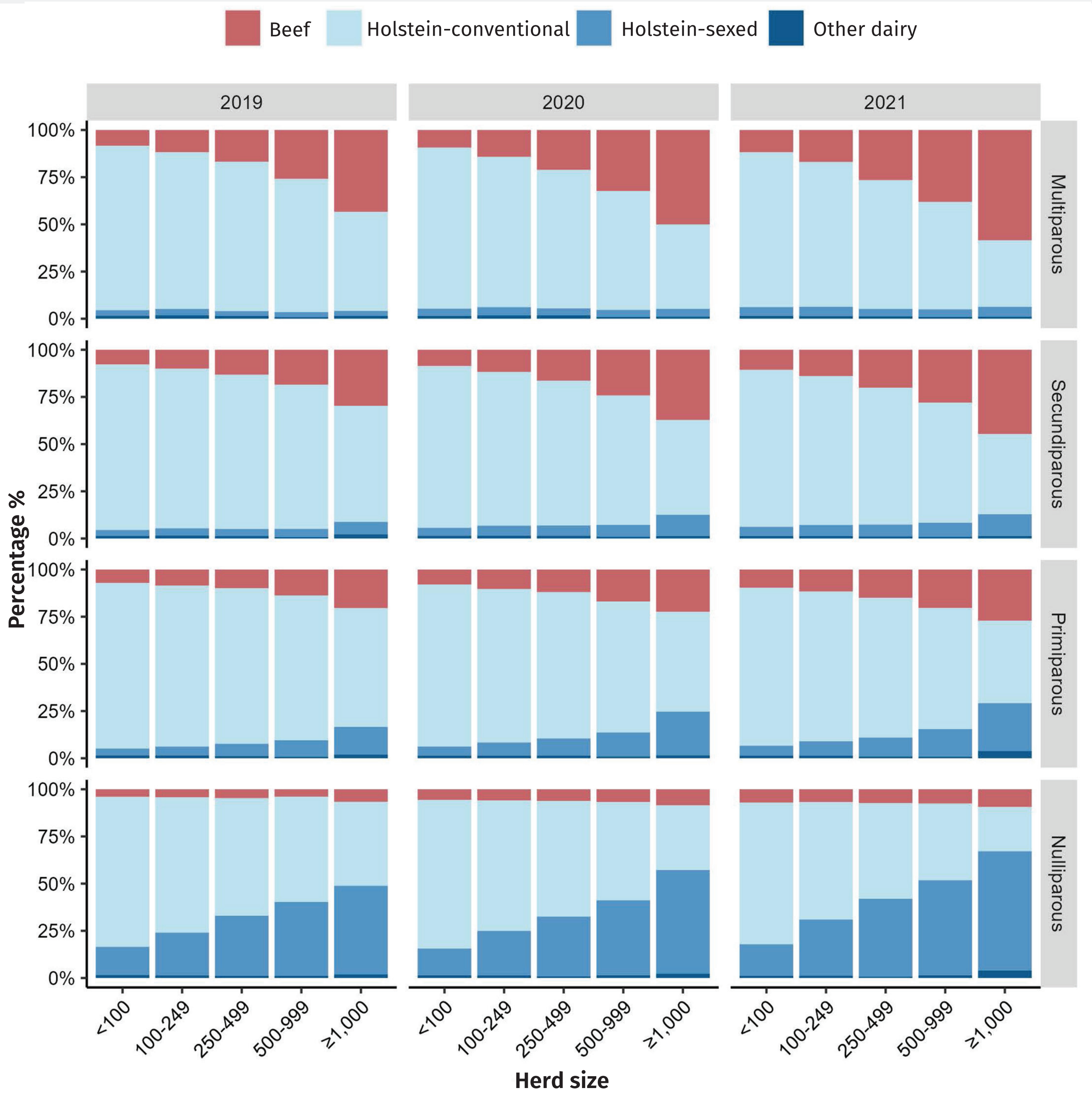


Figure 5: Relative frequency of beef, conventional Holstein, sexed Holstein, and other dairy semen inseminations of U.S. Holstein females (nulliparous to multiparous) by herd size (< 100 cows to ≥ 1,000 cows) from 2019 to 2021 (adapted from Lauber et al., 2023).[62]



the week, the recipient utilization rate for cows submitted to a synchronized estrus was less than cows submitted to a Double-Ovsynch protocol (50.0 vs. 93.0%).[47] Cows submitted to a synchronized estrus for ET had a greater cost per pregnancy of $150.12 than cows submitted to Double-Ovsynch protocol ($461.50 vs. $311.38, respectively).[47] This dairy farm received $400 per live calf; thus the only profitable recipient synchronization strategy was the Double-Ovsynch protocol. The effect of hCG treatment was investigated in a follow-up experiment with the same dairy farm and embryos using the Double-Ovsynch protocol because of the decreased recipient utilization and increased cost per pregnancy of cows submitted to a synchronized estrus.

In the follow-up experiment, lactating multiparous Jersey cows (n = 368) were submitted to a Double-Ovsynch protocol and randomized to be untreated controls (n = 192) or treated with 2,500 IU of hCG at ET (n = 194).[47] Treatment of cows with hCG at ET increased P4 concentrations and total luteal volume 7 d after ET than untreated controls.[47] Interestingly, P/ET at d 26, d 33 and d 61 and the pregnancy loss from d 26 to 33, d 33 to 61 and d 26 to 61 did not differ between treatments (Table 5).[47] In a previous experiment with this dairy farm, lactating multiparous cows submitted to a Double-Ovsynch protocol and inseminated with beef semen had 58.0% P/AI 61 d after AI.[59] Thus, a strategy to implement the transfer of IVP embryos could be after a resynchronization protocol to maximize fertility to first insemination and increase the value of replacements or nonreplacements by transferring IVP embryos. More randomized-controlled studies are needed to understand IVP embryo development and strategies to decrease pregnancy losses for increased adoption by dairy farms.

Conclusion

The increased reproductive performance of lactating dairy cows over the past 2 decades is due to improvements in periparturient management and the adoption of fertility programs. The Ovsynch protocol increased the service rate, but not P/AI compared with detection of estrus. Fertility programs (the Presynch-Ovsynch and Double Ovsynch protocols for TAI) that increase the service rate and P/AI compared with detection of estrus evolved from randomized-controlled experiments to understand the endocrinology and physiology of lactating dairy cows. Submission of heifers to a synchronized ovulation protocol was previously associated with poor reproductive performance compared with detection of estrus. Randomized-controlled experiments generated several modifications creating the 5-d CIDR-Synch protocol that has similar and more P/AI with conventional and sexed semen, respectively, compared with detection of estrus. Dairy farms have a hierarchy of reproductive needs that require fulfillment before new needs emerge. A high 21-d pregnancy rate is the first need required to be fulfilled before dairy farms can adopt advanced reproductive technologies. Adopting these technologies provides benefits for dairy farms, but challenges with these technologies persist and need to be addressed in randomized-controlled studies to maximize these benefits.

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Table 5: Effect of human chorionic gonadotropin (hCG) treatment on pregnancies per embryo transfer (P/ET) and pregnancy loss for recipient lactating multiparous Jersey cows of frozen, grade 1, stage 7 in vitro produced beef embryos (adapted from Hincapie et al.,unpublished).[47]

	Treatment[1]		
Item	**Control**	**hCG**	***P*-value**
Pregnancies per ET (%)			
d 26[2]% (no./total)	47.9 (92/192)	48.7 (94/193)	0.87
d 33% (no./total)	36.4 (70/192)	38.6 (75/194)	0.65
d 61% (no./total)	33.3 (64/192)	34.5 (67/194)	0.80
Pregnancy Loss (%)			
d 26 - 33[2]% (no./total)	23.9 (22/92)	20.2 (19/94)	0.54
d 33 - 61% (no./total)	7.2 (5/69)	10.6 (8/75)	0.48
d 26 - 61% (no./total)	29.6 (27/91)	28.7 (27/94)	0.88

1 At embryo transfer (ET), 8 d after the last GnRH (G2), cows with a corpus luteum (CL) ≥ 13 mm were randomly assigned to be untreated controls (CON) or treated with 2,500 IU of hCG.

2 On d 26 after G2, P/ET were determined based on plasma pregnancy-specific protein B (PSPB) concentration with a threshold of 1.27 ng/mL indicating pregnancy.

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