1   **Dairy cattle fertility after 12 years of genomic selection: Lessons learned, current applications, and**

2   **future development**

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13   **TAKE HOME MESSAGE**

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15   • Genomic selection was a critical tool for stopping the decline in Holstein cow fertility, and the

16   fertility of the Holstein cow is now slowly but steadily improving.

17   • You can improve fertility in your herd by using bulls with high genomic breeding values for

18   fertility traits, and you will see those changes in one generation.

19   • The availability of low-cost, high-density genetic marker panels and whole-genome sequence

20   data has allowed us to identify genes associated with large effects on fertility, and to dissect the

21   biology of gametogenesis, fertilization, and development.

22   • Continued development of reproductive technologies such as sexed semen, oocyte pickup, and

23   embryo transfer has provided dairy farmers with new tools to increase rates of genetic gain.

24 • Other current and anticipated advances in reproductive management, including timed artificial

25 insemination and development of remote sensing technologies, will allow farmers to improve the

26 fertility of their herds and increase their profitability.

27

28 **WHAT WAS THE STATE OF DAIRY COW FERTILITY BEFORE GENOMICS?**

29

30 Before the introduction of genomic selection in 2009 (Wiggans et al., 2017), the U.S. Holstein breed had

31 experienced a prolonged decline in fertility due to the negative association with yield (Hansen, 2000).

32 Daughter pregnancy rate had decreased from a high of 39.5% in 1959 to a low of 24.1% in 2005 (Council

33 on Dairy Cattle Breeding, 2020). This represents an increase of 1.3 days open per year. The situation was

34 not acceptable to farmers, who tried many different strategies, such as advanced reproductive

35 technologies, crossbreeding, and switching breeds entirely. Fertility was first added to the Lifetime Net

36 Merit selection index in 2003 with a weight of 7% (VanRaden and Seykora, 2003). Today, the fertility

37 traits (daughter pregnancy rate, heifer conception rate, and cow conception rate) receive 10% of the

38 weight in the index (VanRaden et al., 2018).

39

40 A major challenge when trying to improve fertility through genetics is the question of how to measure it.

41 For genetic improvement, we want to record observations for as many animals as we can, as cheaply as

42 possible. The DPR evaluations are based on observations of days open, while heifer (**HCR**) and cow

43 conception rates (**CCR**) are based on breeding and pregnancy-check data. The low heritabilities of these

44 traits indicate that the environment plays a much bigger role in their expression than does genetics.

45 Among other things, each of these traits is affected by management decisions made by the farmer. Age at

46 first calving is also evaluated as early first calving (**EFC**), (Hutchison et al., 2017) but may be a more

47 general measure of the onset of puberty than fertility *per se*. This leaves us with an important question:

48 are our measures of fertility very good? Are we measuring things that have a strong correlation with

49 reproductive physiology, or are we simply measuring things that are easy to record?

HOW DID GENOMICS CHANGE THINGS?

The genetic trend for three fertility traits in U.S. Holsteins – daughter pregnancy rate and heifer and cow conception rates – are shown in Figure 1. The first animals to be genotyped were bulls. As the cost of testing decreased, many farmers began routinely testing their cows and there are now more than 3.7 million genotyped Holsteins in the National Cooperator Database maintained by the Council on Dairy Cattle Breeding (Bowie, MD). With the help of genomic selection, we are improving genetic merit for fertility faster than we were before that tool was available.

However, the rapid adoption of genomic selection has resulted in some big changes in genetic improvement programs. Perhaps the most notable of these is that the generation interval has decreased by almost half (García-Ruiz et al., 2016), which means that the rate of genetic gain has increased substantially. This is driven largely by a shift from predominant usage of 5-7 -year-old progeny-tested bulls in AI programs to usage of young bulls under 4 years of age (Hutchison et al., 2014). One consequence of these changes is that few bulls receive a conventional progeny test anymore. By the time second-crop daughters come in, a bull is 2-3 generations behind the young bulls in the population.

Some people are worried that the genetic evaluations are not as stable now as they used to be, meaning that bull evaluations can change a lot from one genetic evaluation run to another, but that is not the case. Early in the development of genomic selection, there were sometimes large changes from one run to another, but the statistical methodology is now mature and such changes are rarely seen. As a reminder, there were sometimes large changes in traditional (non-genomic) evaluations when, for example, lots of daughter records were added at one time to a bull's proof.

75   You might wonder if going faster is actually better – we went in the wrong direction for a long time, how

76   can we be sure that we are actually on the right path today? A recent study of 4,445 Holsteins from 4

77   commercial dairies showed that animals with genomic PTA for DPR in the top quartile had higher

78   pregnancy rates, lower days open, and fewer services to conception than cows with PTA in the bottom

79   quartile (Lima et al., 2020). Similarly, cows with PTA for DPR > 1 (n = 1,285) had higher pregnancy rate

80   at first service, fewer services per conception, and fewer days open that cows with a DPR $\leq$ -1 (n = 989)

81   (Ortega et al., 2017a). These results are important because they confirm that animals predicted to have

82   high fertility generally do, and animals predicted to perform poorly usually do.

83

84   **WHERE ARE WE TODAY?**

85

86   **Knowledge of the Molecular Genetics of Reproductive Biology is Advancing Rapidly**

87

88   Improved breeding values may be the most visible product of the genomic revolution in dairy cattle but

89   there is much more to the story than that. Over the last 12 years, we have dramatically improved our

90   understanding of the reproductive biology of the cow. With this knowledge has come identification of the

91   specific genes controlling reproductive processes. Candidate markers were identified and validated in

92   genes associated with reproductive processes (Cochran et al., 2013a; b), which resulted in the addition of

93   39 SNP to the genomic evaluation panel used by the Council on Dairy Cattle Breeding (**CDCB**; Bowie,

94   MD). Addition of these markers increased the accuracy of prediction by 0.20% (Ortega et al., 2016),

95   which may seem small, but the increase from 50 k to 777 k SNP genotypes increased accuracy by only

96   0.40% (VanRaden et al., 2013). This shows that a carefully targeted approach to identify new DNA

97   markers can be much more efficient than selection of markers without using functional information. A

98   single change in *COQ9* had favorable effects on cellular oxygen metabolism, body weight changes, and

99   ovarian function (Ortega et al., 2017). We also now know that the use of bulls only, cows only, or a mix

100   of the two in association studies identify different sets of genes that often do not overlap (Gaddis et al.,

101   2016). The use of embryo transfer continues to grow, and there is evidence that several aspects of that

102   process are under some degree of genetic control (Jaton et al., 2016; Parker Gaddis et al., 2017), although

103   genetic effects are very small for some traits.

104

105   As the example of *COQ9* illustrates, it is likely that much of the genetic variation affecting reproduction

106   is not caused by mutations affecting function or expression of genes that evolved specifically for a

107   function in the embryo or reproductive tissues, but rather that play a role in both reproductive and non-

108   reproductive cells alike. Embryo-lethal mutations in housekeeping genes, such as the mutation in *CWC15*

109   responsible for the JH1 fertility haplotype in Jerseys (Sonstegard et al., 2013), are a reminder that lethal

110   recessive alleles are an important cause of infertility and that inbreeding reduces reproductive function

111   (Martikainen et al., 2018).

112

113   **There is a Renewed Appreciation of Andrology**

114

115   After many years of dormancy, research into the reproductive biology of the male is accelerating. At one

116   level, the race for obtaining semen from the best bulls is increasing the pressure for early puberty in bulls.

117   In addition, however, there are opportunities for improving bull fertility through genetic selection,

118   management or post-harvest processing of semen. The use of sexed semen has become commonplace in

119   commercial dairy production (e.g., Burnell, 2019), but recent research suggests that sorted semen have

120   different motility profiles and reduced rates of embryonic development than conventional semen (Steele

121   et al., 2020). This is consistent with findings of Ortega et al. (2018) that bulls with high (favorable) sire

122   conception rates (**SCR**) produce more fertilized oocytes and fewer degenerate embryos than do low-SCR

123   bulls. Interactions between the sperm and the female reproductive tract are crucial for maintaining sperm

124   survival in the reproductive tract (Pollard et al., 1991; Kumaresan et al., 2019). The availability of

125   genotypes for virtually all bulls entering artificial insemination service is also being used to identify

126   causal variants for genetic differences in fertility among bulls (Hiltpold et al., 2020). Lastly, recent

127   advances in flow cytometry and machine learning may increase the accuracy of evaluating bull fertility

128   (Bucher et al., 2019).

129

130   **Dairy Farmers Have Rapidly Embraced New Reproductive Tools**

131

132   *Sexed Semen.* The rapid adoption of sexed semen as a common tool in reproductive management has

133   been driven by rapid improvements in the technology. While initially used principally on heifers due to

134   lower fertility of sexed semen (e.g., Norman et al., 2010), it is not commonly used on heifers and older

135   cows, and there is little difference in fertility between conventional and sexed semen. This has allowed

136   dairies to adopt more complex breeding strategies because they are assured of a surplus of heifer calves as

137   replacements.

138

139   *Beef-on-Dairy.* The increasing use of beef bulls on dairy cows to produce higher-valued calves for the

140   packing sector is not, strictly speaking, a fertility issue, but it is enabled by the surplus of heifer calves

141   resulting from widespread use of sexed semen. Sales of beef semen have increased rapidly in the US over

142   the last few years (Geiger, 2020; National Association of Animal Breeders, 2020), which can increase

143   farm profitability (Ettema et al., 2017) and is consistent with anecdotal reports of a shift in breeding

144   strategy.

145

146   *Who Benefits?* There is some evidence that farmers with larger herds are more likely to adopt new

147   technologies than those with smaller herds (Gargiulo et al., 2018), which appears to be driven largely by

148   labor costs on large farms. This leads one to wonder if new reproductive technologies are just more tool

149   that benefits the farmer with 1,200 or 12,000 cows, and not the producer with 120 cows. However, one

150   notable difference between precision technologies, such as automated milking systems, and beef or sexed

151   semen is that the former requires substantial capitalization and the latter do not. It is certainly possible

152 that a very large dairy may negotiate a slightly more favorable per-unit price due to volume purchases, but

153 it is not clear that such advantages make this semen off-limits for the smaller farm.

154

155 **WHERE ARE WE GOING?**

156

157 ***What Should We Do About Genetic Diseases?*** It is inevitable that undesirable genetic defects will arise

158 in any population, and the widespread use of artificial insemination in dairy cattle means that we will

159 sometimes have to manage such conditions (Cole et al., 2016). Currently, there are 20 such haplotypes

160 being monitored in the US dairy population (Cole et al., 2018), and there are more being reported in other

161 countries (e.g., Fritz et al., 2018; Häfliger et al., 2020). Due to the complexity of interpreting these data,

162 genetic diseases are best managed using a computerized mating system (Cole, 2015; Upperman et al.,

163 2019). However, this is always a moving target because, as one disease or another is brought under

164 control, new ones will arise. MacArthur et al. (2012) have reported that human genomes typically contain

165 ~100 genuine loss-of-function variants, with ~20 genes being completely inactivated. Statistics are likely

166 similar in the bovine, and a lethal mutation can rapidly increase in frequency when it is inherited by a

167 genetically elite bull. For example, the Holstein bull Carlin-M Ivanhoe Bell (007HO00543;

168 HOUSA000001667366), who has more than 80,000 offspring, was the carrier of both the bovine

169 leukocyte adhesion deficiency (Shuster et al., 1992) and complex vertebral malformation (Agerholm et

170 al., 2001) defects.

171

172 ***How Should We Introduce or Multiply Desirable Genes?*** There is growing interest in strategies for

173 spreading desirable genetic variants, such as the slick mutation in the prolactin receptor gene (Hansen,

174 2020a) and polled allele (Mueller et al., 2019), through dairy cattle populations. As is the case with beef-

175 on-dairy, this is not a exactly an advance in reproductive biology, but most strategies for introgressing

176 these alleles incorporate IVF and embryo transfer (**ET**) at some stage in the process (Cole, 2019). In that

177 sense, improvements in embryo technologies which increase the use of ET as a genetic selection tool will

178   also benefit these schemes (Hansen, 2020b), and have the potential to increase the value of carrier

179   embryos in the ET marketplace. Gene editing is now routine and the advent of livestock with mutations

180   designed by humans is on the near horizon. How such animals are accepted by the public will be an

181   important determinant of how quickly the technology sees application in the United States.

182

183   *New Reproductive Traits.* The advantage of measuring traits like CCR or DPR is that millions of records

184   can be obtained relatively easily using records collected on farms. Unfortunately, these traits are affected

185   to an extraordinary degree by environment, including management decisions of the dairy farmer, and the

186   heritabilities of these traits is very low. Other reproductive traits may be more heritable. As pointed out as

187   far back as 8 years ago (Boichard and Brochard, 2012), development of new devices for measuring

188   reproductive physiology of cows may make it feasible to incorporate new traits into selection indices for

189   reproduction. It remains to be seen whether other physiological measurements of reproduction can be

190   found that are both more heritable than traits like CCR or DPR and feasible for wide-scale measurement.

191   Concentrations in blood of anti-Müllerian hormone are very heritable (Nawaz et al., 2018) and the

192   intervals from calving to estrus and calving to elevated progesterone concentrations were moderately

193   heritable (Ismael et al., 2015; Tarekegn et al., 2019). In contrast, heritabilities were low for duration and

194   strength of estrus (Ismael et al., 2015), progesterone concentrations during the luteal phase (Tarekegn et

195   al., 2019) and embryo survival after ET (Parker-Gaddis et al., 2017).

196

197   *More Embryo Transfer?* While ET has been common in some parts of the world, notably in Brazil where

198   the large yield of oocytes from *Bos indicus* increases the efficiency of in vitro production systems, ET is

199   now increasing in importance on US dairies. While mostly limited to high-end breeders in the past, the

200   potential of ET to increase the number of offspring produced by genetically elite females is of growing

201   importance (e.g., Fleming et al., 2018). What will be essential to make use of ET more widespread is to

202   reduce the costs of embryo production and increase the effectiveness of the procedure for achieving

203   pregnancies (Hansen, 2020b). A recent reported advance in cryopreservation of embryos produced in

204   vitro (Gómez et al., 2020), if repeated, could be an important step in that direction.

205

206   ***Should We Continue to Depend on Reproductive Hormones in the Long Run?*** Timed AI, which is now

207   very common on US dairy farms, is dependent on the use of exogeneous reproductive hormones. There is

208   some evidence that effectiveness of hormonal interventions to increase fertility depends on interactions

209   with specific mutations in genes associated with reproduction (Zolini et al., 2019). It is possible,

210   therefore, that timed AI, like any other management intervention, could lead to a change in frequency of

211   specific alleles.

212

213   While American consumers have generally been more accepting of biotechnological innovations in the

214   food chain, a change in consumer sentiment could rapidly change the regulatory landscape (Ufer et al.,

215   2019). Timed AI offers several advantages to the farmer including reducing the need for estrus detection

216   and improvement in fertility as compared to induced estrus (Carvalho et al., 2018). It can also be

217   considered a method for improving animal welfare since, as pointed out by JEP Santos (personal

218   communication), an open cow often ends up as a dead cow. It is likely that application of precision

219   agricultural technologies to dairy cattle management will lead to new methods for detecting estrus and

220   pregnancy diagnosis. If this occurs, some of the rationale for timed AI will dissipate.

221

222   **CONCLUSIONS**

223

224   Dairy cow fertility has been steadily improving since the introduction of genomic selection in 2008. In

225   addition to more accurate genetic evaluations, genomics has led to a deeper understanding of gamete and

226   cow physiology, as well as enabled more sophisticated strategies for herd management. While we are

227   unlikely to see another tool that provides such a rapid change in cow performance, we will continue to

228   benefit from discoveries enabled by genomic selection for a very long time.

229

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407

408　**Figure 1.** Trends in U.S. Holstein breeding values (BV) for daughter pregnancy rate (DPR; red lines), heifer conception rate (HCR; blue lines),

409　and cow conception rate (CCR; black lines) for bulls (solid lines) and cows (broken lines) for animals born 1957—2019.[1]
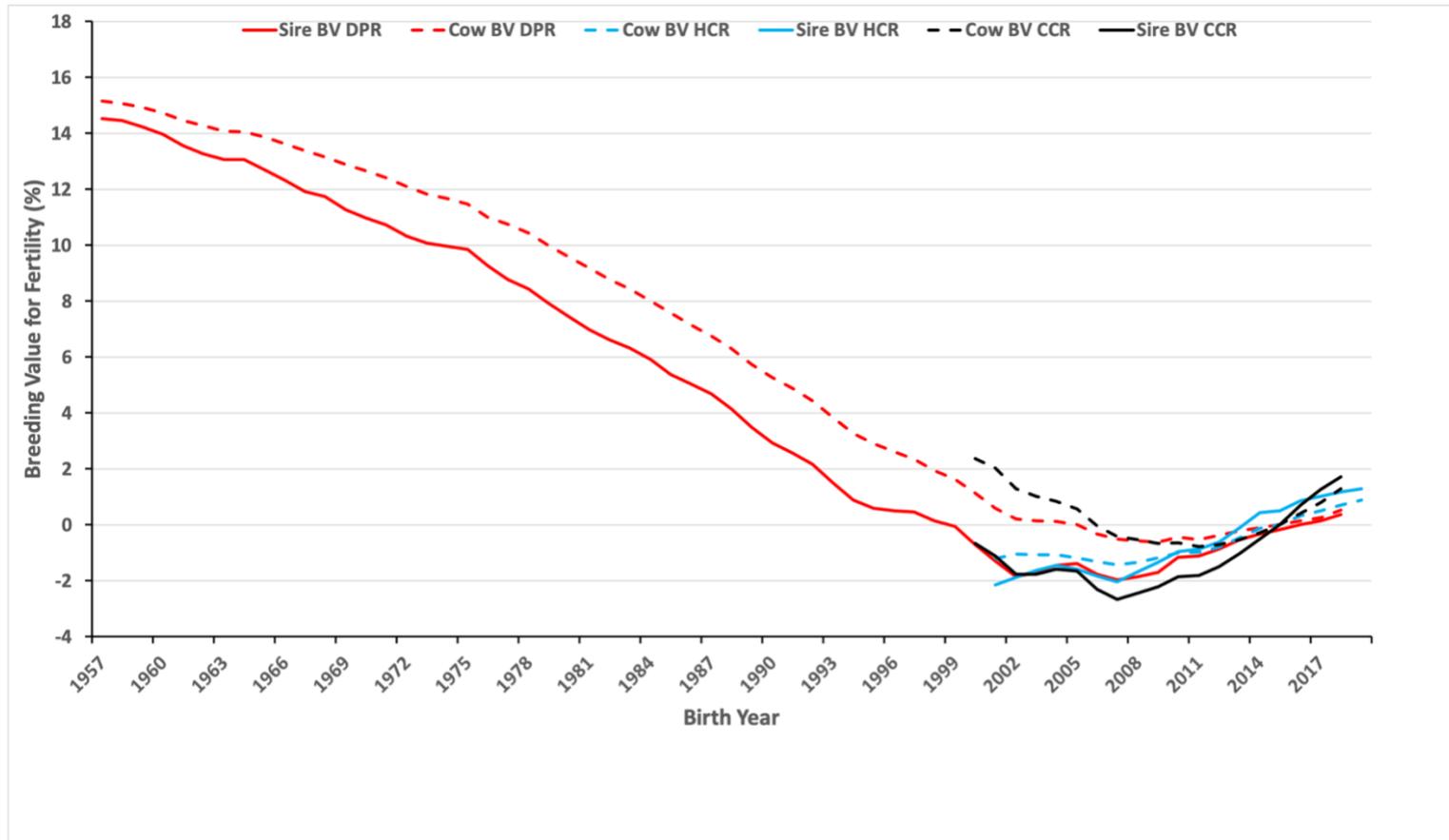
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411　[1]Data were taken from the Council on Dairy Cattle Breeding's August 2020 genetic and phenotypic trends page

412　(https://queries.uscdcb.com/eval/summary/trend.cfm).