A single ultrasound determination of ≥ 25 follicles ≥ 3 mm in diameter in dairy heifers is predictive of a reduced productive herd life

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ABSTRACT

Fertility and productive herd life (time in herd after birth of first calf) are inferior in dairy cows with relatively low compared with intermediate but not high numbers of follicles growing during ovarian follicular waves. The present study, therefore, tested the hypothesis that fertility and productive herd life are lower in dairy heifers with high follicle numbers compared with age-matched herdmates with fewer follicles. To test this hypothesis, 11 to 15 mo old Holstein heifers were subjected to a single ultrasound measurement of the number of follicles ≥ 3 mm in diameter. Heifers were classified into a high- (≥ 25 follicles), mid- (16–24), or low-range (≤ 15) follicle number group (FNG). All heifers not removed from the herd before first calving ($n = 408$) had the opportunity to start their fifth or sixth lactation after birth of their first calf. During this time, performance and health parameters for each individual were recorded daily by herd managers. Results showed that heifers in the high-range FNG had a 180-d shorter productive herd life, reduced survival rate, and greater probability of being culled after birth of the first calf, as well as fewer lactations compared with heifers in the low-range FNG. Cows in the high- compared with the mid- or low-range FNG also had greater involuntary culling rates, days open, and services per conception, and lower pregnancy rates during the first, second, or third lactations. We concluded that dairy heifers with ≥ 25 follicles ≥ 3 mm in diameter have suboptimal fertility and a shorter productive herd life compared with herdmates with fewer follicles.

Key words: dairy heifers, single ultrasound determination of follicle numbers, single antral follicle count, culling rate, productive herd life

INTRODUCTION

Number of follicles growing during follicular waves [antral follicle count (AFC)] and circulating concentrations of anti-Müllerian hormone (AMH), which are positively correlated with AFC (Ireland et al., 2008), are biomarkers predictive of the ovarian reserve (total number of morphologically healthy oocytes/follicles in ovaries; Ireland et al., 2008), ovarian progesterone and androgen production (Jimenez-Krassel et al., 2009; Mossa et al., 2010; Scheetz et al., 2012), egg quality (Ireland et al., 2009), response to superovulation (Ireland et al., 2007), fertility (Mossa et al., 2012; Ribeiro et al., 2014), and productive herd life (time in herd after birth of first calf; Jimenez-Krassel et al., 2015) in cattle. These observations, coupled with the recent finding follicle numbers are a moderately heritable genetic trait in dairy cows (Walsh et al., 2014), support the potential usefulness of AFC or AMH as candidate biomarkers to identify individuals with superior herd longevity.

Reproductive performance and productive herd life, however, may not be enhanced in dairy cows with relatively high numbers of follicles growing during ovarian follicular waves. For example, although pregnancy rate is greater and number of services per conception are lower for dairy cows with an intermediate compared with a low AFC, neither pregnancy rate nor number of services per conception differ between dairy cows with high versus a low AFC (Mossa et al., 2012). In addition, survival rate and productive herd life are greater for quartiles of heifers with intermediate (quartiles 2 and 3) compared with low (quartile 1) AMH concentrations. However, survival rate, productive herd life, and pregnancy rate do not differ ($P > 0.05$) for the quartile of heifers with the highest (quartile 4) compared with the lowest AMH concentrations (Jimenez-Krassel et al., 2015). One explanation for the equivocal nature of this relationship is the possibility reproductive performance and herd longevity are suboptimal in some individuals classified as having a high AFC or high

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AMH concentrations. Indeed, it is well established that women with polycystic ovarian syndrome have a relatively high AFC (Wiser et al., 2013) and are subfertile (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2008) compared with their counterparts without this disorder. However, whether fertility and herd longevity are negatively affected in cattle with a high AFC is unknown. Thus, the present study tested the hypothesis that heifers with a high AFC are subfertile and, therefore, removed from a herd for poor reproductive performance at a greater rate and have a shorter productive herd life compared with age-matched herdmates with a lower AFC.

Accurate determination of peak follicle number growing during follicular waves to precisely identify all cattle in herd that have a high AFC is labor-intensive, requiring daily ovarian ultrasonography throughout multiple follicular waves of a large number of individuals. Although a single measurement of circulating AMH concentration is positively correlated with AFC in cattle (Ireland et al., 2011), the assays are expensive. Moreover, the exact relationship between AMH concentration and high AFC has not been established. The objective of the present study, therefore, was not only to test the aforementioned hypothesis, but determine if individuals with high follicle numbers (≥ 25), utilizing a single ultrasound measurement of number of follicles ≥ 3 mm in diameter, exhibited suboptimal reproductive performance and a reduced productive herd life compared with herdmates with fewer follicles.

MATERIALS AND METHODS

All experiments involving cattle were approved by the Institutional Animal Care and Use Committee at Michigan State University. Our project was initiated September 5, 2008, and ended December 25, 2014.

Measurement of Follicle Numbers

Cycling Holstein heifers ($n = 440$, 11–15 mo old, located on Green Meadow Farms Inc., Elsie, MI) were subjected to 2 intramuscular injections of $\text{PGF}_{2\alpha}$ administered 11 d apart to synchronize estrous cycles. At 96 h after the last $\text{PGF}_{2\alpha}$ injection, a single ultrasound measurement, rather than daily ovarian ultrasonography throughout consecutive follicular waves as used in our previous studies (Burns et al., 2005), was used to count number of follicles ≥ 3 mm in diameter (Burns et al., 2005). Cattle were then classified into follicle number groups (**FNG**) based on number of follicles as follows: low-range (≤ 15 follicles), mid-range (16–24), and high-range (≥ 25)

Reproductive Management and Herd-Removal Protocols

After completion of the $\text{PGF}_{2\alpha}$ -induced estrous cycle, heifers were observed for signs of estrus 2 times/d and then subjected to AI the next morning after standing estrus, and heifers returning to estrus were reinseminated up to 6 times. Diagnosis of pregnancy was done by palpation of uterine contents or ultrasonography between 45 to 60 d after AI. Heifers diagnosed as not pregnant were subjected to AI as described.

After calving, lactating cows were subjected to a Presynch-11/Ovsynch program (Galvão et al., 2007). Briefly, beginning 47 to 53 DIM, all lactating cows received 2 injections of $\text{PGF}_{2\alpha}$ spaced 14 d apart. Cows exhibiting standing estrus were subjected to AI (at approximately 63 DIM) after the second $\text{PGF}_{2\alpha}$ injection. Detection of estrus was performed 3 times/d. Cows not receiving AI after the second $\text{PGF}_{2\alpha}$ injection were subjected to Ovsynch beginning 11 d after the second $\text{PGF}_{2\alpha}$ injection. The Ovsynch program starts with a GnRH injection and is followed by a $\text{PGF}_{2\alpha}$ injection 7 d later, then a second GnRH injection 2 d later followed by AI at a fixed time interval 16 h later (Pursley et al., 1995). Pregnancy status was assessed by palpation or ultrasonography of uterine contents at ~ 35 d after AI. Cows returning to estrus before pregnancy diagnosis were inseminated at standing estrus. Animals diagnosed not pregnant were subjected to AI at standing estrus after either a single $\text{PGF}_{2\alpha}$ injection if a corpus luteum (CL) was present or rechecked a week later if a CL was not present. Cows were administered $\text{PGF}_{2\alpha}$ and observed for estrus if a CL was present and Ovsynch plus timed AI if not. This breeding regimen in cows was repeated, if necessary, up to 5 times.

Records on reproductive performance, level of milk production, health, and reasons for culling of each individual animal were maintained on-the-farm using the DairyCOMP 305 software program (VAS, 2011). Relevant information for each cow was recorded daily by managers and selected employees. The following 16 involuntary terms were used at the farm to describe why cows were removed or culled from the herd: reproductive problems (≥ 4 inseminations without pregnancy, cystic follicles, excessive fat around ovaries making palpation and breeding difficult, abortions), mastitis, injury or accident, calving injury, metritis or retained placenta, displaced abomasum, death, lameness, metabolic syndrome, infections, lost records, and unknown. Three voluntary terms were also used to describe why cows were removed or culled: low milk production, poor conformation, and sold as replacement to other dairy farmers. All culling decisions were made without knowledge of follicle numbers of animals involved in the

present study. Whether the proportion of cows removed from the herd for each of the aforementioned involuntary or voluntary reasons was associated with follicle numbers in the same heifers between 11 and 15 mo old was determined at various times before and after the end of each lactation. Animals voluntarily sold as dairy replacements to other producers after follicle number measurements were not included in the analyses.

Statistical Analysis

All statistical analyses were done using SAS procedures (SAS, 2010). Continuous variables were log-transformed before analyses when the assumptions of normality and homogeneity of variance were not satisfied. Analysis of variance was used to determine if significant ($P \leq 0.05$) overall differences existed among different FNG for all variables, such as number of AI, days open, level of milk production as determined by 305-d mature equivalents, number of lactations started and completed, and productive herd life. Lactations started indicate number of animals that calved, whereas lactations completed indicates number of animals with dry periods at the end of each lactation. Productive herd life was defined as the time period between first calving and culling or the end of the study. If the ANOVA was significant ($P < 0.05$), Dunnett's *t*-test was used to determine which means of continuous variables for low- or mid-range FNG differed ($P \leq 0.05$) from high-range FNG.

During survival analyses, censored records represent cows that were culled for various reasons, sold, or died, whereas uncensored cows remain in the herd. Proportion of cows remaining in the herd (survival rate) was evaluated using Kaplan-Meier curves for each FNG and differences between FNG were analyzed using Cox proportional hazard model. The hazard ratio is defined as the probability (relative risk) that low- and mid-range FNG of cows would be culled compared with the high-range FNG of cows after birth of the first calf (Pankowski et al., 1995). Survival was expressed as the relative hazard of a cow being culled at day *t*, given that it had not culled at day *t* - 1, in the exposed group relative to the reference group (high-range). Therefore, a hazard ratio (HR) of 1 represents an equal likelihood of a cow being culled at time *t* compared with a contemporary in high-range group, whereas a greater HR indicates an increased likelihood of an animal being culled at time *t* compared with the high-range FNG.

The correlation between number of antral follicles and level of milk production was determined by Pearson correlation analysis. The 19 different voluntary and involuntary culling reasons used by management at Green Meadow Farms were combined into the following

5 primary herd removal (culling) reason for statistical analysis: poor reproductive performance (≥ 4 AI without pregnancy, cystic ovaries, fat around ovaries, abortions), death, health (mastitis, lameness, metritis or retained placenta, infection, metabolic syndrome, displaced abomasum), voluntary (low milk production and poor conformation), and other (injury or accidents, unknown). A multinomial logistic regression analysis tested whether the frequency of the 5 major reasons individuals were removed from herds differed ($P < 0.05$) among the different FNG by the end of each lactation using CATMOD procedure of SAS (SAS, 2010), as reported by others (Nuñez-Dominquez et al., 1991). Logistic regression analyses were used to determine if overall culling rates, pregnancy rate to the first AI, and total percentage of cows pregnant differed among FNG during each of the subsequent lactations.

RESULTS

Distribution of heifers by follicle number is shown in Figure 1, and mean for follicle number in each FNG of heifers is in Table 1. Dairy cows classified into the high-range FNG as heifers had fewer ($P < 0.02$) lactations started and completed compared with the cows in the low-range FNG (Table 1). Cows in the high-range FNG also had, on average, a 180-d shorter ($P < 0.01$) productive herd life compared with cows in the low-range FNG (Table 1). We also noted a tendency ($P < 0.09$) for cows in the high- compared with mid-range FNG to have a shorter productive herd life. Age of animals at their first PGF_{2α} injection, first service, and at each calving did not differ ($P \geq 10$) among the different FNG (data not shown).

Results of survival analysis showed that the proportion of cows remaining in the herd (survival rate) differed ($P = 0.05$) among the FNG (Figure 2), and the probability of being culled after birth of the first calf was 33% (HR = 1.33, $P = 0.05$) greater for cows in the high- compared with low- but not mid-range FNG (Figure 2).

Survival rate, which is proportion of cows remaining in the herd, and thus the inverse of culling rate, was lower ($P = 0.05$) for cows in the high- compared with the low-range FNG (Figure 2). However, we found no differences ($P > 0.2$) in culling rates of heifers by 24 mo of age among the FNG (8.4, 7.2, and 4.7% for low- mid- or high-range FNG, respectively). Moreover, culling rates did not differ at the end of each lactation among FNG (data not shown).

We also evaluated culling rates at various time periods (after birth of the first calf) during each lactation when divergence between survival curves was the greatest among the different FNG (Figure 2). When involun-

Table 1. Summary of number of follicles, number of started and completed lactations, and productive herd life for Holstein cows in each follicle number group (FNG)¹

Item	Low-range		Mid-range		High-range		ANOVA <i>P</i> -value
	n	Mean	n	Mean	n	Mean	
Antral follicle count	218	10.98 (1–15) ²	129	19.14 (16–24)	61	30.85 (25–51)	
Lactation ± SEM							
Started	218	2.9 ± 0.1 ^a	129	2.7 ± 0.1 ^{ab}	61	2.5 ± 0.1 ^b	0.02
Completed	218	2.3 ± 0.1 ^a	129	2.0 ± 0.1 ^{ab}	61	1.7 ± 0.2 ^b	0.02
Productive herd life (d)	218	970 ± 39 ^a	129	865 ± 45 ^{ab†}	61	790 ± 70 ^b	0.01

^{a,b}Different superscripts in a row indicate means for high- differed ($P < 0.05$) from low- or mid-range FNG.
¹Number of follicles was measured in 11- to 15-mo-old Holstein heifers, and animals were divided into FNG based on number of follicles ≥ 3 mm in diameter (low-range = 1 to 15, mid-range = 16 to 24, and high-range = 25 to 51). Productive herd life is defined as days in herd after birth of first calf.
²Range of number of follicles ≥ 3 mm in diameter.
[†] $P < 0.09$

tary (reproduction, death, health, other) and voluntary (low milk production, poor conformation) culling rates were combined (All reasons, Table 2), culling rates differed ($P \leq 0.03$) among FNG at d 586, 1,006, and 1,296 after first calving, which corresponded with the second, third, and fourth lactation, respectively; culling rates tended ($P = 0.08$) to differ among FNG at d 193, which

corresponds with the first lactation. These culling rates (all reasons) were greater ($P \leq 0.05$) at d 586, 1,006, and 1,296 and tended ($P < 0.10$) to be greater at d 193 in the high- compared with mid- or low-range FNG (Table 2). However, although the involuntary culling rates differed ($P \leq 0.03$) among the different FNG at d 193 and 586, and were greater ($P \leq 0.05$) in the high

Figure 1. Frequency distribution for number of Holstein heifers with different number of follicles ≥ 3 mm in diameter determined by a single ultrasound determination of follicle numbers. Follicle number ranged from 3 to 51. Each bar depicts a range in different number of follicles in increments of 3. For example, the first bar reflects number of animals that had an antral follicle count between 1 and 3, whereas the next bar reflects number of animals that had an antral follicle count between 4 and 6.

compared with the mid- or low-range FNG, none of the individual culling reasons differed ($P \geq 0.05$) among the FNG during these times (Table 2). In addition, voluntary culling rates did not differ ($P > 0.23$) among FNG (Table 2).

Level of milk production, as assessed by 305-d mature equivalents, did not differ ($P > 0.19$) among FNG during any lactation (data not shown). In addition, correlations between number of antral follicles and milk production were not significant ($P > 0.1$; data not shown).

We also analyzed whether reproductive performance differed among FNG for heifers and during different lactations. Although no differences were observed for any reproductive performance measure for heifers or for cows when all lactations were combined (data not shown), days open, services per conception, and total percent pregnant did differ ($P \leq 0.05$) among FNG for several different lactations (Table 3). During the first lactation, days open were greater ($P \leq 0.05$), whereas total percent pregnant was lower ($P \leq 0.05$) for cows in the high- compared with mid- or low-range FNG (Table 3). We also noted a tendency ($P < 0.09$) during the fourth lactation for services per conception to be greater in the high- compared with low-range FNG.

DISCUSSION

The FNG classification scheme used in the present study relied on a single ultrasound measurement of follicle numbers at unknown stages of a follicular wave; therefore, it differs from the AFC classification scheme used in our previous studies (Burns et al., 2005). Our previous studies used daily ovarian ultrasonography throughout most days of consecutive follicular waves to classify cattle based on peak number of antral follicles ≥ 3 mm in diameter growing during a follicular wave (Burns et al., 2005). Based on peak AFC during follicular waves, cattle were classified into low (≤ 15 follicles), intermediate (16 to 24), or high (≥ 25 follicles) AFC groups. The single ultrasound determination of AFC used in the present study can, however, accurately identify an unknown proportion (but not all) of the cattle with a consistently very high AFC during follicular waves, provided the cutoff for follicle numbers is ≥ 25 follicles ≥ 3 mm in diameter, as for the high-range FNG in the present study. For example, even if the single ultrasound determination is made at the nadir for AFC during a follicular wave, which can be 50% lower than the peak, an individual with an AFC of 25 or more must have had a consistently high (likely

Figure 2. Kaplan-Meier plots of survival curves for proportion of Holstein cows in high-, mid-, or low-range follicle number group (FNG) remaining in the herd after birth of their first calf. An asterisk (*) indicates survival rate differed ($P = 0.05$) among FNG, and probability of being culled after birth of the first calf was 33% greater ($P = 0.05$) for cows in the high- compared with low-range FNG. Color version available online.

very high) peak AFC during follicular waves. Moreover, it is likely the high-range FNG contained individuals with a very high AFC during follicular waves, especially because the probability of a single ultrasound measurement coinciding with peak AFC, which lasts only 1 to 2 d of a 7 to 10 d follicular wave (Burns et al., 2005), is relatively low. Thus, many individuals with consistently a high peak AFC (≥ 25 follicles) during follicular waves, but not at peak AFC when the ultrasound measurement was made, would not have been included in the high-range FNG in the present study.

In contrast to ultrasound measurements >25 follicles, any single determination of AFC <25 follicles cannot

be used reliably to classify cattle as having consistently a low, intermediate, or high AFC as in our previous studies (Burns et al., 2005). For example, a cow with an AFC of 8 after a single ultrasound measurement could be in the low (≤ 15 follicles ≥ 3 mm in diameter) or intermediate (16–24 follicles) AFC group because the AFC measurement could have been made at the nadir or peak (or in between) for AFC during a wave. In addition, a cow with an AFC of 24 after a single measurement could have been in the high or intermediate AFC group for the same reason. Consequently, in the present study, the low- and mid-range FNG contain a mixture of cattle with consistently a low, intermediate, and high

Table 2. Culling rates at different days after birth of the first calf, when divergence in survival rates during each lactation is greatest between cows in the low-, mid- and high-range follicle number group (FNG)¹

	Low-range		Mid-range		High-range		ANOVA <i>P</i> -value
Culling reason	n	%	n	%	n	%	
First lactation (d 193) ²							
Total number	218		129		61		
Involuntary	19	8.7 ^a	11	8.5 ^a	12	19.7 ^b	0.03
Reproduction	2	0.9	2	1.6	2	3.3	0.39
Death	10	4.6	7	5.4	6	9.8	0.28
Health	7	3.2	2	1.6	4	6.6	0.19
Other	—	—	—	—	—	—	—
Voluntary	8	3.7	7	5.4	1	1.6	0.43
All reasons	27	12.4 [†]	18	14.0 [†]	13	21.3	0.08
Second lactation (d 586)							
Total number	218		129		61		
Involuntary	55	25.3 ^{ab}	24	18.6 ^a	22	36.1 ^b	0.03
Reproduction	26	11.9	10	7.8	9	14.8	0.29
Death	18	8.3	12	9.3	8	13.1	0.51
Health	11	5.1	2	1.6	5	8.2	0.09
Other	—	—	—	—	—	—	—
Voluntary	9	4.3	10	7.8	5	8.2	0.27
All reasons	64	29.4 ^a	36	27.9 ^a	27	44.3 ^b	0.03
Third lactation (d 1,006)							
Total number	218		129		61		
Involuntary	101	46.3	62	48.1	36	59.0	0.21 ³
Reproduction	50	22.9	32	24.8	18	29.5	
Death	31	14.2	21	16.3	11	18.0	
Health	20	9.2	5	3.9	7	11.5	
Other	0	0	4	3.1	0	0	
Voluntary	13	5.9	14	10.9	6	9.8	0.23
All reasons	114	52.3 ^a	76	58.9 ^{ab}	42	68.9 ^b	0.02
Fourth lactation (d 1,296)							
Total number	218		129		61		
Involuntary	133	61.0	86	66.7	45	74.8	0.15
Reproduction	65	29.8	43	33.3	21	34.4	
Death	40	18.4	31	24.0	15	24.6	
Health	26	11.9	7	5.4	9	14.8	
Other	2	0.9	5	3.9	0	0	
Voluntary	18	8.3	17	13.2	8	13.1	0.27
All reasons	151	69.3 ^a	103	79.8 ^{ab}	53	86.9 ^b	0.01

^{a,b}Different superscripts ($P \leq 0.05$) within a row indicate that the high- differs from mid- or low-range FNG after multinomial logistic regression analyses.

¹Survival curves are depicted in Figure 2. Days after birth of first calf for analysis of culling rates was chosen as the time of the greatest divergence between survival curves at each lactation between different FNG.

²Days after birth of first calf.

³Statistical differences between means for individual culling reasons were not determined unless involuntary/voluntary culling reasons differed ($P \leq 0.05$) among FNG.

[†] $P = 0.10$.

Table 3. Reproductive parameters in Holstein heifers and cows at each lactation

Item ¹	Low-range		Mid-range		High-range		<i>P</i> -value ²
	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	
Heifer							
SC	218	1.99 ± 0.1	129	2.1 ± 0.1	61	1.93 ± 0.2	0.76
CR (%)	218	47 ± 3	129	47 ± 4	61	48 ± 6	0.99
TPP (%)	217	100 ± 0	128	100 ± 0	61	100 ± 0	NA
First lactation							
DO (d)	204	140 ± 6 ^b	123	136 ± 7 ^b	55	168 ± 18 ^a	0.05
SC	196	2.4 ± 0.1	117	2.3 ± 0.2	53	2.7 ± 0.3	0.41
CR (%)	196	39 ± 4	117	44 ± 5	53	38 ± 7	0.56
TPP (%)	196	88 ± 2 ^a	117	86 ± 3 ^{ab}	53	77 ± 6 ^b	0.04
Second lactation							
CaI (d)	170	395 ± 4	101	396 ± 6	41	402 ± 10	0.71
DO (d)	165	157 ± 6	97	162 ± 10	41	153 ± 13	0.83
SC	157	2.9 ± 0.1	94	2.8 ± 0.2	37	2.8 ± 0.3	0.96
CR (%)	157	27 ± 4	94	25 ± 4	38	26 ± 7	0.88
TPP (%)	157	85 ± 3	94	77 ± 4	38	76 ± 7	0.08
Third lactation							
CaI (d)	132	410 ± 5	68	396 ± 6	29	406 ± 12	0.30
DO (d)	119	146 ± 8	61	164 ± 13	27	168 ± 15	0.30
SC	111	2.3 ± 0.1	54	2.8 ± 0.2	23	2.8 ± 0.3	0.05
CR (%)	111	38 ± 5	54	30 ± 6	23	17 ± 8	0.06
TPP (%)	111	78 ± 4	54	72 ± 6	23	61 ± 10	0.09
Fourth lactation							
CaI (d)	79	388 ± 5	37	404 ± 10	13	389 ± 12	0.28
DO (d)	66	147 ± 8	27	215 ± 33	9	164 ± 25	0.04
SC	58	2.6 ± 0.2 [†]	21	3.2 ± 0.4	8	3.6 ± 0.7	0.09
CR (%)	58	29 ± 6	21	23 ± 9	8	25 ± 16	0.87
TPP (%)	58	78 ± 5	21	57 ± 11	8	88 ± 13	0.07
Fifth lactation							
CaI (d)	37	398 ± 9	10	401 ± 21	6	441 ± 25	0.08
DO (d)	35	187 ± 19	7	161 ± 54	6	167 ± 40	0.81
SC	27	2.7 ± 0.4	6	2.7 ± 0.9	6	2.3 ± 0.6	0.92
CR (%)	27	22 ± 8	6	33 ± 21	6	17 ± 17	0.78
TPP (%)	25	56 ± 10	5	60 ± 25	4	75 ± 25	0.78

^{a,b}Different superscripts in a row indicate means differed from high-range follicle number group (FNG; $P < 0.05$). Note, although overall P -values were significant for SC ($P < 0.05$) during the third lactation and DO ($P < 0.04$) during the fourth lactation, and corresponding means for SC and DO in low- vs. mid-range FNG differed ($P < 0.05$), none of these means differed from the high-range FNG.

¹SC = services per conception; CR = conception rate at first AI; TPP = total percent pregnant; DO = days open; CaI = calving intervals.

²SC, DO, and CaI analyzed by ANOVA, whereas CR and TPP were analyzed by logistic regression.

[†] $P < 0.09$.

AFC during follicular waves. We, therefore, tested the hypothesis by using a single ultrasound determination of AFC in heifers to determine if fertility and longevity were lower in cows in high- compared with the mid- or low-range FNG. The most significant finding of the present study demonstrated for the first time that a single ultrasound measurement of ≥ 25 follicles ≥ 3 mm in diameter in dairy heifers is positively linked to a reduced survival rate, fewer lactations, a greater probability of being culled after birth of the first calf, reduced fertility, and a 180-d shorter productive herd life, on average, compared with heifers with ≤ 15 follicles. This observation is important, especially when combined with results of our previous studies (Mossa et al., 2012), because it provides new evidence that cattle with the highest AFC do not exhibit greater fertility or greater productive herd life compared with cattle with a lower AFC. For example, productive herd life for quartile 1

of dairy cows with the lowest AMH concentrations as heifers is ~ 180 d shorter compared with quartile 2 or 3, but not quartile 4 dairy cows, which have the highest AMH (Jimenez-Krassel et al., 2015). The present study shows that cows within the high-range FNG as heifers, which presumably also would have had the highest AMH concentrations (similar to quartile 4 heifers in our previous study), also had productive herd life ~ 180 d shorter compared with herd mates with fewer follicles as heifers. Taken together, these observations imply that a relatively low or high number of follicles growing during follicular waves are both linked to significantly reduced potential productive herd life of dairy heifers.

Days open were also longer ($P < 0.05$) whereas pregnancy rate was lower ($P < 0.04$) for cows in the high- compared with low- or mid-range FNG during the first lactation in the present study. Although statistical analyses were limited due to small number of

animals in each grouping and lactation, results imply that fertility may be suboptimal in cows with a very high AFC during follicular waves as heifers compared with herdmates with fewer follicles. This finding contrasts with our previous study (Mossa et al., 2012) because several measures of reproductive performance (e.g., pregnancy rate at first service, number of services overall) in dairy cows with a high AFC during follicular waves did not differ from individuals with low or an intermediate AFC, whereas other measures of fertility (e.g., calving to conception interval, overall pregnancy rate at the end of the breeding season, and odds ratio for overall pregnancy rate) were greater in cows with high compared with a low AFC. These results (Mossa et al., 2012) and those of our present study, however, cannot be compared directly for 2 reasons. First, our previous study (Mossa et al., 2012) classified dairy cows into AFC groups based on the average of 2 consecutive ultrasound measurements during the first follicular wave, whereas the present study used a single determination of follicle numbers to classify cattle into FNG. Second, our previous study (Mossa et al., 2012) used dairy cows of mixed ages (up to 8 parities) whereas our present study used 11 to 15 mo old heifers. The present study shows that herd longevity is shorter for heifers with a very high (high-range FNG) compared with a low AFC during follicular waves (low-range FNG). Taken together, these studies support the likelihood that classification of cattle based arbitrarily on high AFC, or on the quartile of individuals with the highest AMH concentrations as in our previous studies (Mossa et al., 2012; Jimenez-Krassel et al., 2015), may have to be redefined to better identify superior female cattle, especially because these existing classification schemes for cattle with a high AFC or high circulating AMH concentrations also identify some individuals with suboptimal fertility and a reduced herd longevity. The reason reproductive performance may be inferior in heifers with a very high AFC, similar to women with polycystic ovarian syndrome and a high AFC (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2008; Wiser et al., 2013), is unknown.

As previously reported (Jimenez-Krassel et al., 2015), economic value for herd longevity or productive life of cows is \$24 to \$35 per month based on values for predicted transmitting ability for productive life in the USDA Net Merit Index (Cole and VanRaden, 2009; VanRaden and Cole, 2014). Thus, another significant finding of the present study is the observation that a single ultrasound determination of ≥ 25 follicles ≥ 3 mm in diameter in young adult heifers may be a simple diagnostic method to identify inferior heifers to remove from the herd and replace with superior heifers, thereby improving herd longevity, productive herd life, and

profitability of the dairy operation (Jimenez-Krassel et al., 2015). Similar to results for AMH in our previous study (Jimenez-Krassel et al., 2015), level of milk production was not correlated with follicle numbers in the present study. Moreover, voluntary culling rates, which included level of milk production (usually a major reason for culling dairy cows from herds; Bascom and Young, 1998), did not differ among FNG in the present study. Thus, removal of cows with a very high AFC from herds and replacement with superior heifers would not compromise milk production.

Even though the proportion of cows remaining in the herd (which is the inverse of culling rate) or survival rate was lower for cows in the high- compared with low-range FNG, culling rates at the end of each lactation did not differ among FNG. However, when other time points during each lactation were examined culling rates differed among FNG. Although the reason for this finding is unknown, it may be because culling decisions and removal of cows from the herd are not made by management at fixed times relative to age or stage of lactation.

Survival rate and productive herd life were lower whereas probability of being culled and overall culling rates during (but not at the end of) each lactation were greater for cows in the high- compared with low-range FNG in the present study. However, even though overall involuntary culling rates were greater during the first and second lactation for cattle in the high-range compared with low- or mid-range FNG, the precise reason (poor reproduction, death, health, and other) these cattle were culled could not be established in the present study, likely because of the limited number of animals in each FNG during the different lactations and absence of rigorously adhered to quantitative reasons to remove cows from the herd by management (Lehenbauer and Oltjen, 1998). Nevertheless, the significantly diminished herd longevity for the high-range FNG cows in the present study explained why these individuals also had fewer completed lactations.

Because our study was conducted in a single herd with limited animal numbers ($n = 408$), it is premature to make an industry-wide recommendation to remove inferior dairy heifers in a herd based on single AFC measurements. Nevertheless, our findings support the conclusion that dairy heifers with ≥ 25 follicles ≥ 3 mm in diameter have suboptimal fertility and a shorter productive herd life compared with herdmates with fewer follicles.

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