Scientific Papers. Series D. Animal Science. Vol. LXII, No. 1, 2019
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

VARIABILITY IN NUMBER OF ANTRAL FOLLICLES IN HOLSTEIN FRIESIAN HEIFERS AND ITS ASSOCIATION WITH REPRODUCTIVE AND PRODUCTIVE PERFORMANCES AFTER THE END OF FIRST LACTATION

Constantin GĂVAN

Agricultural Research and Development Station Șimnic - Craiova, România

Corresponding author email:scda_simnic@yahoo.com

Abstract

In cattles the small antral growing follicles develop from a reserve of primordial follicles constituted early in life. The antral follicle count (AFC, follicles ≥ 3 mm in diameter) is determined by ovarian ultrasound measurement. In this study 13 to16 months old Holstein Friesian heifers were subjected to a single ultrasound measurement of the number of follicles ≥3 mm in diameter. Heifers were classified into a low (≤ 5 follicles) mid (16-24 follicles) and high (≥25 follicles) follicle number group (FNG). We then compared the reproductive and productive performances at the end of first lactation. Results showed that heifers in the high FNG had a higher pregnancy rate, a shorter calving to conception interval, and a higher culling rate, compared with heifers in the low FNG. Because this study was made in a single herd with limited animal numbers (n=100) it is premature to make an industry-wide recommendation to select the dairy heifers in a herd based on single AFC measurements.

Key words: *antral follicles, ultrasound measurement, reproductive performances, culling rate.*

INTRODUCTION

Recently, there have been an increasing interest in studies concerning antral follicle count (AFC) and its association with reproductive performances in dairy cattle (Ireland et al., 2011; Pontes et al., 2011; Rico et al., 2012; Silva Santos et al., 2014; Morotti et al., 2015). The AFC represents the member of follicles visualized by ultrasound evaluation in the ovaries.

In cattle, calculations based on the number of granulose cells in follicles of various classes and from the time required to double the number of cells within a follicle, indicate that a follicle takes 27 days to grow from 0.13 to 0.67 mm, 6.8 days from 0.68 to 3.67 mm and 7.8 days from 3.68 to 8.56 mm, indicating that growth rates varied with the size of the follicle (Lussier et at., 1987). A period equivalent to oestrus cycles would therefore be required for a follicle to grow through the antral phase, i.e. from 0.13 mm to preovulatory size .

Antral follicles grow in a wave-like pattern (Ginther et al., 1989; Fortune et al., 1991). In cows there are two or three waves of follicular development during an oestrus cycle (Fortune et al., 1991; Ginther et al., 1996).

The numbers of follicles ≥ 3 mm in diameter recruited in each wave have been counted on different days of the oestrus cycle, in dairy heifers and postpartum dairy cows (Singh et al., 2004; Ireland et al., 2011). The AFC is highly variable among animals but very highly repeatable (0.85 to 0.95) within individuals (Jimenez-Krassel et al., 2009; Mossa et al., 2010).

This persistence in AFC in the same individual becomes a good information for the classification of females as low-, intermediate- or high AFC by a simple ultrasound evaluation during follicular waves. (Burns et al., 2005; Ireland et al., 2007, 2011; Mossa et al., 2012.)

Studies have shown that AFC can be affected by maternal environmental conditions, such as health and nutritional status during pregnancy or by lactation status and milk quality. The relationship between AFC and genetic characteristics in dairy cattle is an issue that needs to be better understood.

In this paper the hypothesis that AFC is positively associated with fertility in Holstein Friesian cattle was tested. The objective was to determinate if AFC, utilizing a single ultrasound measurement of number of follicles ≥ 3mm in diameter at puberty age, was associated with several measures of reproductive and productive efficiency after the end of first lactation.

MATERIAL AND METHODS

Animals

Dairy cattle used in this experiment were located at Agriculture Research and Development Station (ARDS) Simnic Craiova Romania.

The experiment was performed in compliance with the European Union Directive 86/609/EC, on Holstein – Friesian cattle- that belong to a long large genetic improvement program.

Holstein Friesian (HF) heifers with adequate body condition score (BCS) and normal health status were selected for the experiments .This project was initiated in January 10, 2015.

Measurement of Follicle Numbers

Cycling NF heifers (n=107) 13-15 month old were subjected to 2 intramuscular injections of PGF 2∝ administrated 15 mounts old were subjected to 2 intramuscular injections of PGF2α administrated 11 days apart to synchronize estrous cycle.

At 96 hours after the last PGF2∝ injection, a single ultrasound measurement was used to count number of follicles ≥ 3mm in diameters (Burns et al, 2005). Ovaries in each heifer were scanned with Veterinary Ultrasound scanner Ecoson 80+.

Each ovary was scanned from end to end to identify the positions of the antral follicles and the corpus luteum. Video images for each ovarian section were captured on a computer monitor. The lactations of each antral follicle ≥ 3 mm in diameter in each section were drown an ovarian map (Burns et al., 2005). Two separate of diameters were averaged for each follicle and recorded on each ovarian map. Total number of antral follicle ≥ 3mm in diameter per pair of ovaries for each animal was determined by counting the number of follicle ≥ 3 mm in diameter on each map for each animal. Heifers were classified into follicular number group (FNG) based on number of follicles as follows: low (13-15 follicles) mid (16-24follicles) and high (≥ 25 follicles).

Reproductive management

After induced estrous cycle, heifers were observed for signs of oestrus 3 times per day, and then were subjected to artificial insemination (AI) after standing oestrus. Diagnosis of pregnancy was done by palpation of uterine contents between50 to 60 day after AI. The heifers diagnosed as not pregnant were subjected again to AI.

After first calving, lactating primiparous cows, were subjected to AI, after a voluntary waiting period (VWP) of 60 days postpartum. The cows diagnosed with metritis (n=3) were treated with prostaglandin $F_2\alpha$ ($PGF_2\alpha$) at 23 ± 3 days postpartum.

Cows returning to estrus before pregnancy diagnosis were inseminated at standing oestrus. Pregnancy status was assessed by rectal palpation of uterine contents at 60 days after AI. The primiparous cows diagnosed non pregnant were subjected to Ai at standing estrus, after either a single $PGF_2\alpha$ injection if a corpus luteum (CL) was present or rechecked 7 days later if a CL was not present. This breeding procedure in cows was repeated for same animals up to 5 times.

The reproductive performance, level of milk production, health, and reasons why animals were removed or culled from the herd, of each individual cow were recorded daily.

The involuntary terms used to describe why cows were removed or culled from the herd were: reproductive problems (≥ 5 inseminations without pregnancy, cystic follicles, abortions), mastitis, calving injury, retained placenta or metritis, displaced abomasum, death, lameness, metabolic syndrome, infections.

The voluntary terms used were: low milk production, poor conformation and sold to other dairy farmers.

The proportion of cows removed from the herd for each involuntary or voluntary reason was associated with each FNG.

To study the association between AFC and fertility, the following reproductive performance were calculated and analyzed: pregnancy rate at first service, pregnancy rate after all services (up to 5) and number of services per conception as heifers, and pregnancy rat at first service, pregnancy rate after all services (up to 5), mean length of the service period, and number of services per conception as primiparous cows.

Statistical analysis

All statistical analysis were done using Microsoft Office Excel procedures. Analysis of variance was used to determine if significant ($p \leq 0.05$) overall differences existed among different FNG for all reproductive parameters. The correlation between number of ancestral follicles and level of milk was determined by Pearson correlation analysis.

RESULTS AND DISCUSSIONS

Based on the AFC, utilizing a single ultrasound measurement of number of follicles ≥ 3mm in diameter, distribution of heifers is shown in figure 1.

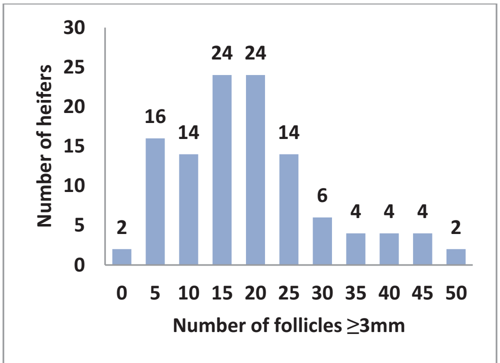

Figure 1. Freqvency distribution for number of heifers with different number of follicles ≥ 3mm in diameter determined by a single ultrasound examination

Table 1. Characteristics at single ultrasound measurement of number of follicles ≥ 3 mm in diameter and reproductive performances in each follicular number group of Holstein Friesian young animals

Item	Follicle number group00			
	Low	Mid	High	Level of significance
Characteristics at scanning:				
Number of heifers (%)	42 (42%)	36 (36%)	22 (22%)	-
Mean antral follicle count (mm)	10.47^{a}	19.38^{b}	34.72^{c}	< 0.001
Range (mm)	3-15	16-24	25-50	-
Reproductive performance as heifers:				
Pregnancy rate at first service (%)	62.2^{a}	75.4^{b}	68.1^{ab}	< 0.005
Pregnancy rate after all services (%)	84.4^{a}	88.6^{ab}	96.1^{b}	<0.05
Number of services per conception	2.2	1.8	1.8	NS
Reproductive performance as primiparous cows:				
Pregnancy rate at frst service (%)	52.2	65.1^{ab}	68.0^{b}	<0.05
Pregnancy rate after all service (%)	72.8	86.8^{ab}	90.0^{b}	<0.05
Mean length of service period (days)	118^{a}	104^{ab}	100^{b}	<0.05
Number of services per conception (nr)	2.4	2.2	2.0	NS

[a] [b] different superscripts within a row indicate. That the high-differs from mid- or low - FNG groups: NS = non significant

Table 2. Productive performances and culling rates and reason of culling during first lactation in each follicle number group of Holstein Friesian young animals

Item	Follicle number group						
	Low		Mid		High		Level of signi ficance
	n	%	n	%	n	%	
Milk production with 3,5 % fat corrected milk (kg/d)	42	29.4	36	31.2	22	30.8	NS
Culling reason:							
Involuntary	2	4.7^{a}	2	5.6^{a}	3	13.6^{b}	< 0.05
Reproduction	1	2.3	1	2.8	1	4.5	NS
Death	-	-	1	2.8	-	-	-
Health	1	2.3^{a}	-	-	2	9.1^{b}	< 0.05
Voluntary	2	4.7	2	5.5	1	4.5	NS
All reason	4	9.4^{a}	4	11.1^{a}	4	18.1^{b}	< 0.05

[a] [b] different superscripts within a row indicate that the high differ from mid ar low FNG groups. NS = non significant

Based on the AFC heifers were assigned to the low group (≤ 15 follicles) 42 (42%) to the mid group (16 to 24 follicles) 36 (36%), and 22 (22%) to the high (≥ 25 mm) group (Table 1). The association between AFC and fertility of heifers is summarized in Table 1. The actual pregnancy rates of heifers at first service were 62.2%, in the low group, 75.4% in the mid group and 68.1% in the high group. The actual pregnancy rates of heifers at first service were 62.2%, in the low group, 75.4% in the mid group and 68.1% in the high group. The actual pregnancy rates overall were 84.4% in the low group, 88.6% in the mid group and 96.1% in the high group of heifers, and number of services per conception was 2.2, 1.8 and 1.8 in the low, mid and high groups respectively. The association between AFC and fertility in the first lactation is summarized in Table 1. As primiparens cows the actual pregnancy rates at first service was 52.2%, 65.1% and 68% in the low, mid and high groups respectively. After all services the actual pregnancy rates was 8.4%, 88.6% and 96.1% in the low, mid and high groups respectively. The mean, length of service period was 118 days, 104 days and 100 days in the low, mid and high groups respect-tively (table 1). Primiparous cows with e low AFC received more services per concepttion compared with primiparous cows with mid and high AFC (2.6 vs. 2.2 and 2.0 respectively).

The association between AFC and productive performances and culling rates after first lacta-tion of dairy cows is summarized in table 2.

No difference was evident in milk production with 3.5% fat corrected milk per day among group (Table 2).

The FNG classification scheme used in this study relied on a single ultrasound mea-surement of follicle numbers at unknown stage of follicular wave. In a previous study, Burns et al., (2005), used daily ovarian ultrasound evaluation throughout most days of consecutive follicular waves to classify cattle based on peak number of antral follicles ≥ 3mm in diameter into low (≤ 15 follicles, mid (16-24) or high (≥ 25 follicles) AFC groups. A single ultrasound measurement could have been made at the nadir or peak (or in between) for AFC during a wave. If the single ultrasound determination is made at the nadir for AFC during follicular wave, which can be 50% lower than the peak, an individual with an AFC of 25 or more must have a consistently high peak AFC. A cow with an AFC of 8 after a single determination could be in the low (≤15 follicles ≥3 mm in diameter) or mid (16-24 follicles) AFC group. A cow with an AFC of 24 after a single measurement could have been in the high or mid AFC group. The low and mid FNG contain a mixture of animals with a low, intermediate or high AFC during follicular waves. In our study we compared high FNG with the mid or low FNG.

In this study animals with high AFC were superior in several characteristics related to fertility, such as high pregnancy rate, or short length of service period as heifers, and as primiparous cows. This result is consistent with some previous reports (Mossa et at., 2012, Koyama et al., 2018).

In the present study culling rates the end of first lactation were greater for cows in the high, compared with low FNG (18.1% vs. 9.4%). Because the proportion of cows with an AFC ≥ 25 follicles was 22% of the cows enrolled in this study, we could not assess the adverse effects of the higher AFC on dairy cows. The high variation in follicle numbers may not only be reflective of reproductive disorders, but could be the evidence to indicate that it may be associated with alterations in the function of other non-reproductive systems that may have effects on animals health.

CONCLUSIONS:

We confirmed that the reproductive perfor-mance of cows with an AFC of ≥ 25 follicles was better than of cows with mower AFCs.

Because this study was made in a single herd with limited animal numbers (n=100) it is premature to make an industry-wide recom-mendation to select the dairy heifers in a based on single AFC measurements.

Future studies should, therefore, verify if the high variation in follicle numbers in dairy cows could be associated with alterations in the function of non-reproductive systems.

REFERENCES

Burns, D.S., Jimenez-Krassel, F., Ireland, J.L.H., Knight, P.G., Ireland, J.J. (2005). Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum folicle-stimulatig hormone concentrations. *Biol.Reprod*., 73: 54-62.

Fortune, J.E., Sirois, J., Turzillo, A.M., Lavoir, M. (1991). Follicle selection in domestic ruminants. *J.Reprod.Fertil*., 43 (suppl.1), 187-198.

Ginther, O.J., Kastelic, J.P., Knopt, L. (1989). Composition and characteristics of follicular waves during the bovine oestrous cycle. *Anim.Reprod.SCI*, 20, 187-200.

Ginther, O.J., Kot, K., Kulick, I.J., Martin, S., Wiltbank, M.C. (1996). Relationships between F.S.H. and ovarian follicular waves during the last six months of pregnancy in cattle. *J.Reprod.Fertil*., 108, 271-279.

Ireland, J.J., Jimenez-Krassel, F., Ireland, J.L.N., Smith, G.W., Lonergan, P., Evans, A.C.O. (2007). Follicle numbers are highly repeatable within individual animals, but are inversely correlated with F.S.H. concentrations and the proportion good-quality embryos after ovarian stimulation in cattle. *Hum. Reprod*., 22, 1687-1695.

Ireland, J.L.H., Scheets, D., Jimenez-Krassel, F., Themmen, A.P.N., Word, F., Lonergan, P., Smith, G.W., Perez, G.I., Evans, A.C.O., Ireland, J.J. (2008). Antral follicle count reliably predicts number of morphologically health oocytes and follicles in ovarian of young cattle. *Biol. Reprod*., 79, 1219-1225.

Ireland, J.J., Smith, G.W., Scheetz, D., Jimenez-Krassel, F., Folger, J., Ireland, J.L.H., Mossa, F., Lonergan, P., Evans, A.C.O. (2011). Does size matter in females ? On overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti - Mullerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reprod.Fertil.Dev*., 23, 1-14.

Jimenez –Krassel F., Scheetz, D.M., Neuder, L.M., Irleand, J.L.H., Smith, G.W., Han, X., Davis, J.S., lonergan, P., Evans, A.C.O., Ireland, J.J. (2009). Evidence that high variation in ovarian reserves of healthy young adults has a negative impact on the corpus luteum and endometrium during reproductive cycles of single – ovulating species. *Boil. Reprod*., 80, 1272-1281.

Koyama K., Koyama T., Sugimoto, M. (2018). Repeatability of antral follicle count according parity in dairy cows. *Journal of Reproduction and Development*, 64 (6), 535-539.

Lussier J.G., Matton, P., Dufour, J.J. (1987). Growth rates of follicles in the ovary of the cow. *Journal of production and Fertility*, 81, 301-307.

Morotti, F., Barreiros, T.R.R., Machado, F.Z., Gonzalez, S.M., Marinho, L.S.R., Seneda, M.M. (2015). Is the number of antral follicles an interesting selection criterium for fertility in cattle. *Anim. Reprod*., 12 (3), 479-486.

Mossa, F.F., Krassel, J., Folger, J.K., Ireland, J.L., Smith, G., Lonergan, P., Evans, A.C.O., Ireland, J.J. (2010). Evidence that high variation in antral follicle count during follicular waves is linked to alternations in ovarian production in cattle. *Reproduction*, 140, 713-720.

Mossa F., Walsh, S.W., Butter, S.T., Berry, D.P., Carter, F., Lonergan, P., Smith, G.W., Ireland, J.J., Evans, A.C.O. (2012). Low numbers of ovarian follicles ≥3mm in diameter are associated with low fertility in dairy cows. *J. Dairy Sci*., 95, 2355-2361.

Pontes J.H.F., Melo-Sterza, F.A., Basso, A.C., Ferreira, C.R., Sanches, B.V., Rubin, K.C.P., Seneda, M.M. (2011). Ovum pickup, in vitro embryo production and pregnancy rates from a larg-scale commercial program using Nelore cattle (*Bos indicus*) donors. *Theriogenology*, 75, 1640-1646.

Rico, C.L., Drauilhet, P., Salvetti, R., Dalbies-Tran, P., Jarrier, J.L., Touze, E., Pil, I., Ponsart, C., Fabre, S., Manniaux, D. (2012). Determination of anti – Mulesian hormone concentrations in blood as a tool to select Holstein donor cows for embrio production: from the laboratory to the farm. *Reprod. Fertile. Dev*., 24, 934-944.

Silva-Santos, K.C, Santos, G.M.G., Koetz junior, C., Morotti, F., Siloto, L.S., Marcantonio, T.N., Urbano, M.R., Oliverio R.L., Lima, D.C.M., Seneda, M.M. (2014). Antral follicle populations and embryo production in vitro and in vivo of Bos indicus-taurus donors from weaning to yearling ages. *Reprod. Domest Anim*., 49, 228-232

Singh, J., Dominguez, M., Jaiswal, R., Adams, G.P. (2004). A simple ultrasaund test to predict the superstimulatory response in cattle. *Theriogenology*, 62, 227-243.