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A farm-level comparison of health disorders and reproductive performance in Holstein-Gyr and Holstein dairy cows

Isabelle Matos Macedo [a,b], Maria Lucia Gambarini [a,*], Paulo César de Oliveira Filho [a], Arthur Araújo Peixoto [a], Ozana de Fátima Zacaroni [a], Fabio Soares de Lima [b,**]

[a] Center for Studies and Research in Animal Reproductive Biology (NEP-BRA), College of Veterinary and Animal Science, Federal University of Goiás, Rodovia Goiânia - Nova Veneza, km 8, Campus Samambaia, CEP 74690-900, Goiânia, Goiás, Brazil

[b] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA, 95616, United States

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ABSTRACT

Holstein-Gyr crossbred dairy cows are commonly used in Central and South American countries to combine the perceived benefits of heat and health disorders tolerance of Gyr cows with the milk production of Holstein cows. The objectives of this study were to compare the incidence of health disorders and reproductive performance in Holstein-Gyr and Holstein cows managed and housed identically in a single herd. The animals were followed up to 300 days in milk, and health and reproductive data were retrieved from farm records. Among the diseases diagnosed, breed affected only metritis, with a higher incidence in Holstein cows. Cows that calved in the cool season had an increased incidence of dystocia and hyperketonemia compared to calving in the warm season. Cows calving in the warm season had a higher incidence of stillbirth, retained fetal membranes (RFM), metritis, and multimorbidity than cows calving in the cool season. Multiparous cows had a greater incidence of retained fetal membranes, uterine diseases, purulent vaginal discharge, hypocalcemia, and morbidity than primiparous cows. An interaction between breed category and calving season was present for RFM, with Holstein cows in the warm season having a higher incidence than Holstein-Gyr. Primiparous cows had a greater pregnancy rate at first service and fewer services than multiparous cows. Morbidity and multimorbidity were associated with prolonged open days, independent of breed. In summary, Holstein-Gyr and Holstein cows housed and managed similarly experienced similar incidences of health disorders and reproductive performance, with morbidity negatively impacting the interval from calving to pregnancy in both groups.

1. Introduction

The dairy industry in Brazil has distinct characteristics that set it apart from the world's highly productive countries. Most of the country is characterized by a tropical climate with two seasons: a cool, dry season from fall to late winter and a hot season in the spring and rains near or during the summer months [1]. Dairy farms in Brazil predominantly have a grazing component, with 58.6 % primarily being grazing, followed by an integration of grazing and confinement (40.4 %), while primarily confinement is used for only 1.0 % of the farms [2]. Also, the breed composition of Brazilian herds is mainly comprised of Holstein animals crossed with Zebu, particularly Gyr, resulting in Holstein-Gyr, known as Girolando cows in Brazil. Crossbred cows, mostly Holstein-Gyr, represent a large contingent of the dairy cows used for milk production in the country, including being present in approximately 25 % of the top 100 herds in the country [3–6].

Being the fifth-highest milk producer with the second-largest herd worldwide [7], Brazil's dairy sector is still very heterogeneous, with numerous small farms utilizing varying production practices and management strategies [6]. The demographics of the dairy sector help explain the relatively low milk production per cow per day, ranging from 4.1 kg in farms milking cows once to 8.7 kg in farms milking two or three times per day [2,6]. However, a noteworthy upward trajectory in the top 100 diaries indicated that from 2017 to 2022, the average milk production per cow increased from 27.0 kg to 31.0 kg per cow per day [8]. Improving the productivity of individual animals has been a critical aspect of enhancing livestock production. However, highly productive cows demand a well-managed environment to optimize production.

* Corresponding author.
** Corresponding author.
E-mail addresses: marialucia@ufg.br (M.L. Gambarini), falima@ucdavis.edu (F.S. Lima).

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Following the world trend for housing dairy cows, 82 % of Brazil's most productive dairy farms have confinement systems [8]. Compared to other milk production systems, compost-bedded pack barns have gained popularity as an alternative for prioritizing cow comfort by improving health, longevity, and milk production [9].

It has been suggested that crossbreeding animals can be a helpful strategy in tropical countries, such as Brazil because it combines the local adaptability to diseases and heat stress of zebu breeds like Gyr with the productivity of Holstein cows [10,11]. Indeed, there have been studies reporting that zebu cattle breeds (*Bos taurus indicus*) are more tolerant to high-temperature conditions, resistant to certain diseases (e.g., parasites, mastitis, and puerperal diseases), and more adapt to grazing [3,11–13]. Several successful cases of farmers having successfully crossbred Holstein and Gyr animals to generate cows with high milk production (~15 kg/d) and adaptability to tropical conditions have been reported [5,14]. Holstein-Gyr cattle can have excellent productivity and high fertility [13], which made the breed popular amongst Brazilian dairy farms despite the lower persistency of lactation yield when compared with Holstein cows [14].

Although several studies have characterized health disorders' incidence and prevalence in dairy herds [15–18], few have evaluated crossbreed herds and assessed the impact of disease incidence on subsequent reproductive performance. Another aspect that has been scarcely investigated is the combined effects of breed, parity, and calving season on health and reproduction in crossbred dairy cows housed and managed in the same environment, especially in the confined system that are the most common choice of newer and expanding dairy farms in Brazil. Therefore, more information is necessary to characterize the incidence of health disorders and reproductive performance of crossbred Holstein-Gyr housed under a confinement system in tropical areas.

The objectives of this study were to evaluate and compare at a herd level the incidence of health disorders and reproductive performance in Holstein-Gyr and Holstein dairy cows, and to assess interactions of breed with parity, and calving season impacting health and reproductive performance.

2. Materials and methods

2.1. Farm, animals, and nutritional management

The data for this retrospective study was obtained from a commercial dairy farm in the south region of Goiás State, Brazil. The farm has ~650 lactating cows, with around half being Holstein and the other half being crossbred Holstein-Gyr animals (with genetic compositions varying from 1/2 to 15/16 Holstein). The cows were milked thrice daily in a composted bedding pack barn housing system, producing an average of 35 kg of milk/cow/day.

Cows were fed with a total mixed ration three times a day composed of corn silage, Tifton hay, cottonseed, soybean hulls, distiller's dried grains with solubles (FlexyPRO®), finely ground corn, corn rehydrated, soybean meal, and the additive RONOZYME® (RumiStar™), adjusted by production according to each pen. All diets were formulated to meet the National Research Council (NRC, 2001) requirements, and minerals and water ad libitum at every pen.

All cows were equipped with a leg sensor (AfiAct II, Afimilk Inc., Kibbutz Afikim, Israel), and data was obtained from the AfiFarm database (Afimilk Agricultural Cooperative Ltd., Kibbutz Afikim, Israel). The database included cow identification, calving data, health occurrences, reproduction, and survival data.

2.2. Health management and monitoring

The vaccination program for the cows included yearly vaccines for foot-and-mouth disease (Bovicel®) and respiratory diseases (Bovilis® Vista® Once SQ or CattleMaster GOLD FP® 5). At the beginning of the dry period, mastitis (J-Vac®) and clostridial diseases (Clostrisan® 11) vaccines were administered, followed by a calf diarrhea vaccine (Rotavec® Corona) and the second shoot against mastitis (J-Vac®) at the prepartum. Cows were also dewormed twice a year, and the drugs used were alternated. The cows were treated with bovine somatotropin (bST-Lactotropin®, Agener União, Brazil) starting at 60 days postpartum for Holstein-Gyr and 150 for Holsteins every 14 days. The treatment was stopped 14 days before the expected dry-off date.

The length of the voluntary waiting period (VWP) was 55 days. All the cows at 7 ± 3 days pos-partum received a gynecological exam (palpation and vaginal discharge evaluation through Metricheck™ device), clinical inspection, and blood samples were collected to assess β-hydroxybutyric acid (BHBA) concentration using an electronic handheld meter and electrochemical test strips (FreeStyle Precision Neo, Abbott Diabetes Care, Witney, UK). Also, the cows were checked daily at the AfiFarm system for heat detection, calving alert, and health monitoring (low production) and at the milking parlor for any clinical signs (vaginal discharge, lameness, and any abnormal behavior). Animals identified by the farm workers were examined and treated adequately on the same day by the veterinary team (Fig. 1).

The occurrence of diseases was diagnosed and recorded during all lactation. Dystocia was defined as any difficult birth resulting in assisted extraction of the calf at birth. Stillbirth was considered any case of calf death within 48 h after parturition, as described [19]. To diagnose retained fetal membranes (RFM), cows were evaluated immediately after calving and during the first 24 h postpartum. Those who did not release the fetal membranes within the first 12 h after fetal expulsion were considered positive cases of RFM. Metritis was defined as fetid, watery, reddish-brownish discharge within 21 days after calving, and purulent vaginal discharge (PVD) was considered as the presence of purulent (or mucopurulent) vaginal discharge after 21 d postpartum, as previously described [20]. An animal was considered to have uterine diseases if it had metritis or PVD. A cow was considered hypocalcemic if she could not rise following calving or remained prostrated with cold extremities and decreased rectal temperature (<38 °C) and responded to intravenous calcium treatment within 72 h. Hyperketonemia was diagnosed routinely at day 7 ± 3 days postpartum or when presented with depressed appetite based on elevated blood BHBA (≥1.2 mmol/L). Metabolic disorders were considered any animal that experienced hypocalcemia or hyperketonemia.

Mastitis diagnoses were made at every milking by farm-trained staff. Following the milking routine, the teats were immersed in a pre-dipping solution and cleaned with a paper towel. Next, the forestripping was performed to identify signs of abnormal milk (e.g., clots, flakes, or watery appearance), and other signs such as udder edema, heat, hardness, redness, or pain were also observed. The milk machine was attached and removed when the milk flow stopped, and the post-dipping solution was submerged in each teat. In the case of clinical mastitis (CM), a milk sample was collected for culture and microbiological diagnosis (AccuMast®, FERA, College Station, EUA). Milkers registered all CM cases in spreadsheets, including animal identification, CM diagnosis date, culture result, treatment start date, affected teat(s), and drug(s) used for treatment. Cows with at least one documented disorder were considered as having an event of morbidity.

2.3. Reproductive management

Cows without clinical signs and reproductive tract disorders at the end of the VWP were eligible to start the reproductive program. The farm used two reproductive programs: artificial insemination (AI) with heat detection and timed AI (TAI) protocol. At the end of the VWP (55 ± 3 DIM), all animals were inspected by palpation and ultrasonography to diagnose uterine and ovarian conditions (Fig. 1). For cows with a corpus luteum (CL) at the exam, an intramuscular injection of prostaglandin $PGF_{2\alpha}$ (Sincrocio®, Ourofino Animal Health) was made, and the AI was performed after heat detection. For cows with no CL or with high milk

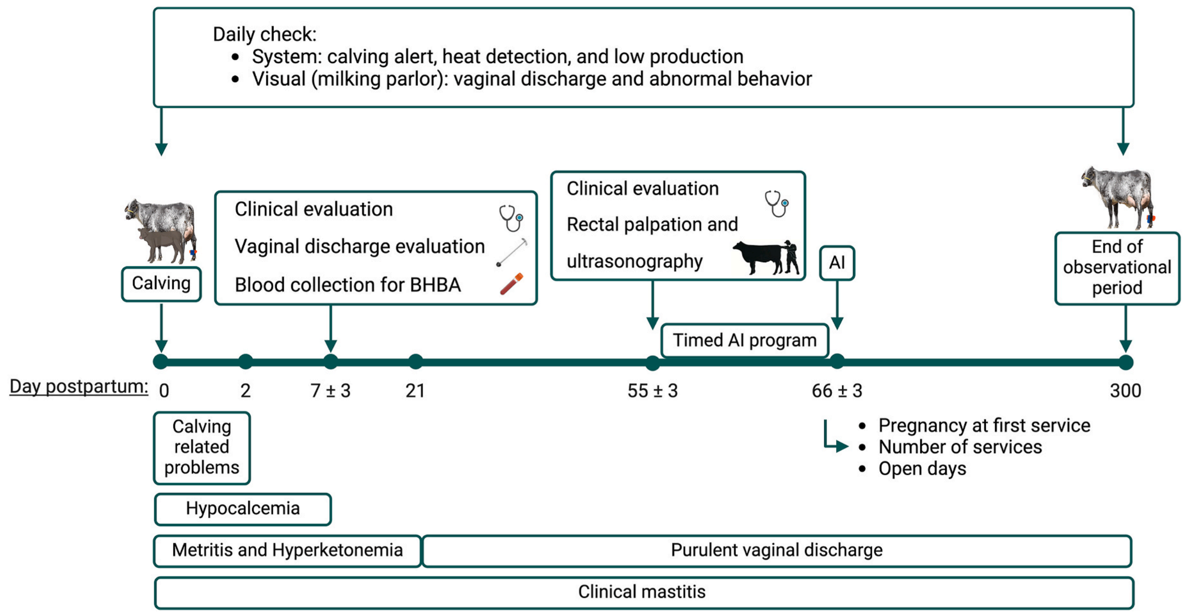

Fig. 1. Diagram of activities, health disorders diagnosed (closed boxes), and fertility responses evaluated. BHBA = β-hydroxybutyric acid; AI = artificial insemination. The timed AI protocol (d 55 GnRH injection, estradiol benzoate injection, and progesterone implant, d 62 PGF2α injection, d 64 PGF2α injection, estradiol cypionate, and implant removal, and AI at heat detection or d 66) started when cows averaged 55 ± 3 d postpartum, and AI was performed when cows averaged 66 ± 3 d postpartum.

production, the TAI protocol (d0 - GnRH injection, estradiol benzoate injection and progesterone implant, d7 - PGF2α injection, d9 - PGF2α injection, estradiol cypionate and implant removal, d11 - AI) was initiated. Semen straws from different Holstein bulls were used for AI, and pregnancy diagnosis was performed 33 ± 2 days after AI by an ultrasound examination. The presence of an embryo within the amniotic vesicle with a normal heartbeat was the criterion to determine pregnancy. The cows detected in estrus before the expected date of pregnancy diagnosis were inseminated 12 h after heat detection. Cows diagnosed as non-pregnant without a CL were re-enrolled in the TAI protocol, while cows diagnosed as non-pregnant with CL received prostaglandin treatment and were re-inseminated after being heat detected.

2.4. Data collection

Research team members visited the herd monthly to check the health diagnosis and reproductive process standardization and collect the data. Between October 01, 2020, and September 30, 2021, all calving events were analyzed, and each cow-related event was followed up and registered until 300 DIM. Animals with two calving events during this period were considered only the first calving event, and cows without breed information or with missing data were not included in the study. The herd management software (AfiFarm®, Brazil) was used to record all the data. Mastitis incidents were documented in spreadsheets, including milk culture results and treatments.

In the present study, the dependent variables that were evaluated related to reproductive performance include the pregnancy at first service, number of services per conception, and days open (DO). Additionally, the number of disease cases (none, one, or two or more cases) and the incidence of dystocia, stillbirth, RFM, metritis, PVD, clinical hypocalcemia, hyperketonemia, and CM were assessed. Cows with at least one documented health disorder were considered to have morbidity, while those with two or more health disorders were considered to have multimorbidity.

Calving events between September 22 and March 20 were defined as the warm season, and calving between March 21 and September 21 was defined as the cool season. Retrospective weather data were collected from the Fazenda Brasilanda's Weather Station (FieldClimate – METOS® by Pessl Instruments), located 1 km from the dairy farm. The average daily temperature for the warm season was 23.2 °C, with a maximum of 34.1 °C and a minimum of 16.1 °C, and the average relative humidity was 76.8 %, with a maximum of 99.4 % and a minimum of 24.3 %. The average daily temperature for the cool season was 20.7 °C, with a maximum of 33.1 °C and a minimum of 7.6 °C, and the average relative humidity was 60.0 %, with a maximum of 98.5 % and a minimum of 17.0 %. The THI for the warm season was 71.72, and the THI for the cool season was 66.74. The monthly rainfall average for the warm season was 134.9 mm, and for the cool season was 24.1 mm.

A total of 583 lactating dairy cows were used for the study, consisting of 276 Holstein-Gyr and 307 Holsteins cows. Among them, 359 were multiparous, while 224 were primiparous. Additionally, 256 animals gave birth during the warm season, while 327 calved during the cool season.

2.5. Statistical analyses

All data were recorded and organized in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) before statistical analysis. For all analyses, breed was treated as the independent variable, and health events and reproductive outcomes were treated as the dependent variable.

For categorical data (dystocia, stillbirth, RFM, metritis, PVD, hyperketonemia, hypocalcemia, clinical mastitis, pregnancy 1st AI, number of services, morbidity, and multimorbidity) were analyzed using multivariable logistic regression considering binary distribution on JMP statistical software (SAS Institute Inc., Cary, NC). All models include the effect of breed (Holstein vs. Holstein-Gyr), parity (multiparous vs. primiparous), calving season (cool vs. warm), and their interactions. Each dependent variable was individually analyzed in a stepwise regression model, where the stipulations stopped when the lower AIC (Akaike information criterion) value was reached.

The effects of breed, morbidity, and multimorbidity on time to pregnancy and proportion were analyzed using survival analysis with Cox's proportional hazard model on JMP. For time from calving to pregnancy, cows that did not become pregnant were censored when they left the herd or at 300 DIM, whichever occurred first. The survival analysis on JMP was used to generate the least squares means ± standard error of the mean and median days to the event, while the survival curves were generated using the Kaplan-Meyer survival curves of MedCalc version 9.2 for Windows (MedCalc Software bvba, Mariakerke, Belgium).

In all analyses, the significance level was set at $P \leq 0.05$, and P values between 0.05 and 0.1 were considered a tendency.

3. Results

3.1. Descriptive data

During the observational period, 316 animals (54.2 %, 316/583) were affected by at least one health disorder (morbidity), including 84 cows (14.4 %, 84/583) that presented two or more disorders (multimorbidity). For calving-related problems, the incidence of dystocia, stillbirth, and RFM was 8.6 % (50/583), 4.3 % (25/583), and 7.0 % (41/583), respectively. Among metabolic diseases, the incidence of clinical hypocalcemia was 2.4 % (14/583) cases, and the incidence of hyperketonemia was 3.4 % (20/583). In addition, 22.8 % (133/583) of animals had metritis, 11.7 % (68/583) had PVD, and 23.3 % (136/583) had at least one clinical mastitis case during lactation.

3.2. Health disorders

Holstein cows had a higher incidence of metritis than Holstein-Gyr ($P = 0.001$) and tended ($P = 0.07$) to have a greater incidence of uterine diseases (Table 1). However, no differences in the incidence of dystocia, stillbirth, RFM, PVD, metabolic disorders, hypocalcemia, hyperketonemia, and mastitis were identified between Holstein and Holstein-Gyr cows (Table 1). Cows calving in the cool season had a lower incidence of stillbirth and RFM and a higher incidence of dystocia and hyperketonemia than cows calving in the warm season (Table 1). Multiparous cows had a higher incidence of RFM, uterine diseases, PVD, and hypocalcemia than primiparous cows (Table 1).

There were no interactions between breed category and parity (Table 2), but an interaction between breed category and calving season was present for RFM, with Holsteins having a higher incidence of RFM than crossbred cows in the warm season. In the cool season, there were no differences between the breed categories (Table 3).

For morbidity, there was a higher incidence (P = 0.01) in multiparous than primiparous (76.7 ± 3.7 vs. 57.9 ± 4.7). There was a tendency (P = 0.09) for Holstein-Gyr to have a lower incidence of morbidity (62.2 ± 4.4 vs. 72.3 ± 4.1) than Holstein cows. Likewise, there was a tendency for cows calving in the warm season to have a higher incidence of morbidity than cows calving in the cool season (72.8 ± 4.5 vs. 61.9 ± 4.0), nor were there interactions between breed category and season and breed category and parity (Fig. 2A and B). However, there were no interactions between breed category and season or breed category and parity. Cows in the warm season had greater incidence (P = 0.01) of multimorbidity than herdmates in the cool season (62.2 ± 4.4 vs. 72.3 ± 4.1), but no effects of the breed category, nor interactions between breed category or season and breed category and parity were present (Fig. 2C and D).

3.3. Reproductive performance

There were no differences in the distribution of type insemination (estrus detection or timed AI) between breeding group, parity, or calving season (Table 4). The pregnancy for the 1st AI was not different ($P = 0.74$) between breed categories (Holstein-Gyr = 41.7 % vs. Holstein = 44.0 %), and the number of services was also not different ($P = 0.42$) between breed categories (Holstein-Gyr = 2.55 vs. Holstein = 2.66). Primiparous cows had greater ($P < 0.001$) pregnancy for the 1st AI than multiparous cows (Fig. 3A), but there were no effects of calving season ($P = 0.20$) in pregnancy for the 1st AI (Fig. 3A and B). There were no interactions between breed and parity ($P = 0.14$), nor between breed and calving season ($P = 0.69$) in pregnancy for the 1st AI (Fig. 3A and B).

Multiparous cows had a greater ($P < 0.01$) number of services per pregnancy when compared with primiparous cows (Fig. 3C), but breed category ($P = 0.42$) and calving season ($P = 0.38$) did not impact the number of services per pregnancy (Fig. 3C and D). There were no interactions between breed and parity ($P = 0.22$), nor between breed and calving season ($P = 0.94$) for the number of services per pregnancy (Fig. 3C and D).

There were no differences ($P = 0.69$) between breed categories for pregnancy interval (Fig. 4). The median and mean days to pregnancy for Holstein-Gyr were 100 d (95 % CI = 89 to 108) and 124.4 ± 4.3 d, respectively, and for Holstein cows were 95 d (95 % CI = 80 to 105) and 127.9 ± 4.5 d, respectively.

There was a breed-by-morbidity interaction (P < 0.001) for pregnancy interval between categories (Fig. 5). Holstein-Gyr with multiple health disorders had higher time from calving to pregnancy (Mean: 165.0 ± 14.0 d; Median: 149.5 d, 95 % CI = 106 to 231), followed by Holstein cows with at least one health disorder (Mean: 142.3 ± 7.6 days; Median: 106.5 d, 95 % CI = 95 to 129), Holstein with multiple health disorders (Mean: 135.6 ± 9.9; Median: 149.5 d, 95 % CI = 106 to 231), Holstein-Gyr with at least one health disorder (Mean: 134.4 ± 6.8; Median: 108.5 d, 95 % CI = 98 to 139), Holstein with none health disorder (Mean: 105.3 ± 5.4; Median: 74 d, 95 % CI = 70 to 84), and Holstein-Gyr with none health disorder (Mean: 101.4 ± 4.6; Median: 78 d, 95 % CI = 73 to 92).

4. Discussion

This study's main objective was to compare two of the main breeds (Holstein vs. Holstein-Gyr crossbreed) used in Brazil's dairy farms under the same management and production system. Overall, when managed and housed similarly, the breed category had a minor impact on the

Table 1
Incidence (%) of health disorders (least squares mean and standard error) by breed, calving season (CS), and parity. Asterisks (*) indicate significant differences ($P \leq 0.05$). RFM = retained fetal membranes; PVD = purulent vaginal discharge; CS = calving season.

Health disorder	Breed		Calving season		Parity		*P*-value		
	Holstein-Gyr	Holstein	Warm	Cool	Primiparous	Multiparous	Breed	CS	Parity
Dystocia	8.9 ± 1.8	7.5 ± 1.6	5.4 ± 1.8	11.0 ± 1.6	8.5 ± 1.9	7.8 ± 1.5	0.57	0.02*	0.77
Stillbirth	3.8 ± 1.3	3.8 ± 1.3	6.6 ± 1.3	2.9 ± 1.1	5.2 ± 1.4	4.3 ± 1.1	0.29	0.03*	0.63
RFM	5.8 ± 1.5	8.1 ± 1.5	10.1 ± 1.6	3.8 ± 1.4	4.2 ± 1.7	9.6 ± 1.3	0.29	0.003*	0.01*
Uterine diseases	30.2 ± 3.0	37.7 ± 2.8	36.9 ± 3.0	30.9 ± 2.7	29.7 ± 3.2	38.1 ± 2.5	0.07	0.13	0.04*
Metritis	19.0 ± 2.6	28.4 ± 2.4	27.3 ± 2.6	20.0 ± 2.4	24.4 ± 2.8	22.7 ± 2.2	0.001*	0.04*	0.60
PVD	11.2 ± 2.0	9.3 ± 1.9	9.6 ± 2.0	10.9 ± 1.8	5.1 ± 2.2	15.4 ± 1.7	0.50	0.61	0.0002*
Metabolic disorders	5.4 ± 1.5	5.0 ± 1.4	4.7 ± 1.5	5.8 ± 1.3	4.2 ± 1.6	6.3 ± 1.2	0.84	0.57	0.30
Hyperketonemia	3.4 ± 1.2	3.2 ± 1.1	1.5 ± 1.2	5.0 ± 1.0	3.7 ± 1.2	2.8 ± 1.0	0.87	0.02*	0.58
Hypocalcemia	2.0 ± 1.0	2.1 ± 0.9	3.1 ± 1.0	1.0 ± 0.9	0.5 ± 1.0	3.7 ± 0.8	0.94	0.10	0.01*
Clinical mastitis	22.0 ± 2.7	24.3 ± 2.5	24.1 ± 2.7	22.2 ± 2.4	21.1 ± 2.9	25.1 ± 2.3	0.53	0.59	0.27

Table 2
Incidence (%) of health disorders (least squares mean and standard error) according to parity in Holstein and Holstein-Gyr animals. Asterisks (*) indicate significant differences ($P \leq 0.05$). RFM = retained fetal membranes; PVD = purulent vaginal discharge.

Health disorder	Primiparous		Multiparous		*P*-value		
	Holstein-Gyr	Holstein	Holstein-Gyr	Holstein	Breed	Parity	Bred*Parity
Dystocia	8.5 ± 2.9	8.6 ± 2.4	9.2 ± 2.1	6.4 ± 2.1	0.57	0.77	0.55
Stillbirth	5.4 ± 2.1	4.9 ± 1.8	2.2 ± 1.5	6.4 ± 1.6	0.29	0.63	0.17
RFM	3.3 ± 2.6	5.2 ± 2.2	8.2 ± 1.9	11.0 ± 1.9	0.29	0.01*	0.85
Uterine diseases	26.9 ± 4.9	32.4 ± 4.1	33.4 ± 3.5	42.9 ± 3.6	0.07	0.04*	0.62
Metritis	22.6 ± 4.3	26.6 ± 3.6	15.3 ± 3.1	30.1 ± 3.2	0.001*	0.60	0.13
PVD	4.3 ± 3.3	5.9 ± 2.8	18.0 ± 2.4	12.8 ± 2.4	0.50	0.0002*	0.21
Metabolic disorders	3.2 ± 2.4	5.2 ± 2.0	7.6 ± 1.7	4.9 ± 1.8	0.84	0.30	0.24
Hyperketonemia	3.1 ± 1.9	4.4 ± 1.6	3.7 ± 1.3	1.9 ± 1.4	0.87	0.58	0.32
Hypocalcemia	0.1 ± 1.6	0.8 ± 1.3	3.9 ± 1.1	3.5 ± 1.2	0.94	0.01*	0.69
Clinical mastitis	21.5 ± 4.4	20.7 ± 3.7	22.4 ± 3.1	27.9 ± 3.3	0.53	0.27	0.39

Table 3
Incidence (%) of health disorders (least squares mean and standard error) according to the calving season in Holstein and Holstein-Gyr animals. Asterisks (*) indicate significant differences ($P \leq 0.05$). RFM = retained fetal membranes; PVD = purulent vaginal discharge; CS = calving season.

Health disorder	Warm		Cool		*P*-value		
	Holstein-Gyr	Holstein	Holstein-Gyr	Holstein	Breed	CS	Breed*CS
Dystocia	7.5 ± 2.5	3.3 ± 2.5	10.2 ± 2.4	11.7 ± 2.1	0.57	0.02*	0.22
Stillbirth	4.4 ± 1.8	8.7 ± 1.8	3.3 ± 1.7	2.6 ± 1.5	0.29	0.03*	0.14
RFM	6.8 ± 2.3	13.4 ± 2.3	4.8 ± 2.2	2.8 ± 1.9	0.29	0.003*	0.04*
Uterine diseases	31.0 ± 4.3	42.8 ± 4.2	29.3 ± 4	32.5 ± 3.5	0.07	0.13	0.27
Metritis	19.6 ± 3.7	35.1 ± 3.7	18.4 ± 3.5	21.6 ± 3.1	0.001*	0.04*	0.08
PVD	11.4 ± 2.9	7.8 ± 2.8	11.0 ± 2.7	10.9 ± 2.4	0.50	0.61	0.51
Metabolic disorders	5.3 ± 2.1	4.0 ± 2.1	5.5 ± 2.0	6.1 ± 1.7	0.84	0.57	0.63
Hyperketonemia	1.4 ± 1.6	1.7 ± 1.6	5.4 ± 1.6	4.6 ± 1.4	0.87	0.02*	0.73
Hypocalcemia	3.9 ± 1.4	2.3 ± 1.4	0.1 ± 1.3	1.9 ± 1.1	0.94	0.10	0.17
Clinical mastitis	22.0 ± 3.8	26.1 ± 3.8	21.9 ± 3.6	22.4 ± 3.2	0.53	0.59	0.60

incidence of health disorders and no differences in reproductive performance. An interesting fact aligned with previous studies using only Holstein cows or Holstein [18] and other crossbreed cows [15] was that morbidity was a significant burden for reproductive performance, prolonging the time from calving to pregnancy. Those findings highlight the importance of dairy farms developing a robust program to mitigate herd health disorders and their detrimental impact on animal performance independent of the breeding category.

Overall, our study found morbidity and multimorbidity incidences were similar between breeds and resembled the range found in other studies, with mostly Holstein cows in the U.S. and Japan [18,21]. Amongst the specific diseases investigated, metritis was the only disease with differences in incidence between the breed categories, with Holstein cows having a higher incidence than Holstein-Gyr cows. These findings are aligned with previous epidemiological studies that suggest a lower range of metritis for crossbreds such as Holstein-Jersey (between 5.5 and 10.9 % [15,22]) than Holstein cows (between 20 and 40 % of incidence [23]). Metritis is a complex multifactorial disease with several risk factors widely described in previous studies, such as parity, haptoglobin, dystocia, twins, stillbirth, abortion, uterine prolapse, retained placenta, and negative energy balance [24] Some of the potential explanations for the higher incidence of metritis might be a predisposition for dystocia due to the higher frequency of larger calves [25] or potentially greater immunological challenges related to metabolic challenges associated with higher milk production. Additionally, we found an interaction between breed and season for RFM, meaning a higher incidence of RFM during the warm season for Holstein cows than Holstein-Gyr cows, which likely contributed to their higher metritis incidence. Heat stress is another relevant factor, as it can impair immune function and increase disease risk [26,27]. It has been suggested that crossbreed animals such as Holstein-Gyr might have thermotolerance that confers better comfort during the hotter months [11] and have lower milk yield compared to Holstein cows [28]. The presence of a high proportion of Holstein genes and milk yield could be associated with lower immune capacity and higher risk for infectious disease cases, reducing the combined stress of heat and metabolic load. These physiological differences may help explain the lower metritis incidence in Holstein-Gyr cows observed in this study.

Multiparous cows had higher morbidity, incidence of uterine-related disorders (RFM and PVD), and hypocalcemia than primiparous. Cows with multiple parturitions have higher metabolic demands to produce milk and experience a decrease in immunocompetence, making them more susceptible to diseases [29]. Additionally, calcium homeostasis is more likely to be a problem, and calcium is an essential mineral for the immune system [30]. Regarding calving season, the incidence of stillbirth, RFM, and metritis was higher during the warmer months (October–March). High temperatures can trigger signs of heat stress in dairy cows, leading to reduced feed intake and immune competence [26], thereby increasing vulnerability to postpartum diseases. The results for incidence are aligned with those of Molinari et al. [31], who observed more metritis cases during the months of high THI in Florida.

On the other hand, the decrease in temperatures generally leads to an increase in voluntary food intake, a rise in resting metabolic rate, and an increase in metabolic capacity [32], which can support immune competence and reduce some health risks compared to heat stress conditions. Multimorbidity, dystocia, and hyperketonemia were found to have a higher incidence during the cool season (March–September). As feed intake increases during the colder season, the live body weight of cows and the blood flow to the uterus increase, leading to heavier calf birth weight, and contributing to a greater risk of dystocia during the cool season [33,34]. Dystocia and the disruption of the energetic balance were described as risk factors for clinical ketosis, once it can lead to decreased feed intake during the postpartum [35].

As anticipated, primiparous had increased pregnancy per AI for the first service and reduced the number of services than multiparous [36]. However, there was no interaction between breed and parity and breed and season for reproductive responses, suggesting that in the same environment and management, Holstein and Holstein-Gyr are impacted

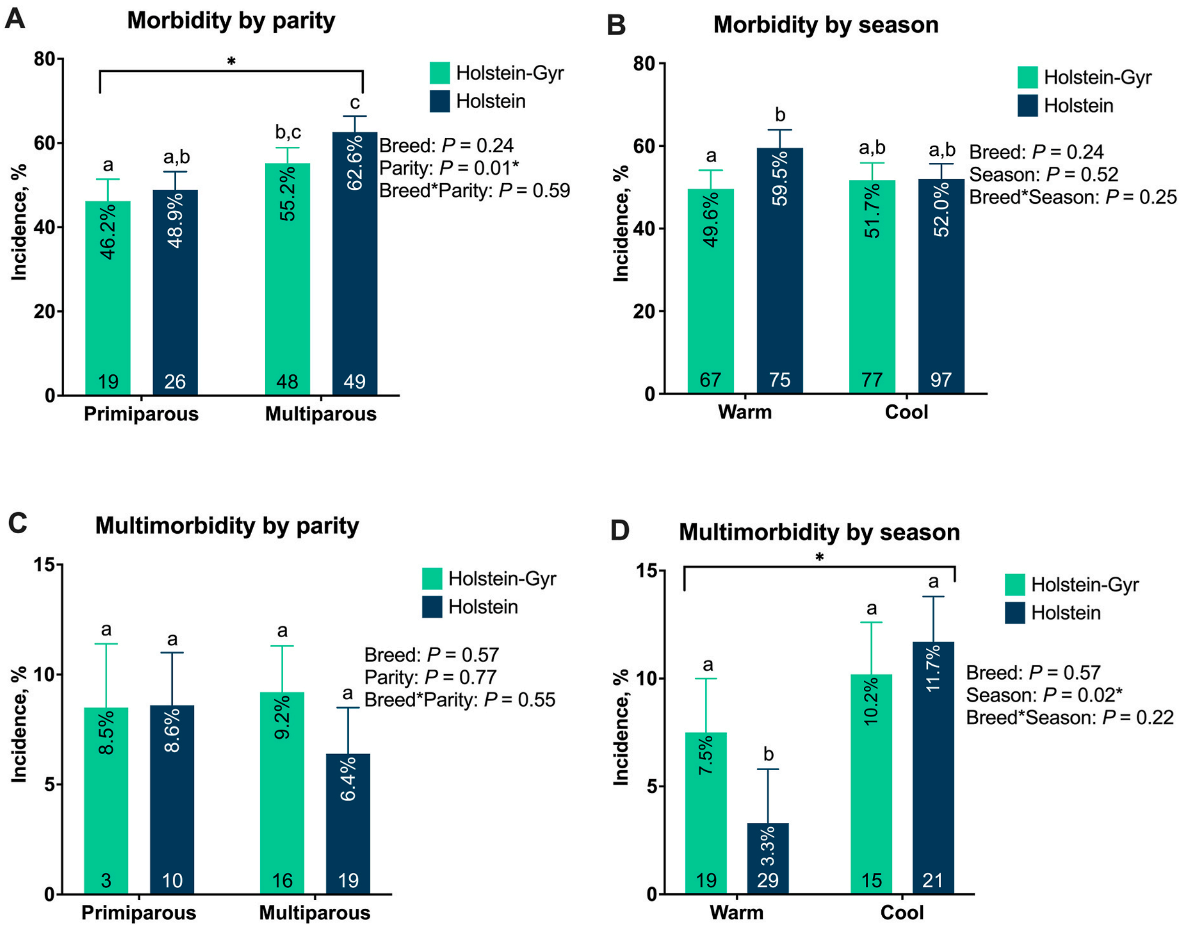

Fig. 2. Bar graph illustrating the results for morbidity (at least one case of health disorder; A and B) and multimorbidity (two or more cases of health disorders; C and D) by parity and calving season. Error bars represent the standard error of the mean. Bars represent the estimated marginal mean ± S.E.M. The proportion is listed above the bar graph, and the number of cows per group is within the bar at the bottom. Brackets and asterisk (*) indicate significant differences (P ≤ 0.05) between groups. Different superscripts indicate significant differences (P ≤ 0.05) between categories within and between groups.

Table 4
Incidence (%) of health disorders (least squares mean and standard error) according to the calving season in Holstein and Holstein-Gyr animals. Asterisks (*) indicate significant differences ($P \leq 0.05$). RFM = retained fetal membranes; PVD = purulent vaginal discharge; CS = calving season.

	Warm		Cool		*P*-value		
	Holstein-Gyr	Holstein	Holstein-Gyr	Holstein	Breed	CS	Breed*CS
Primiparous							
Estrus detected	72.1 % (31/43)	82.8 % (48/58)	80.0 % (40/50)	82.8 % (48/58)	0.29	0.03*	0.14
Timed AI	27.9 % (12/43)	17.2 % (10/58)	20.0 % (10/50)	17.2 % (10/58)	0.29	0.003*	0.04*
Multiparous							
Estrus detected	72.1 % (31/43)	82.8 % (48/58)	66.3 % (63/95)	82.8 % (48/58)	0.001*	0.04*	0.08
Timed AI	27.9 % (12/43)	17.2 % (10/58)	33.7 % (32/95)	17.2 % (10/58)	0.50	0.61	0.51

by parity and season similarly. In the present study, diseases significantly impacted the increase of days open for Holstein and Holstein-Gyr animals. The morbidity effect on reproduction performance is well established and reported previously [15,18] The occurrence of inflammatory disorders, especially during the post-partum, has been associated with reduced fertilization of oocytes, impaired early conceptus development, caused inflammation-like changes in conceptus cell gene expression, and increased odds of pregnancy loss [37]. Macmillan et al. [38] also reported that multimorbidity impacted the reproduction performance of cows more than one or no disease cases. In his study, healthy cows and cows diagnosed with one postpartum health disorder had 1.4 and 1.3 times, respectively, greater risk of pregnancy up to 150 DIM compared to those diagnosed as having two or more postpartum health disorders. Similarly to this find, Ribeiro et al. [15] reported that 60.3 % of cows with no disease during the postpartum period were pregnant on day 65 after the first insemination, while 48.8 % of the cows with single clinical disease were pregnant, and 29.3 % of cows with multiple clinical diseases were pregnant.

5. Conclusion

This study evaluated and compared the incidence of health disorders and reproductive performance between Holstein-Gyr and Holstein cows and assessed interactions with parity and calving season. Under the same management and housing conditions, both breeds showed similar overall health and reproductive outcomes, with breed influencing only the incidence of metritis. In contrast, parity and calving season had a greater impact on health disorders and reproductive performance.

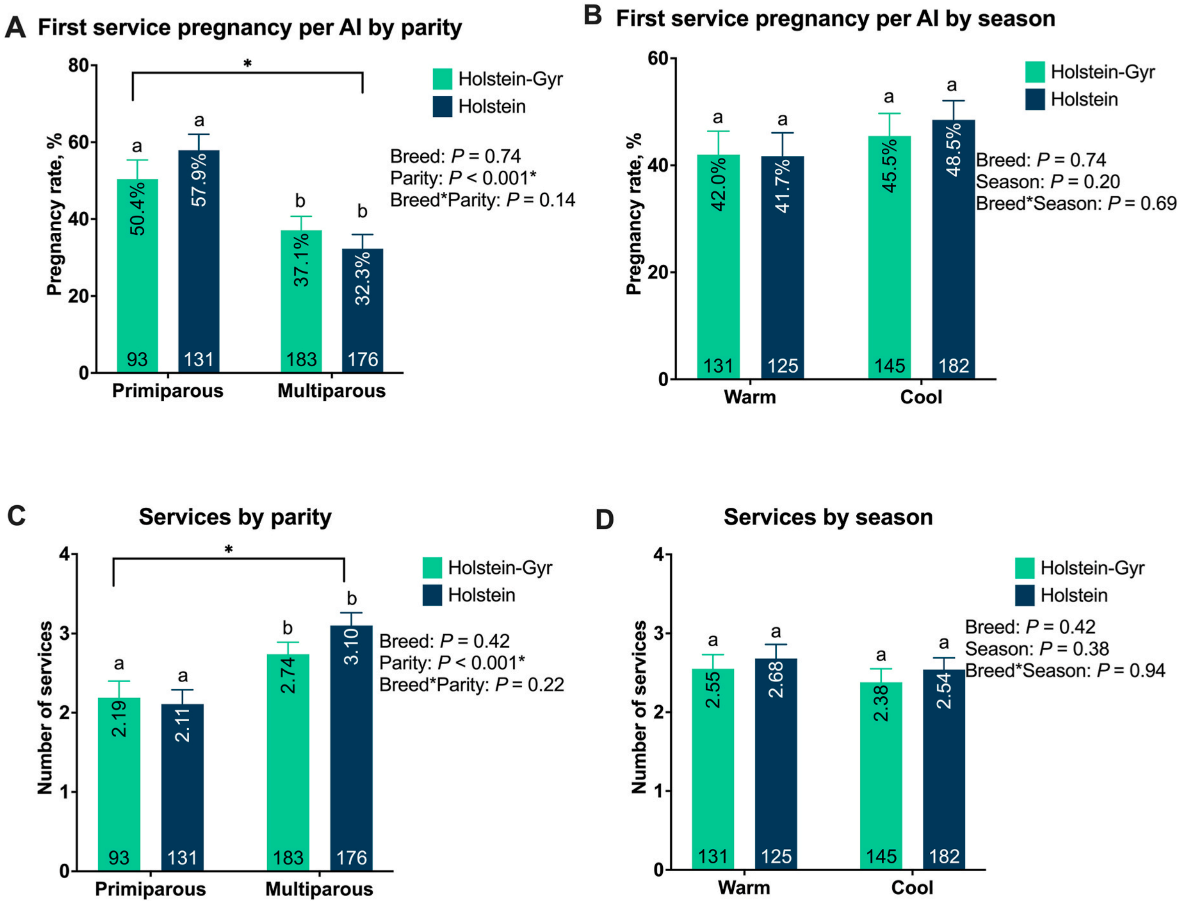

Fig. 3. Bar graph illustrating the results for first service pregnancy per AI (A and B) and services (C and D) by parity and calving season. Bars represent the estimated marginal mean ± S.E.M. The proportion is listed above the bar graph, and the number of cows per group is within the bar at the bottom. Brackets and asterisk (*) indicate significant differences (P ≤ 0.05) between groups. Different superscripts indicate significant differences (P ≤ 0.05) between categories within and between groups.

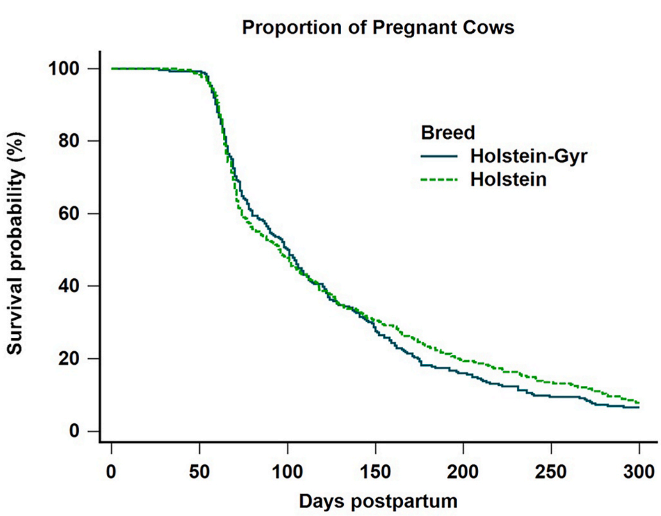

Fig. 4. Survival curves for non-pregnant cows (%) related to days postpartum according to breed (Holstein-Gyr and Holstein) in lactating dairy cows (P = 0.66). The mean and median interval to pregnancy for Holstein-Gyr was 124.4 ± 4.3 d and 100 d (95 % CI = 89 to 108), respectively. The mean and median interval to pregnancy for Holstein was 127.9 ± 4.5 d and 95 d (95 % CI = 80 to 105), respectively. The proportion remaining not pregnant is shown on the y-axis.

Therefore, these findings underscore that in well-managed systems, Holstein and Holstein-Gyr cows have similar health disorders and reproductive performance, and improving management practices to reduce disease incidence and overcome seasonality may have a greater impact on herd health and reproductive efficiency than breed selection alone.

CRediT authorship contribution statement

Isabelle Matos Macedo: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Lucia Gambarini:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Paulo César de Oliveira Filho:** Investigation. **Arthur Araújo Peixoto:** Investigation. **Ozana de Fátima Zacaroni:** Investigation. **Fabio Soares de Lima:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Conceptualization.

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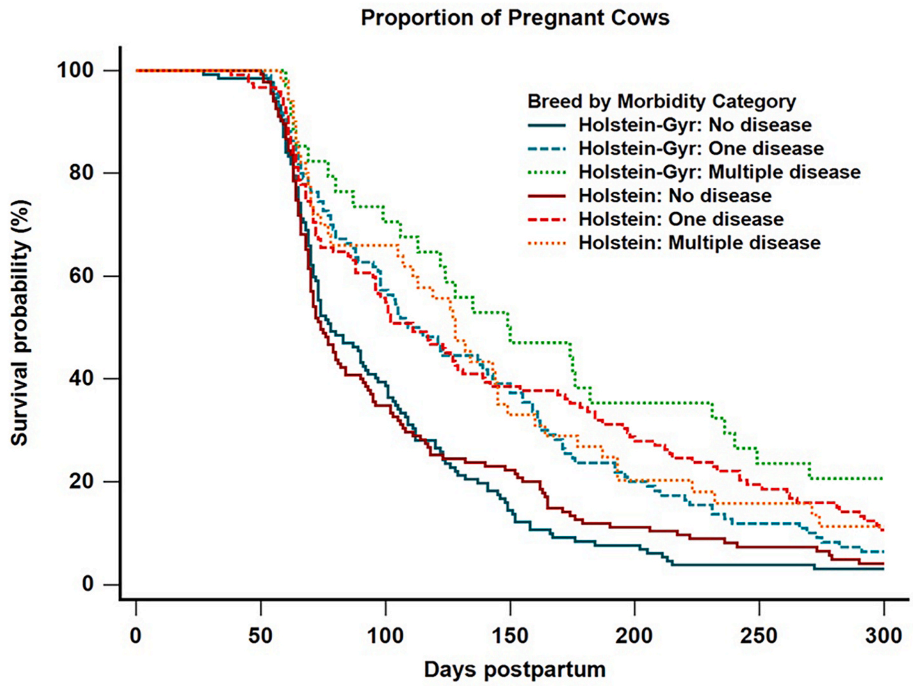

Fig. 5. Survival curves for non-pregnant cows (%) related to days postpartum according to morbidity (none, one, or two or more health disorders) in lactating Holstein and Holstein-Gyr dairy cows ($P < 0.001$). The mean and median interval to pregnancy for Holstein-Gyr without morbidity was 124.4 ± 4.3 d and 100 d (95 % CI = 89 to 108), respectively. The mean and median interval to pregnancy for Holstein-Gyr with one disease was 101.4 ± 4.6 d and 100 d (95 % CI = 89 to 108), respectively. The mean and median interval to pregnancy for Holstein-Gyr with multiple diseases was 165.0 ± 14.0 d and 100 d (95 % CI = 89 to 108), respectively.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the article titled "A farm-level comparison of health disorders and reproductive performance in Holstein-Gyr and Holstein dairy cows."

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