Endocrine and reproductive parameters in sick and healthy primiparous and multiparous dairy cows

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To investigate the association of health status and parity with hormone profiles during the transition period and reproductive parameters in Holstein dairy cows, a prospective observational cohort study was carried out including only healthy primiparous (PP, n = 116) and multiparous (MP, n = 172) cows at the beginning of the study. A subset of 120 healthy and sick cows was randomly selected for insulin, IGF-I, leptin and adiponectin determination. Primiparous cows had greater IGF-I and adiponectin concentrations (P < 0.05) and tended (P = 0.07) to have greater insulin concentrations than MP cows. While healthy and sick MP and sick PP cows presented a sharp decrease in IGF-I concentrations after calving, healthy PP cows maintained them. Postpartum adiponectin concentrations were lower in sick than in healthy MP cows. A greater percentage of healthy cows ovulated during the first 7 weeks after calving when compared to sick cows (67.9% vs 50%, P = 0.002) and a similar trend was found for MP vs PP cows (64% vs 53%, P = 0.01). More healthy cows were inseminated in comparison to sick cows (94% vs 76.5%, P < 0.01) and more PP than MP cows (90.4% vs 82.7%, P < 0.05). Similarly, healthy cows presented a greater proportion of pregnancy than sick cows (75% vs 54%, P < 0.01) and the proportion of pregnancy was higher in PP than in MP cows (74% vs 56%, P = 0.04). Health status interacting with parity yielded different endocrine profiles, which may partially explain the differences in reproductive performance.

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1. Introduction

Due to intensive genetic selection in addition to improvements in nutrition and management of dairy herds, milk production has increased substantially over the last decade [1]. This increase in milk production demands a correct nutritional balance in order to fulfill lactation requirements despite the decrease in voluntary dry matter intake (DMI) around calving [2,3]. The uncoupling of the somatotrophic axis (high growth hormone [GH] vs low insulin-like growth factor-I [IGF-I] concentrations), the increase in hepatic gluconeogenesis, lower glucose utilization by peripheral tissues, and lipolysis are homeorhetic adaptations for increasing energy substrates availability [4,5].

Also, the interrelationship of the endocrine, metabolic and immune system may induce a depressed immune system around calving by activating local and systemic defense mechanisms that induce inflammation [6]. This increases the chances of getting sick as it has been shown that 30%–50% of the cows are affected by some form of metabolic or infectious disease around calving [7–9] in addition to reproductive problems or/and increased culling rates [6,10,11]. Additionally, it is known that peripartum diseases are interrelated; e.g., retained placenta to hypocalcemia [12] (which is considered a gateway disease [13]) predisposing an animal to metritis and mastitis [14,15] or affecting reproductive performance [16,17]. Indeed, reproductive failure is still an important reason for involuntary culling in dairy herds [18].

Furthermore, parity affects metabolic adaptation to lactation; primiparous cows, which have not reached their adult body size...
and continue to grow during pregnancy and lactation, present metabolic differences compared to older cows [19]. The competing demands of the mammary gland are superimposed on growth requirements, and both insulin and IGF-I stimulate growth. The profiles of these anabolic hormones and metabolites (such as non-esterified fatty acids [NEFA]) during the transition period according to parity have been inconsistent [20,21]. IGF-I and insulin concentrations are low around calving (because of homeorhetic adaptation), affecting the reproductive axis at both central (hypothalamus-pituitary gland) and peripheral (gonads, reproductive tract, and embryo) level [22]. Indeed, a positive relation between IGF-I and the first postpartum ovulation has been demonstrated [23,24]. Moreover, low IGF-I concentrations were reported in cows developing postpartum diseases, but because of the limited number of cows used, the authors concluded that a reliable association could not be established [25].

Adiponectin and leptin, because of their insulin sensitizing actions and association with body condition, could be potential regulators of metabolism during the transition from pregnancy to lactation [26]. Even if no relationship between leptin and the first postpartum luteal activity could be demonstrated, higher leptin concentrations were associated with shorter intervals to the first observed estrus [27]. Adiponectin is not only a metabolic hormone, as has also been linked to inflammation. Indeed, low adiponectin concentrations around calving have been proposed to be linked to inflammatory disease pathophysiology [28] and could be another mechanism that contributes to poor fertility in dairy cows [26]. As far as we know, there are no reports on endocrine (insulin, IGF-I, leptin and adiponectin) profiles during the transition period in sick and healthy primiparous and multiparous cows and their association with reproductive success.

Taking these findings into account, the objectives of this study were to assess whether health status interacted with parity affecting hormone profiles (IGF-I, insulin, adiponectin, and leptin) during the transition period, and if they were associated with reproductive performance in primiparous (PP) and multiparous (MP) Holstein cows.

2. Materials and methods

2.1. Cows and herd management

The study was conducted in a commercial dairy free stall (low-housing system) herd in Rio Grande do Sul, southern Brazil. All procedures were carried out in accordance with regulations of the Animal Experimentation Committee (SIPPEE 10.059.15 CEUA 0522017 UNIPAMPA, Brazil).

From a 700-cow herd, 288 Holstein dairy cows (n = 116 PP and n = 172 MP) were selected, with an approximate milk yield of 8000 kg per lactation and two calving seasons; spring/summer (October 2014 to January 2015) and autumn/winter (May to August 2015).

Cows were kept on paddocks without significant pasture allowance from day –21 until calving, separated by parity, and were offered 13.3 kg/d of DM as total mixed ration (TMR) including anionic salts according to close-up requirements (NRC, 2001) twice a day at 8:00 and 16:30 hs. After calving, all cows were kept together in a compost barn until 3 days in milk (DIM) and were fed a TMR 3 times daily for ad libitum intake in collective feeders, at 5:30, 10:00 and 15:00 hs, and were milked 2 times daily. They did not have access to pasture. The total daily offer of DM was 21.5 kg/d in the spring calving season (2014) and 28.9 kg/d in the autumn calving season (2015). Close-up and fresh cow diet composition has been reported previously [9]. Cows had ad libitum access to water and they received 300 mL of propylene glycol, 250 g of calcium propionate diluted in 1L water orally and 40 g Calcium gluconate subcutaneously at calving. After 3 DIM, cows were translated to a free-stall and were fed the same TMR, but milked 3 times daily (DeLaval rotary 80 stations). Data for 305-d milk yield were obtained from DairyPlan C-21 software (GEA).

2.2. Study design, disease recording and sample collection

This is a prospective observational cohort study and only healthy cows were enrolled at the beginning of the study. Peripartum diseases were diagnosed by a single trained veterinarian as reported before [9], using the following criteria: Clinical hypocalcemia was defined as any recumbent cow within 72 hs after parturition exhibiting anorexia, nervous symptoms, staggering, varying degrees of unconsciousness, and good response to intravenously administered calcium. Clinical mastitis was characterized by the presence of abnormal milk or by signs of inflammation in 1 or more quarters, evaluated by the milking person from calving to 30 DIM at the start of milking. Retained placent (RP) was defined as failure to expel the placenta within 24 h after parturition. Between 3 and 21 ± 1 DIM, all cows were monitored for metritis twice weekly, every Tuesday and Friday by a veterinarian, using a manual vaginal examination. Presence of abnormal vaginal secretion associated with blood haptoglobin (Hp) concentration > 1 mg/dL, was used for diagnosis of metritis. Lameness was diagnosed weekly using the scale from 1 to 5, where grade 1 had no alteration in gait and at grade 5, the cow was severely limping, without supporting the member on the floor. Cows with lameness were considered those with locomotion score ≥3. Left displaced abomasum (LDA) was diagnosed based on an auscultation of a “ping” during percussion of the left side of the abdomen. During these health checks, the presence of any other clinical disease(s) was recorded, as well as deaths and culling because of health problems.

Cows were classified according to their health status in healthy cows (without clinical events) and sick cows (one or more clinical events) in order to analyze endocrine and reproductive variables. From the 288 cows evaluated, 45.8% (n = 132) became sick and 1.9% (n = 6) were culled or died within the first 30 DIM. Health status was not affected by parity and the proportions of healthy and sick cows were for MP: 52.9% and 47.0% respectively and for PP: 56.0% and 43.9% respectively.

2.3. Reproductive management

From 50 days after calving, cows were managed with a timed artificial insemination (AI) synchronization protocol consisting in 2 mL of estradiol benzoate (Sincrodioil, Ourofino Saúde Animal Ltda, Brazil), 1 mL of gonadotropin-releasing hormone (GnRH Geastr, ARSA S.R.L, Buenos Aires, Argentina) and an intravaginal progesterone (P4) device (CIDR, Zoetis, Brazil), followed seven days later by an injection of 2 mL prostaglandin (Estron Agener Uniao, Brazil), removing at this time the intravaginal device, and an injection of 0.5 mg estradiol cipionate (ECP, Zoetis, Brazil) [29]. All cows were inseminated 48 h later (47–50 h). Pregnancy diagnosis was performed on day 45 by ultrasound (DVF60vet, Oxson Technology, Brazil). Non-pregnant cows were submitted to the same synchronization protocol.

2.4. Blood sample collection and analyses

From week –2 until week +7, blood samples were collected from the coccygeal vein into 10-mL sterile heparinized tubes. Samples were centrifuged at 3000 x g for 20 min and plasma was stored frozen (−20 °C) until further analysis. In all cows (n = 288) plasma P4 concentrations were measured weekly after calving.
using a solid-phase 125I radioimmunoassay (RIA) kit (MP, Biomedicals, ICN Biomedicals, Inc. California). The sensitivity of the assay was 0.01 ng/mL, and the intra and inter-assay coefficients of variation (CVs) were not greater than 10.6%. Days to first ovulation (resumption of ovarian cyclicity) was defined as the interval between calving and the first day in which plasma P4 concentrations were ≥1 ng/mL.

To analyze the endocrine profiles and their relationship with reproductive parameters, a subset of 120 cows that included 57 PP cows (healthy n = 28, sick n = 29) and 63 MP cows (healthy n = 28, sick n = 35) was randomly selected. Insulin, IGF-I, leptin, and adiponectin concentrations were determined on weeks −2, −1, +1, +3, and +5 related to calving (a total of 600 samples for each hormone). IGF-I and insulin concentrations were measured using immunoradiometric assays (IRMA) with commercial kits. Plasma IGF-I concentrations were determined using the IGF-I RIA kit (Cis Bio International, GIF SUR YVETTE CEDEX, France). The assay's sensitivity was 1.54 ng/mL, and the intra and inter-assay CVs for control 1 (8.3 ng/mL) were 9% and 11.9%, respectively. Plasma insulin concentrations were determined using the INS-IRMA kit (DIA Source Immune Assays S.A., Belgium). The assay's sensitivity was 1.3 μU/mL, and the intra- and inter-assay CVs for control 1 (19.4 μU/mL) were 5.5% and 8.6%, respectively, and for control 2 (65.6 μU/mL) 4.4% and 4.9%, respectively. Leptin concentrations were determined with a liquid phase RIA using a commercial Multi-Species Leptin kit (RIA kit, Millipore, USA). The RIA had a sensitivity of 2.8 ng/mL, and the intra- and inter-assay CVs for control 1 (19.4 μU/mL) were 5.5% and 8.6%, respectively, and for control 2 (65.6 μU/mL) 4.4% and 4.9%, respectively. Leptin concentrations were determined with a liquid phase RIA using a commercial Multi-Species Leptin kit (RIA kit, Millipore, USA). The RIA had a sensitivity of 2.8 ng/mL, and the intra- and inter-assay CVs for control 1 (47.5 ng/mL) were 8.2% and 8.5%, respectively, and for control 2 (429 ng/mL) 9.9% and 11.9%, respectively. Plasma insulin concentrations were determined using the INS-IRMA kit (DIA Source Immune Assays S.A., Belgium). The assay's sensitivity was 1.3 μU/mL, and the intra- and inter-assay CVs for control 1 (19.4 μU/mL) were 5.5% and 8.6%, respectively, and for control 2 (65.6 μU/mL) 4.4% and 4.9%, respectively. Adiponectin concentrations were measured using an RIA kit (HADP-61 HK, Millipore, USA) with undiluted plasma samples. The assay's sensitivity was 1.54 ng/mL, and the intra and inter-assay CVs for control 1 (8.4 ng/mL) were 9.1% and 8.5% respectively and for control 2 (81.5 ng/mL) 7.9% and 11.1% respectively.

2.5. Statistical analysis

Insulin, IGF-I, leptin, and adiponectin concentrations were analyzed as repeated measures. The model included the fixed effects of parity (PP vs MP), health status (healthy and sick cows), week, and these factors' interactions. The associations between parity, health status, calving seasons and resumption of ovarian cyclicity, AI, and pregnancy rates were analyzed using 2 parity, health status, calving seasons and resumption of ovarian cyclicity, and these factors' interactions. The associations between each of these factors and the health status, parity, and reproductive parameters were analyzed using multivariable logistic regression (MLR) analysis for percentage of resumption of ovarian cyclicity and pregnancy rates were performed. Data were further evaluated using multivariable logistic regression (MLR) analysis for percentage of resumption of ovarian cyclicity and pregnancy rates were performed. Variables that were not significant (P > 0.05) were removed by manual backward stepwise elimination. Odds ratio (OR) and their respective 95% confidence interval (CI) determined by MLR were used to describe the level of association between the endocrine concentrations, health status, parity and reproductive parameters. Data are reported as least squares means and pooled standard errors. For all statistical analysis P < 0.05 was considered a significant effect, and P ≤ 0.1 as a trend.

The present study was performed to investigate the role of health status and parity on hormone profiles during the transition period and their association with reproductive performance. The sample size calculation for hormone profiles, assuming a SD of 30%, power = 80%, P-value = 0.05, revealed that 24 animals per group are needed to detect a 25% difference in the mean between two compared groups. For percentage of resumption of ovarian cyclicity in the first 7 weeks after calving, assuming 50 vs 70%, with a power = 80% and P-value = 0.05, 93 animals per group are needed (considering a power = 65%, 66 animals are needed). For pregnancy proportion, assuming 65 vs 80%, with a power = 80% and P-value = 0.05, 138 animals per group are needed (the same with a power = 65%, 98 animals are needed). Thus, the sample size considered in this study for endocrine profiles was appropriate, while for reproductive parameters, only moderate differences could be detected.

3. Results

The significance of the fixed effects of the subset groups’ hormonal concentrations (n = 120) is shown in Table 1. IGF-I and adiponectin concentrations were significantly affected by parity; insulin tended to be affected by this factor, while leptin did not reach significance. The evolution of IGF-I and insulin concentrations according to parity and week differed, and a triple interaction parity “status” week was found for IGF-I.

Insulin concentrations tended to be greater in PP than MP (19.5 ± 0.7 vs 17.9 ± 0.6 uU/mL, P = 0.07). In PP cows, insulin concentrations remained constant from week −2 until week +5, while in MP cows, insulin concentrations decreased from the close-up period until week +1 (P < 0.003), increasing thereafter until the end of the study without differences according to health status (Fig. 1 A, B). Overall, IGF-I concentrations were higher in PP than MP cows (136.2 ± 5.3 vs 103.6 ± 5.0 ng/mL, P < 0.0001). Regardless of health status, IGF-I concentrations increased in PP cows from week −2 to week −1 (P < 0.01) and decreased thereafter until week +1 (P < 0.01) with a sharp decrease in sick PP cows (Fig. 1 C). Although concentrations decreased in all MP cows at week +1, healthy MP cows tended to have greater IGF-I concentrations than sick MP cows (P = 0.1). After week +1, IGF-I concentrations in MP cows tended to increase until week +5, but the close-up concentration was not recovered (Fig. 1 D).

Adiponectin concentrations were greater in PP than MP cows (21.9 ± 1.1 versus 19.0 ± 1.0 ng/mL, P = 0.04). In all cows, the concentration decreased from week −2 until the end of the study regardless of parity. Also, healthy MP cows tended to have greater adiponectin concentrations than sick MP cows throughout the fresh cow period (P < 0.10. Fig. 1F). Parity did not reach significance for leptin concentrations (16.4 ± 1.4 and 13.6 ± 1.4 ng/mL for MP

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-value</th>
<th>P-value</th>
<th>P-value</th>
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<td>Insulin</td>
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<td>IGF-I</td>
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<td>&lt;0.0001</td>
<td>0.22</td>
</tr>
<tr>
<td>Adiponectin</td>
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<td>&lt;0.0001</td>
<td>0.17</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.15</td>
<td>&lt;0.0001</td>
<td>0.65</td>
</tr>
</tbody>
</table>

F-tests of fixed effects for hormone concentrations in cows from week −2 until week +5 according to calving in primiparous (n = 57) and multiparous (n = 63) Holstein cows. Fixed effects were parity, health status (Health), week according to calving and their interactions.
and PP cows, respectively, P = 0.15), and concentrations increased after calving (P < 0.05).

During the first seven weeks after calving, from a total number of 288 cows, 59.7% became cyclic, while 40.3% did not. Health status and parity affected the resumption of ovarian cyclicity (P < 0.05) independent of the calving season. A greater percentage of healthy cows reinitiated ovarian cyclicity when compared to sick cows (67.9% [106/156] vs 50% [66/132], P = 0.002). Although a greater percentage of healthy cows presented their first estrus after calving (P < 0.002), this difference was due to sick cows (Table 2). Indeed, no differences according to parity remained in the model (P = 0.15), and concentrations increased after calving (P < 0.05).

Table 2
Reproductive parameters in healthy and sick lactating primiparous (PP) and multiparous (MP) dairy cows.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PP (n = 116)</th>
<th>MP (n = 172)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (n = 65)</td>
<td>Sick (n = 51)</td>
</tr>
<tr>
<td>Resumption of ovarian cyclicity (%)</td>
<td>66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI (%)</td>
<td>97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Pregnancy (%)</td>
<td>82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Conception (%)</td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Within row different letters differ P < 0.05.

4. Discussion

This is the first study associating health status and parity with insulin, IGF-I, leptin and adiponectin profiles during the transition period, and investigating their relation with reproductive parameters. A greater proportion of healthy cows reinitiated ovarian cyclicity during the first 7 weeks after calving compared to sick cows, and the findings for the proportion of insemination and pregnancy were similar. While a lower proportion of PP cows reinitiated their ovarian cyclicity, they presented a greater proportion of pregnancy. Moreover, the significant interaction of health status and parity with IGF-I profiles is novel, and may explain the different reproductive outcome found in healthy and sick primiparous and multiparous cows.

Due to their impact on the reproductive status, endocrine profiles during the transition period were determined [6,11,23]. As a consequence of the increased requirements for milk production and decreased DMI, insulin levels decreased [23,30]. The higher overall IGF-I and insulin concentrations found in PP dairy cows during the transition period have been reviewed previously [19]; milk production is subordinate to growth in PP cows, and these anabolic hormones reveal the difference in metabolic adaptation according to parity. Indeed, the sharp decrease in IGF-I concentrations found in PP cows after calving in contrast to MP cows is an argument for a greater uncoupling of the somatotropic axis in favour of milk production as previously reported [19]. Adiponectin concentration was also affected by parity and was greater in PP cows. No reports of adiponectin concentrations according to parity in dairy cows have been found, but data are consistent with insulin and IGF-I profiles. Indeed, greater adiponectin concentrations have been related to insulin sensitivity [26]. The adiponectin decrease found after calving is consistent with its role in gluconeogenesis suppression [31], which may facilitate an increase in glucose supply to the mammary gland [32]. Leptin was not affected by parity and
concentrations increased after calving in all cows independent of parity, which could be explained by the high concentrate content of the diet in the present study as reported previously [33].

Healthy PP cows maintained greater IGF-I concentrations during the first month after calving, while sick PP cows presented a sharp decrease in response to illness, and this effect was not observed in MP cows. The sharper IGF-I decrease around calving in sick cows was probably associated with a decrease in DMI as previously reported [34,35]. Data suggest a different metabolic adaptation to illness and lactation according to parity. Interestingly, while sick MP cows had greater NEFA concentrations than healthy MP cows, this was not found in PP cows [9]. Thus, the reason for the lower IGF-I in sick PP cows could be the result of calving-induced stress in addition to management and milking routine adaptation at this time [36], and/or a milder NEB associated with a lower milk production when compared to sick MP cows.

Although there was no effect of health status on adiponectin and leptin profiles during the transition period, sick MP cows tended to have lower adiponectin concentrations than healthy cows after calving. Indeed, hypoadiponectinemia has been linked to inflammatory disease pathophysiology [37]. Also, the lower adiponectin concentrations found in sick MP cows after calving are consistent with the lower IGF-I concentrations in this group. Moreover, adiponectin, because of its insulin sensitizing actions, could be a metabolic regulating factor during the transition period [38].

Although the sample size of this study is limited, the reproductive parameters (resumption of ovarian cyclicity, pregnancy and conception rates) were affected by parity. Until day 50 after calving, PP cows presented a lower percentage of resumption of ovarian cyclicity in accordance with previous reports [39]. In dairy cows, the first ovulation after calving has been linked with the severity and duration of NEB [23]. In several studies [19,21,39,40] the NEB in PP cows has been reported to be at its worst one week before calving and early thereafter. However, the lower NEFA and greater IGF-I concentrations in PP cows (this study and [9]) reflect a better energy balance in this category, so that parity-associated factors mentioned before and reported previously [36] could explain the delay of resumption of ovarian cyclicity in PP cows. In contrast, the proportion of pregnant cows was greater in PP than MP cows. Similarly, Santos et al. [39] reported that MP cows were more likely to start ovarian cyclicity earlier than PP cows, but pregnancy rate was higher in PP cows. In our study, the greater milk production of MP cows could explain the lower proportion of pregnancy because of the increased catabolic state, as milk yield was negatively associated with the ability of lactating cows to conceive and maintain pregnancy [39]. However, others reported no association between milk yield and conception or embryonic survival [39,41]. An increase in liver flux is needed to support higher milk production in MP cows, leading to a greater P4 clearance; thus, lower circulating P4 concentrations could explain embryonic loss and the lower pregnancy proportion in this category [42,43].

Health status affected the percentage of resumption of ovarian cyclicity during the first 7 weeks after calving and was lower in sick cows, which is associated with the worse NEB (lower cholesterol, albumin and IGF-I concentrations) of them ([9] and the present study). Sick cows presented lower DMI and lower cholesterol and albumin concentrations [44,45]. As mentioned before, IGF-I stimulates bovine follicular cells and ovulation [46]. Indeed, IGF-I concentrations one week after calving were associated with resumption of ovarian cyclicity. Beam and Butler [47] also reported

Fig. 2. Probability of resumption of ovarian cyclicity and pregnancy in Holstein primiparous (PP, n = 116) and multiparous (MP, n = 172) cows according to health status [healthy cows (●) and sick cows (△)].
greater IGF-I concentrations two weeks after calving in cows that reinitiated ovarian cyclicity in comparison to non-ovulating cows. Interestingly, the negative effect of illness on resumption of ovarian cyclicity in PP cows was more marked than in MP cows, consistent with the sharper decrease in IGF-I pro.

Cyclicity in PP cows was more marked than in MP cows, consistent with the sharper decrease in IGF-I pro. Interestingly, the negative effect of illness on resumption of ovarian cyclicity and lower IGF-I concentrations, the limited number of cows included for reproductive outcomes. Interpretation of the present data should be carefully performed due to effects of diseases on reproduction were observed, suggesting that fertilization and maintenance of pregnancy are important barriers for reproductive success in this category. Nevertheless, the interpretation of the present data should be carefully performed due to the limited number of cows included for reproductive outcomes.

5. Conclusion

Healthy and sick cows had different endocrine profiles during the transition period, which may partially explain the observed reproductive performance. Parity interacted with health status on reproductive performance; sick PP cows showed a delay in the resumption of ovarian cyclicity and lower IGF-I concentrations, while sick MP cows showed a lower proportion of pregnancy.

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References


