Theriogenology 86 (2016) 263–269

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Review article

Associations of insulin resistance later in lactation on fertility of dairy cows

P.S. Baruselli [a,*], L.M. Vieira [a], M.F. Sá Filho [a], R.D. Mingoti [a], R.M. Ferreira [a], M.R. Chiaratti [b], L.H. Oliveira [c], J.N. Sales [d], R. Sartori [c]

[a] Department of Animal Reproduction, FMVZ-USP, São Paulo, São Paulo, Brazil
[b] Department of Genetics and Evolution, CCBS, Federal University of São Carlos, São Carlos, São Paulo, Brazil
[c] Department of Animal Science, ESALQ-USP, Piracicaba, São Paulo, Brazil
[d] Department of Veterinary Medicine (DMV), Federal University of Lavras, Lavras, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 11 February 2016
Accepted 14 March 2016

Keywords:
Cattle
Days open
Metabolic disorder
Oocyte competence
Reproduction

ABSTRACT

The challenge of getting dairy cows pregnant during early lactation is a well-described, worldwide problem. However, specifically in farms with poor reproductive, nutritional, and environmental conditions/management, a low pregnancy rate during early lactation is followed inevitably by an increased number of nonpregnant cows after 150 days in milk, with even more difficulties to achieve pregnancy. Therefore, several studies were designed to understand and develop strategies to mitigate reduced fertility of cows during late lactation. Experiments were performed under tropical regions to determine metabolic status during lactation and association of stage of lactation on oocyte quality and fertility. Lactating cows with extended days not pregnant (e.g.,>150 days in milk) often had systemic metabolic alterations, including development of peripheral insulin resistance and various oocyte alterations, including reduced expression of genes encoding glucose transport proteins, reduced amounts of mtDNA, increased expression of mitochondria-related genes, and increased expression of apoptosis-related genes. Additionally, *in vitro* embryo production and pregnancy per AI were lower in late- versus early-lactation cows in some but not all studies. Notwithstanding, when a normal embryo was transferred to a cow in late lactation, the pregnancy per transfer was reasonable, reinforcing the assertion that fertility problems in late-lactation cows may be associated with oocyte quality, fertilization, and/or failure of early embryo development. In conclusion, insulin resistance may reduce oocyte competence and consequently fertility in late-lactation dairy cows.

1. Introduction

Days to pregnancy after calving can be affected by multiple factors, including nutrition during the dry period, duration of voluntary waiting period, breeding strategy, season, herd size, milk yield, and parity [1,2]. Peripartum diseases such as milk fever, dystocia, retained placenta, and metritis are well-established risk factors for reduced pregnancy rate during early lactation, resulting in prolonged intervals of nonpregnancy [3]. Additionally, compared to first-parity cows, cows in second, third, or greater parities also had higher odds of extended intervals of nonpregnancy [3]. In tropical regions, increased thermal load (combination of heat and humidity) is associated with reduced pregnancy per AI during early lactation and consequently, a greater proportion of dairy cows with prolonged periods of nonpregnancy [1,4]. Also, cows with extended days not pregnant in the previous parity doubled the risk of culling and death around calving [5].

Greater culling risks in cows with an extended interval to achieve pregnancy are often associated with metabolic

* Corresponding author. Tel.: 55 11 3091 7674; fax: 55 11 3091 7412.
E-mail address: barusell@usp.br (P.S. Baruselli).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.04.039

disorders [5–7]. Excessive energy intake is common in late-lactation dairy cows [8]. These cows have increased risk of overconditioning (high body condition score [BCS]) toward the end of lactation, at a stage when milk yield typically is lower, although feed intake is not reduced accordingly [6,9]. Excessive energy intake may alter release and synthesis of reproductive and metabolic hormones, oocyte quality, and ovarian follicular dynamics, resulting in impaired reproductive performance during this stage [9,10].

Among metabolic disorders related to late-lactation cows is development of peripheral insulin resistance [9,11,12], characterized by decreased sensitivity of target tissues to normal circulating insulin concentrations [13], thereby requiring higher insulin concentrations to achieve normal metabolic responses [14]. Females with chronic hyperinsulinemia might have compromised glucose uptake, which leads to cellular apoptosis [15,16]. Furthermore, insulin resistance has negative associations with reproduction of heifers and dry cows, resulting in impaired oocyte quality [17,18].

This review aims to discuss a number of key points related to the potential association of insulin resistance and late-lactation conditions on fertility of dairy cows. Discussion will focus on (1) insulin profile during lactation; (2) association between stage of lactation and fertility; and (3) association of insulin resistance and stage of lactation on oocyte quality.

2. Insulin profile during lactation

Dairy cows typically enter a state of negative energy balance during early lactation when combined energy requirements for maintenance and milk production exceed dietary energy intake. This state is characterized by increased mobilization of body reserves to overcome a shortfall in dietary intake, decreased circulating concentrations of insulin, IGF-I, and glucose, and elevated concentrations of nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) [19]. However, if cows in later stages of lactation are submitted to prolonged intervals of high feed intake with reduced milk yield, BCS will increase, which might lead to development of hyperinsulinemia [20].

Insulin concentrations and blood metabolic responses in dairy cows have been assessed during the dry period and subsequent lactation [21]. During dry-off, plasma insulin concentrations significantly decreased (from ~0.63 [60 days before calving] to 0.26 ng/mL [5 days before calving]), remained low until the seventh month of lactation, and increased thereafter (from ~0.25 [210 days after calving] to 0.69 ng/mL [270 days after calving]). The onset of lactation was characterized by a reduction in plasma glucose concentrations, with a subsequent gradual increase [21]. In other studies, glucose concentrations were low during the first 3 weeks after calving and thereafter only small fluctuations occurred [14]. Similarly, a previous report demonstrated fairly stable glucose concentrations until ~280 days in milk (DIM). Therefore, the pattern of increased insulin during late lactation associated with only slight increase of glucose, reduced milk yield, and increasing BCS might trigger a peripheral insulin resistance status [8].

It was recently hypothesized that dairy cows become increasingly insulin resistant as they proceed through lactation [20]. This hypothesis was tested by performing the glucose tolerance test (GTT) on high-producing dairy cows at various DIM. Holstein cows at 50 (51.5 ± 3.7; n = 30), 100 (102.3 ± 9.4; n = 30), or 150 (154.5 ± 18.9; n = 30) DIM were used, and a GTT was performed after 5 hours of fasting. Milk yield was lower at 150 DIM compared to 50 and 100 DIM (34.2 ± 1.4; 38.0 ± 1.4; and 38.6 ± 1.4 kg/day, respectively; $P \leq 0.05$), and BCS was lower at 50 DIM compared to 100 and 150 DIM (2.8 ± 0.1; 3.0 ± 0.1; and 3.0 ± 0.1, respectively; $P \leq 0.05$). During the GTT, there was no difference among groups for glucose peak = 203.3 ± 7.3, 208.8 ± 6.3, and 194.3 ± 6.0 mg/dL for 50, 100, and 150 DIM, respectively (Fig. 1A). Regardless, cows at 150 DIM had higher peak of insulin (67.9 ± 7.3[a], 67.7 ± 7.0[a] and 88.3 ± 7.2[b] μIU/mL; Fig. 1B), Δ max insulin (54.4 ± 5.4[a], 58.9 ± 5.3[a] and 72.4 ± 5.5[b] μIU/mL) and area under the curve (AUC) between 5 and 60 minutes after glucose infusion (1957.5 ± 173.2[a], 2174.9 ± 168.8[a] and 2633.9 ± 179.8[b] μIU/mL x min) compared to cows 50 or 100 DIM. Insulin resistance can be identified when greater insulin concentrations (peak, Δmax insulin, AUC) are necessary to reduce glucose concentrations to euglycemic levels during the GTT. Therefore, the main hypothesis that cows develop increasing insulin resistance with increasing DIM was substantiated. Another study performed in lactating dairy cows consuming excessive energy (160% of the maintenance energy requirements) verified increased BCS and insulin resistance (GTT) during the experiment (76–286 DIM) compared to the maintenance group [8].

Other studies sought to verify the possible association between a high-energy diet and a hyperinsulinemic state. The model used was nonlactating Holstein cows fed high energy (170% of the maintenance energy requirements) versus a maintenance diet for a prolonged interval (119 days [22]). In that study, cows fed a high-energy diet had peripheral resistance to insulin (based on the GTT). During this test, for all variables related to circulating glucose concentrations, there was no difference between groups (e.g., basal glucose = 67.8 vs. 73.1 mg/dL; P = 0.09; glucose peak = 222.1 ± 12.2 and 235.9 ± 6.5 mg/dL; P = 0.29; metabolism rate = 1.8 ± 0.4 and 2.3 ± 0.2%/min; P = 0.16 for maintenance and high-energy groups, respectively). However, the required insulin concentration to control infused glucose was greater in the high-energy group (basal insulin concentrations = 7.3 ± 1.6 and 23.2 ± 8.9 μIU/mL; P = 0.05; peak of insulin concentrations = 107.9 ± 19.0 and 192.4 ± 29.3 mg/dL; P = 0.03), with peripheral insulin resistance in cows eating a high-energy diet. In another study, nonlactating dairy cows consuming excessive energy had reduced insulin sensitivity (during a GTT) indicative of insulin resistance [23], compared to cows consuming adequate amounts of energy.

On the basis of all these studies, we inferred that dairy cows in later stages of lactation eating high-energy diets are predisposed to development of insulin resistance. Indeed, cows in late lactation (probably receiving more

[a,b] Refer to the statistical difference between the groups ($P < 0.05$).

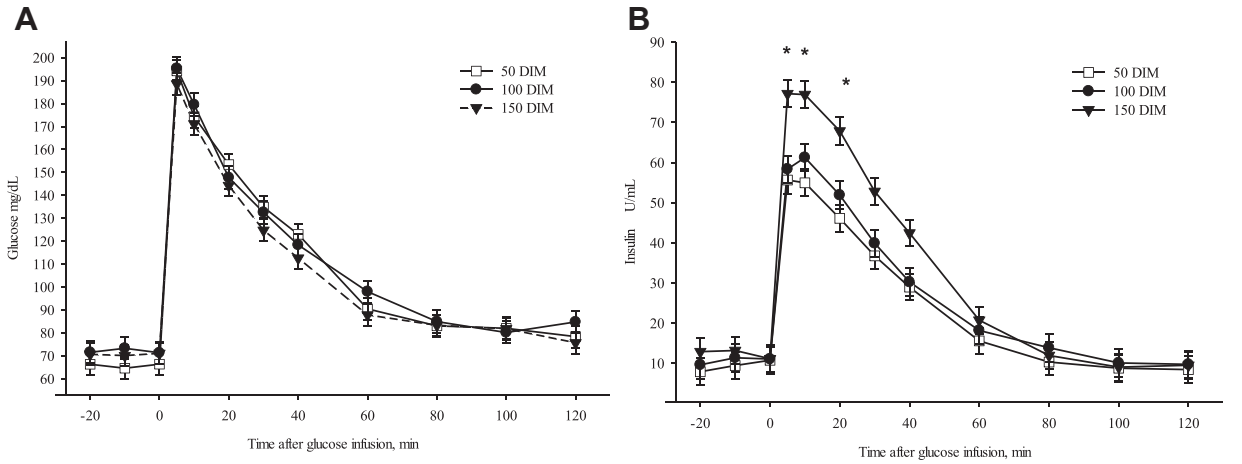

Fig. 1. Plasma concentrations of glucose (A) and insulin (B) in response to the intravenous glucose tolerance test (GTT; 0.3 g/kg of BW of glucose IV) in cows at 50 (DIM50; n = 30), 100 (DIM100; n = 30), or 150 (DIM150; n = 30) days in milk. Fixed effects for the analyses of glucose concentration after the GTT: treatment (P = 0.76); time (P < 0.001); treatment × time (P = 0.03). Fixed effects for the analyses of insulin concentration after the GTT: treatment (P = 0.03); time (P < 0.001); treatment × time (P = 0.86). Within time, an asterisk (*) represents an effect of treatment (P ≤ 0.05). Adapted from Oliveira et al. [20].

energy than required) or those consuming energy in significant amounts above requirements (irrespective of stage of lactation) are likely to develop insulin resistance.

3. Stage of lactation and fertility

Cows that do not become pregnant during early lactation after successive inseminations have been commonly associated with lower risk of pregnancy during late lactation and greater risk of culling [3,5]. These cows are usually classified as repeat-breeder cows. Lower fertilization rate is an important characteristic associated with repeat-breeder cows in summer [24]. The reasons for the lower fertility of late-lactation cows are not fully understood; however, metabolic disorders related to excessive energy intake are a potential factor [9,10].

To investigate this scenario, a prospective experiment was recently performed to assess conception rate of Holstein cows during late (n = 230) and early lactation (n = 206 [25]). It is noteworthy that all cows were treated to be artificially inseminated at a fixed time (TAI) using the same ovulation synchronization protocol, and all were inseminated using only a single batch of semen from a Holstein sire with proven fertility. Cows in early lactation were 100.5 ± 10.3 DIM, had 0.8 ± 0.2 previous AI services; and produced 39.3 ± 0.2 kg of milk/day at the onset of the synchronization protocol for TAI. Conversely, cows in late lactation had greater DIM (339.2 ± 10.1; P < 0.0001), more previous AI services (5.8 ± 0.2; P < 0.0001), and lower milk yield (27.6 ± 1.2; P < 0.0001). Number of previous lactations did not differ between groups (2.0 ± 0.1 and 2.1 ± 0.1 for early- and late-lactation cows, respectively; P = 0.86). Pregnancy per AI 30 days after AI was lower in cows bred during late lactation compared to those bred during early lactation (Fig. 2). Although fertility was reduced in late-lactation cows, effects of different numbers of previous AI services among groups should be considered to avoid misinterpretation.

Although the mechanisms involved in the lower fertility of cows during late lactation are not completely elucidated, metabolic disorders linked to the energy metabolism associated with reduced oocyte competence are significant risk factors that might compromise embryo development. In repeat-breeder cows, decreased fertility has been related to a deleterious effect on oocyte quality [24]. Therefore, embryo transfer could be considered a potential strategy to achieve pregnancy in repeat-breeder cows (i.e., late-lactation cows that remain nonpregnant) [26,27].

A retrospective study using data from a large number of high-producing dairy cows in Brazil confirmed that embryo transfer can be successfully used as a tool to establish

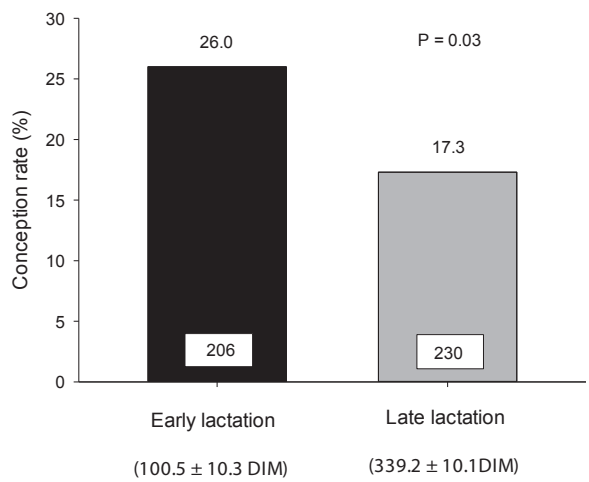

Fig. 2. Conception rate (pregnancy per AI) of Holstein cows inseminated at fixed time during early versus late lactation (n = 206 and 230, respectively). A single batch of semen from a Holstein sire was used to perform all inseminations. Cows eligible to be bred during late lactation were those that not yet become pregnant. Pregnancy diagnosis was performed 30 days after the timed AI. DIM, days in milk. Adapted from Ferreira [25].

pregnancy in late-lactation (i.e., repeat breeder) cows [26]. Although late-lactation cows had lower pregnancy per AI than early-lactation cows, using embryo transfer increased pregnancy establishment in late-lactation cows, bringing it comparable to that obtained for cows subjected to AI or embryo transfer during early lactation (Fig. 3). Furthermore, pregnancy loss was similar, regardless of breeding technique or lactation stage, reinforcing the hypothesis that the fertility problems in late-lactation cows may be associated with oocyte quality, fertilization, and/or failure of early embryo development (reviewed by [26]).

It is important to highlight that the risk of late-lactation cows to have insulin resistance status might be directly associated with poor management. In tropical regions, many dairy farms have environmental conditions compromising cattle welfare (e.g., heat stress) and therefore reduced fertility during early lactation, resulting in more nonpregnant cows during late lactation [28]. Also, inadequate nutritional management (e.g., high-energy diets during late lactation) can promote development of insulin resistance, especially during late lactation, because of reduced energy requirements with reduced milk yield [6,9]. It is noteworthy that if cows are pregnant during late lactation, development of excessive body condition might compromise the transitional period and subsequent lactational performance [29,30]. Conversely, if cows do not become pregnant until late lactation, a common scenario in tropical regions, developing a hyperinsulinemic state might reduce oocyte quality and consequently reproductive performance. However, if there was adequate nutrition and good environment conditions, the situation might be different because the majority of cows will become pregnant during early lactation, and the fewer late-lactation nonpregnant cows are less likely to develop disorder such as insulin resistance. Additionally, supplementing chromium propionate to prevent insulin resistance (by enhancing insulin signaling and promoting greater glucose uptake) in cows consuming excessive energy was recently reported in both lactating [8] and nonlactating dairy cows [23].

4. Association of insulin resistance and stage of lactation on oocyte quality

Although insulin has an important role in cellular metabolism, in excess it may interfere with various metabolic and reproductive processes in dairy cows [11]. During early lactation, low circulating insulin concentrations have been associated with impaired fertility by delaying resumption of cyclicity [31]. Although greater concentrations of insulin are important to restore ovarian cyclicity, it has been shown in heifers that they may also compromise oocyte quality [17] and, therefore, fertility. In that regard, excessive insulin may reduce oocyte quality in heifers [17] and *in vitro* embryo production (IVEP) and gene expression linked to cellular metabolism in nonlactating *Bos indicus*

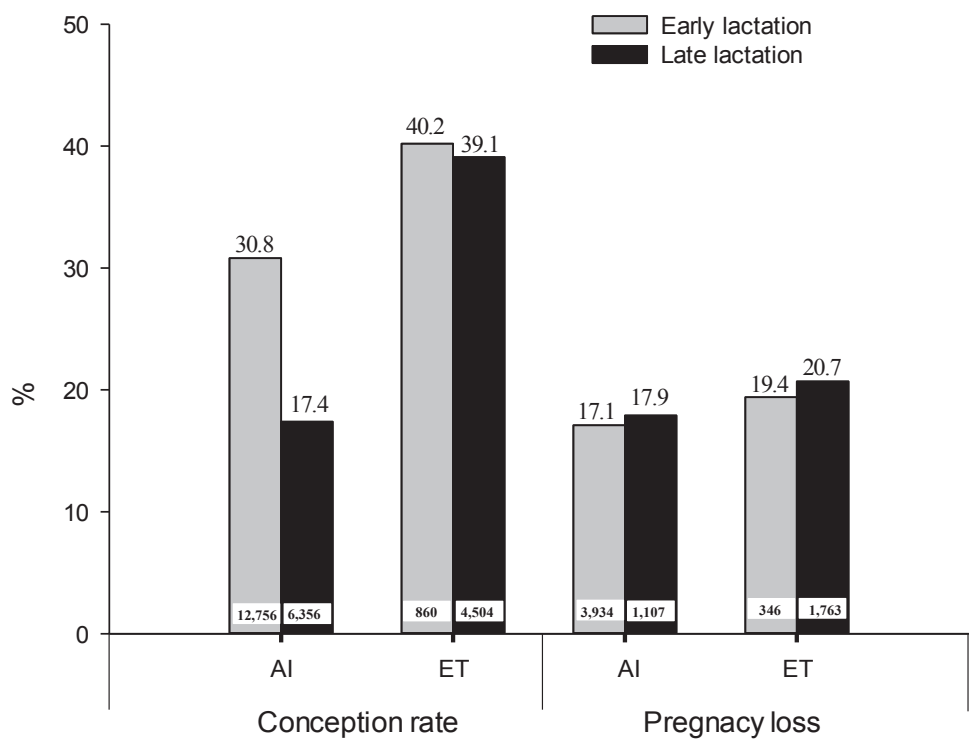

Fig. 3. Retrospective data regarding conception rate (pregnancy per AI or pregnancy per ET) and pregnancy loss of early in lactation (based on the number of AI services [maximum three services] after 60 days of voluntary waiting period; gray bars) and late lactation (≥4 services) Holstein cows (black bars) subjected to AI (n = 19,112) or embryo transfer (ET; n = 5364). There were effects for breeding technique (AI or ET; P = 0.001), animal category (P = 0.001) and their interaction (P = 0.001) on conception rates. Pregnancy loss was not influenced by breeding technique (P = 0.08) or animal category (P = 0.39), and there was no interaction (P = 0.87). Adapted from Baruselli et al. [26].

dairy cows [22]. In the latter study, the negative association of excessive energy intake and increased insulin concentrations on IVEP occurred only after 60 days. Thus, prolonged exposure to a high-energy diet was necessary to compromise oocyte quality. On the basis of "Britt's theory" (i.e., folliculogenesis takes at least 60–80 days until an ovulatory follicle stage [32]), adverse conditions such as excessive energy balance leading to insulin resistance status can affect folliculogenesis leading to subsequent issues of oocyte competence at the time of ovulation. Therefore, negative effects on oocyte quality and fertility might not be apparent at the onset of insulin resistance. Moreover, ovum pick up *in vitro* embryo production (OPU-IVEP) may not be the most adequate method to assess the impact of insulin resistance on fertility. In fact, although lactating cows at 150 DIM were insulin resistant at the GTT, it did not reflect on quality of oocytes aspirated from small- and medium-sized follicles [20].

Although, in some studies, the association of hyperinsulinemia with poor oocyte quality was not detected in cows, reduced expression of genes encoding glucose transport proteins (*GLUTs*) and IGF-receptor in response to high energy in nonlactating *B. indicus* dairy cows (Fig. 4; adapted from Sales [22]) and reduced expression of genes related to cellular metabolism in crossbred heifers [33] were reported. Furthermore, a high plane of nutrition (160% of maintenance energy requirements) increased plasma concentrations of both insulin and IGF1 in heifers [33] and reduced steady state concentrations of mRNA encoding IGF-binding protein-2 and IGF-binding protein-4, insulin receptor, and IGF1 receptor in the follicle [34]. It is noteworthy that GLUT1 oocyte expression may be essential for oocyte maturation [35], and earlier publications reported a negative effect of insulin on *in vitro* embryo development in mice [36] and cattle [37]. However, although several studies hypothesized that the increased bioavailability of IGF1 and IGF2 and/or hyperinsulinemia condition on the follicle-enclosed oocyte have detrimental effects on oocyte development, the precise mechanisms of such effects have not been elucidated.

In another study, early-lactation (110.5 ± 20.8 DIM; n = 70) and late-lactation (425.6 ± 21.0 DIM; n = 67) Holstein cows were subjected to OPU to evaluate oocyte quality and IVEP (Table 1 [25]). In addition to increased number of days not pregnant, late-lactation cows had lower milk yield, greater number of previous inseminations (effect of number of previous inseminations was not controlled), and greater BCS than early-lactation cows (Table 1). Regarding OPU-IVEP, late-lactation cows had greater numbers of recovered and viable oocytes compared to early-lactation cows. However, late-lactation cows had decreased rates of both cleavage (P = 0.08) and blastocyst formation (P = 0.0005). In addition to fewer embryos produced, late-lactation cows had greater peripheral insulin resistance than early-lactation cows, based on homeostasis model assessment of insulin resistance (HOMA-IR; Table 1 [38,39]). The HOMA-IR was calculated according to a formula presented in the previous studies [38,39]:

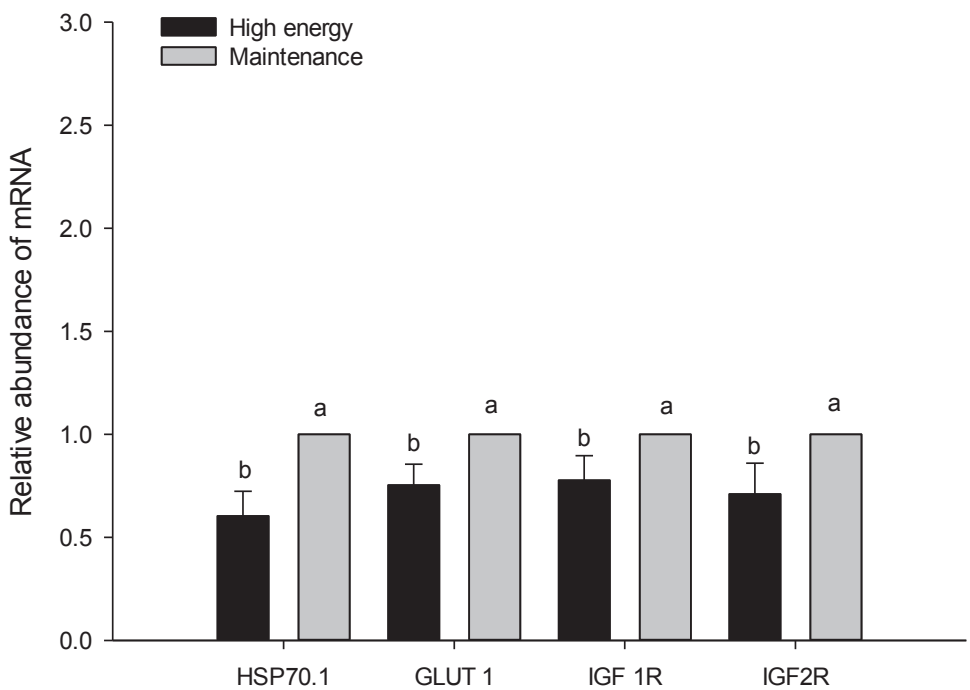

Fig. 4. Relative abundance of transcripts (means ± SEM) associated with cellular stress (PRDX1 and HSP70.1) and metabolism (GLUT1, IGF1R, and IGF2R) were obtained by real-time PCR of oocytes from nonlactating *Bos indicus* dairy cows fed diets to meet 100% (maintenance, n = 90) or 170% (high energy, n = 90) of energy required for maintenance. Maintenance diet data were used as the reference (n = 1) for the relative gene expression and means of high-energy group are shown as mean ± SEM relative to the reference. [a,b]Columns without a common letter differed (P = 0.01). Adapted from Sales [22].

Table 1
Ovum pick up, *in vitro* embryo production and metabolic profile of high production Holstein cows during early or late in lactation.

Item	Phase of lactation		P value
	Early	Late	
Ovum pick up, *in vitro* embryo production and metabolic profile			
General characteristics			
No. of animals	70	67	
DIM, days	110.5 ± 20.8	425.6 ± 21.0	—
Milk production, kg/day	34.3 ± 1.2	23.4 ± 1.2	<0.0001
No. of insemination	0.7 ± 0.2	7.0 ± 0.2	<0.0001
No. of lactation	2.4 ± 0.1	1.9 ± 0.2	0.05
BCS (1–5 scale)	2.79 ± 0.06	3.15 ± 0.07	<0.0001
Ovum pick up			
No. of follicles	14.8 ± 2.4	22.7 ± 2.4	0.0016
Recovery rate, %	46.4 ± 4.4	53.8 ± 4.5	0.10
No. of oocytes	7.3 ± 2.0	14.3 ± 2.0	0.0004
No. of viable oocytes	4.6 ± 1.6	9.7 ± 1.6	0.0010
In vitro embryo production			
No. of cleaved oocytes (D3)	4.7 ± 0.6	3.9 ± 0.6	0.10
Cleavage rate, %	48.0 ± 0.1	41.4 ± 0.1	0.08
No. of blastocyst (D7)	2.2 ± 0.4	1.4 ± 0.3	0.06
Blastocyst rate, %	23.0 ± 0.1	13.3 ± 0.1	0.0005
Metabolites profile			
Total Protein, g/dL	7.9 ± 0.1	7.8 ± 0.1	0.16
Albumin, g/dL	3.2 ± 0.0	3.30 ± 0.0	0.78
Globulin, g/dL	4.6 ± 0.1	4.47 ± 0.1	0.12
Albumin/globulin ratio	0.71 ± 0.0	0.78 ± 0.0	0.14
Urea, mg/dL	36.0 ± 1.6	30.8 ± 1.1	0.18
Creatinine, mg/dL	0.9 ± 0.0	1.0 ± 0.0	0.55
CK, U/L	69.7 ± 5.3	80.1 ± 11.1	0.29
AST, U/L	73.4 ± 3.7	64.3 ± 2.6	0.40
GGT, U/L	22.1 ± 1.6	28.2 ± 4.9	0.30
Triglyceride, mg/dL	15.3 ± 0.4	17.1 ± 0.7	0.10
Cholesterol, mg/dL	156.1 ± 5.4	149.5 ± 5.1	0.98
HDL, mg/dL	51.1 ± 2.1	47.6 ± 1.9	0.52
LDL, mg/dL	102.0 ± 4.4	98.5 ± 4.5	0.89
VLDL, mg/dL	3.1 ± 0.1	3.4 ± 0.1	0.25
NEFA, mol/L	0.45 ± 0.03	0.35 ± 0.02	0.07
BHBA mg/dL	5.11 ± 0.22	4.73 ± 0.18	0.01
Glucose, mg/dL	56.4 ± 0.8	62.0 ± 0.9	0.02
Insulin, μIU/mL	8.4 ± 1.2	21.4 ± 3.0	0.001
Ratio of insulin and glucose	0.15 ± 0.02	0.34 ± 0.05	0.001
HOMA-IR	1.23 ± 0.18	3.36 ± 0.51	0.0001
Oocyte genes expression			
mtDNA amount			
MtDNA	1.0 ± 0.26	0.5 ± 0.13	0.02
Mitochondrial genes			
MTCO1	1.0 ± 0.24	2.7 ± 0.48	0.001
NRF1	1.0 ± 0.20	1.2 ± 0.17	0.19
POLG	1.0 ± 0.33	2.5 ± 0.62	0.008
POLG2	1.0 ± 0.28	1.5 ± 0.26	0.06
PPARG	1.0 ± 0.20	1.8 ± 0.30	0.02
TFAM	1.0 ± 0.20	3.9 ± 1.35	0.003
Apoptotic genes			
BAX	1.0 ± 0.24	1.3 ± 0.18	0.18
BCL2	1.0 ± 0.22	1.2 ± 0.27	0.63
BAX/BCL2	1.0 ± 0.20	2.2 ± 0.41	0.001
ITM2B	1.0 ± 0.26	2.1 ± 0.51	0.02
Maturation genes			
BMP15	1.0 ± 0.15	0.8 ± 0.09	0.34
FGF8	1.0 ± 0.24	1.0 ± 0.15	0.73
FGF10	1.0 ± 0.38	0.5 ± 0.11	0.19
FGF16	1.0 ± 0.20	0.8 ± 0.12	0.72
GDF9	1.0 ± 0.22	0.9 ± 0.14	0.89

Abbreviations: AST, aspartate aminotransferase; BHBA, β-hydroxybutyrate; DIM, days in milk; GGT, gamma glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acids; VLDL, very-low-density lipoproteins.
Adapted from Ferreira [25].

[basal insulin (μIU/mL) × basal glucose (mmol/L)]/22.5. The major purpose of the HOMA-IR is to predict insulin resistance of peripheral tissues based on a single blood sample after an overnight fast. Moreover, late-lactation cows had lower serum concentrations of both NEFA ($P = 0.07$) and BHBA ($P = 0.01$), although there were greater serum concentrations of glucose ($P = 0.02$) and insulin ($P = 0.001$) and a greater insulin-glucose ratio ($P = 0.001$) compared to early-lactation cows. Stage of lactation did not alter other serum metabolites evaluated (Table 1). Therefore, late-lactation cows from the present study might have been consuming energy in excess of requirements. Supporting the previous data, lactating cows consuming excessive energy intake experienced increased insulin resistance and reduced blastocyst rate compared to cows consuming only adequate amounts of energy [8].

Both relative and absolute numbers of copies of mitochondrial DNA (mtDNA) were reduced in oocytes retrieved from late-lactation cows (Table 1), suggesting a disruption of oocyte quality [25]. In addition, expressions of mitochondrial-related genes (*MTCO1*, *POLG*, *POLG2*, *PPARG*, *TFAM*) were increased in late-lactation cows, suggesting the activation of compensatory mechanisms in response to mitochondrial dysfunction (reduced number of copies of mtDNA) aiming to improve the generation of energy (ATP) required during early embryonic development. Furthermore, there was a greater ratio of *BAX*/*BCL2* in late-lactation cows, indicating an apoptotic phenotype of the oocytes from this category (Table 1).

Overall, on the basis of the available data, we inferred there was a possible association between reduced oocyte quality and insulin resistance status, mostly manifested in late-lactation cows fed a diet with excessive energy.

5. Conclusions

Low oocyte competence might be an important contributor to low fertility in dairy cows with insulin resistance status, a common scenario in later stages of lactation in herds with inadequate management (e.g., nutrition, cattle welfare, environmental conditions, and reproductive programs). The data presented herein reinforced existence of several risk factors for extended days not pregnant and, consequently, a greater incidence of nonpregnant cows during late lactation. Cows with an extended interval to become pregnant are commonly those with a previous peripartum disease, dystocia, postpartum uterine infections, or compromised welfare (e.g., heat stress or poor nutrition, both of which are common in tropics). Therefore, after 150 DIM, lactating cows with increased BCS might begin to exhibit metabolic alterations, especially related to the energy metabolism, developing peripheral insulin resistance. Also, late-lactation cows have lower amounts of mtDNA and greater expression of mitochondrial-related and apoptosis-related genes in their oocytes. On the basis of all these data and associations, we inferred that the fertility problems observed in non-lactating dairy cows fed excessive dietary energy and in late-lactation cows may be related to poor oocyte quality associated with an insulin resistance status. Therefore, it is

critical to highlight the importance of providing an adequate periparturient transition period (i.e., minimizing disease, stress, and uterine infections), with adequate energy intake (according to requirements), followed by a systematic and efficient reproductive program using AI and TAI to ensure the maximum number of pregnant cows in early lactation. Additionally, adding chromium propionate supplementation in cows consuming excessive energy can prevent insulin resistance. Furthermore, embryo transfer can be a practical, effective solution to achieve pregnancy in late-lactation, nonpregnant cows (with reduced oocyte quality and fertility to insemination).

Acknowledgments

The authors thank FAPESP (2015/19563–0) and CNPq (303225/2009–2 and 486089/2013-4) for financial support.

References

[1] Oseni S, Misztal I, Tsuruta S, Rekaya R. Seasonality of days open in US Holsteins. J Dairy Sci 2003;86:3718–25.
[2] Gröhn YT, Rajala-Schultz PJ. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci 2000;60:605–14.
[3] Bonneville-Hébert A, Bouchard E, Tremblay DD, Lefebvre R. Effect of reproductive disorders and parity on repeat breeder status and culling of dairy cows in Quebec. Can J Vet Res 2011;75:147–51.
[4] Rensis FD, Scaramuzzi RJ. Heat stress and seasonal effects on reproduction in the dairy cow—a review. Theriogenology 2003;60:1139–51.
[5] Pinedo PJ, De Vries A. Effect of days to conception in the previous lactation on the risk of death and live culling around calving. J Dairy Sci 2010;93:968–77.
[6] Heuer C, Schukken YH, Dobbelaar P. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J Dairy Sci 1999;82:295–304.
[7] Chassagne M, Barnouin J, Chacornac JP. Risk factors for stillbirth in Holstein heifers under field conditions in France: a prospective survey. Theriogenology 1999;51:1477–88.
[8] Leiva T, Cooke RF, Brandão AP, Aboin AC, Ranches J, Vasconcelos JLM. Effects of excessive energy intake and supplementation with chromium propionate on insulin resistance parameters, milk production, and reproductive outcomes of lactating dairy cows. Livestock Sci 2015;180:121–8.
[9] Sinclair KD. Declining fertility, insulin resistance and fatty acid metabolism in dairy cows: developmental consequences for the oocyte and pre-implantation embryo. Acta Scientiae Veterinariae 2010;38:545–57.
[10] Armstrong DG, Gong JG, Webb R. Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. Reprod Suppl 2003;61:403–14.
[11] De Koster JD, Opsomer G. Insulin resistance in dairy cows. Vet Clin North Am Food Anim Pract 2013;29:299–322.
[12] Leiva T, Cooke RF, Brandão AP, Marques RS, Vasconcelos JLM. Effects of rumen-protected choline supplementation on metabolic and performance responses of transition dairy cows. J Anim Sci 2015;93:1896–904.
[13] Boura-Halfon S, Zick Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. Am J Physiol Endocrinol Metab 2009;296:E581–91.
[14] Holtenius P, Holtenius K. A model to estimate insulin sensitivity in dairy cows. Acta Vet Scand 2007;49:29.
[15] Pantaleon M, Kaye P. Glucose transporters in preimplantation development. Rev Reprod 1998;3:77–81.
[16] Chi MM-Y, Schlein AL, Moley KH. High insulin-like growth factor 1 (IGF-1) and insulin concentrations trigger apoptosis in the mouse blastocyst via down-regulation of the IGF-1 receptor. Endocrinology 2000;141:4784–92.
[17] Adamiak SJ, Mackie K, Watt RG, Webb R, Sinclair KD. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biol Reprod 2005;73:918–26.
[18] Sales JNS, Iguma LT, Batista RITP, Quintão CCR, Gama MAS, Freitas C, et al. Effects of a high-energy diet on oocyte quality and in vitro embryo production in Bos indicus and Bos taurus cows. J Dairy Sci 2015;98:3086–99.
[19] Matoba S, O'Hara L, Carter F, Kelly AK, Fair T, Rizos D, et al. The association between metabolic parameters and oocyte quality early and late postpartum in Holstein dairy cows. J Dairy Sci 2012;95:1257–66.
[20] Oliveira LH, Nascimento AB, Monteiro Jr PLJ, Guardieiro MM, Wiltbank MC, Sartori R. Development of insulin resistance in dairy cows by 150 days of lactation does not alter oocyte quality in smaller follicles. J Dairy Sci 2016. Under review.
[21] Accorsi PA, Govoni N, Gaiani R, Pezzi C, Seren E, Tamanini C. Leptin, GH, PRL, insulin and metabolic parameters throughout the dry period and lactation in dairy cows. Reprod Domest Anim 2005;40:217–23.
[22] Sales JNS. Efeito da dieta com alta energia nos parâmetros metabólicos, endócrinos e reprodutivos de vacas *Bos indicus* e *Bos taurus*. PhD Dissertation. São Paulo: University of São Paulo; 2011.
[23] Leiva T, Cooke RF, Aboin AC, Drago FL, Gennari R, Vasconcelos JLM. Effects of excessive energy intake and supplementation with chromium propionate on insulin resistance parameters in nonlactating dairy cows. J Anim Sci 2014;92:775–82.
[24] Ferreira RM, Ayres H, Chiaratti MR, Ferraz ML, Araújo AB, Rodrigues CA, et al. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. J Dairy Sci 2011;94:2383–92.
[25] Ferreira RM. The low fertility of repeat-breeer Holstein (B. taurus) cows during summer heat stress is related to a low oocyte competence. PhD Dissertation. São Paulo: University of São Paulo; 2012.
[26] Baruselli PS, Ferreira RM, Sales JNS, Gimenes LU, Sá Filho MF, Martins CM, et al. Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology 2011;76:1583–93.
[27] Rodrigues CA, Ayres H, Reis EL, Nichi M, Bó GA, Baruselli PS. Artificial insemination and embryo transfer pregnancy rates in high production Holstein breedings under tropical conditions. In: 15th International Congress on Animal Reproduction. Porto Seguro 2004. p. 396.
[28] Orr WN, Cowan RT, Davison TM. Factors affecting pregnancy rate in Holstein-Friesian cattle mated during summer in a tropical upland environment. Aust Vet J 1993;70:251–6.
[29] Vanholder T, Papen J, Bemers R, Vertenten G, Berge ACB. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J Dairy Sci 2015;98:880–8.
[30] Rukkwamsuk T, Kruip TA, Wensing T. Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. Vet Q 1999;31:71–7.
[31] Gong J, Lee W, Garnsworthy P, Webb R. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 2002;123:419–27.
[32] Britt JH. Impacts of early postpartum metabolism on follicular development and fertility. Bovine Pract 1992;24:39–43.
[33] Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CO, Woad KJ, et al. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. Biol Reprod 2001;64:1624–32.
[34] Adamiak SJ. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biol Reprod 2005;73:918–26.
[35] Zhou J, Bievre M, Bondy CA. Reduced GLUT1 expression in Igf1–/– null oocytes and follicles. Growth Horm IGF Res 2000;10:111–7.
[36] Eppig JJ, O'Brien MJ, Pendola FL, Watanabe S. Factors affecting the developmental competence of mouse oocytes grown in vitro: follicle stimulating hormone and insulin. Biol Reprod 1998;59:1445–53.
[37] Laskowski D, Humblot P, Gustafsson H, Bage R, Andersson G, Abraham C, et al. Influence of insulin during oocyte maturation of in vitro produced bovine embryoes. Reprod Domest Anim 2013;48:66.
[38] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment—insulin resistance and beta-cell function from fasting plasma-glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[39] Hackbart KS, Cunha PM, Meyer RK, Wiltbank MC. Effect of glucocorticoid-induced insulin resistance on follicle development and ovulation. Biol Reprod 2013;88:153.