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Intrinsic and extrinsic factors that influence ovarian environment and efficiency of reproduction in cattle

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Abstract

The emergent concepts on ovary environment, reproductive physiology and the development of pharmacology are constantly supporting the advance of assisted reproduction. Within the last years, the biotechnics related to the synchronization of follicular development and the manipulation of bovine estrus cycle have progressed rapidly and consistently. The combined use of timed-artificial insemination (TAI), superovulation (SOV), ovum pick up (OPU), *in vitro* embryo production (IVEP) and timed-embryo transfer (TET) has a great potential to improve reproductive outcomes and disseminate selected genetics, diminishing the interval of generations and improving herds genetic gain. However, several factors can potentially affect the efficiency of these procedures. The knowledge of the particularities of the genetic groups, follicular growth manipulation, follicular population predictors, and metabolic and environmental aspects that interfere with ovarian environment and, consequently, oocyte quantity and quality is crucial to optimize the reproductive programs. This review aims to elucidate some factors that affect the ovarian environment and must be well known in order to improve the efficiency of reproduction in cattle.

Keywords: AMH, follicle, genetic group, heat stress, insulin, oocyte.

Introduction

The increasing knowledge on bovine physiology of the estrous cycle enabled the tight control of follicular growing phases using pharmacological strategies, facilitating the reproductive management and supporting the development of biotechnologies of reproduction (Baruselli *et al*., 2004, 2012; Lamb *et al*., 2010). The strategic reproductive management associate with the use of biotechniques of reproduction can be potentially used to disseminate animals with high genetic merit efficiently. Reproductive tools such as timed-artificial insemination (TAI), superovulation (SOV) of selected donor, *in vivo* embryo production (IVEP), and timed-embryo transfer (TET) had a dramatic growth within the last years, accelerating the selection, multiplication and dissemination of animals with superior genetics e high potential for beef and milk production (Hansen, 2014).

The success of reproductive biotechnologies application, however, is greatly dependent on individual ovarian characteristics (Wise, 1987; Tan and Lu, 1990; Kastrop *et al*., 1991; Pavlok *et al*., 1992; Lonergan *et al*., 1994; Gandolfi *et al*., 1998; Guerreiro *et al*., 2014; Batista *et al*., 2016), genetic particularities (Sartori *et al*., 2001, 2010; Sartorelli *et al*., 2005; Beg and Ginther, 2006; Gimenes *et al*., 2008, 2011) nutritional and metabolic status (Wiltbank *et al*., 2006; Sales *et al*., 2015; Baruselli *et al*., 2016; Ferreira *et al*., 2016b), and environmental factors (Al-Katanani *et al*., 2002; Torres-Júnior *et al*., 2008; Ferreira *et al*., 2011, 2013, 2016a) that may influence the number and quality of the oocytes.

In this context, the present review aims to discuss some key points related to genetics, breed, antral follicle populations, manipulation of ovarian follicular growth, metabolic status (insulin resistance) and environmental factors (heat stress) associated with oocyte and embryo quality.

Physiological factors that influence ovarian characteristics

Influence of genetic group on ovarian characteristics

Several physiological differences between *Bos indicus* and *Bos taurus* cattle related to follicular dynamics have been previously reported. The understanding of these differences has been crucial in developing reproductive strategies specific for each genetic group. The *Bos indicus* cattle are the predominant breeds raised in tropical regions. However, because *Bos indicus* cattle have subtle differences in their reproductive behavior compared with *Bos taurus* breeds (Bó *et al*., 2003; Baruselli *et al*., 2007; Sartori *et al*., 2010), one cannot assume that the physiological parameters observed in *Bos taurus* would be the same as in *Bos indicus* cattle.

In *Bos indicus*, follicle deviation occurred 2.5 to 2.6 days after ovulation (Sartorelli *et al*., 2005; Gimenes *et al*., 2008; respectively), while in *Bos taurus*, follicle deviation occurred 2.8 days after wave emergence (Ginther *et al*., 1996), which means close than one day latter than for *Bos indicus*. The size of the dominant follicle at deviation is smaller in *Bos indicus* (6.0 mm; Sartorelli *et al*., 2005; Gimenes *et al*., 2008) than *Bos taurus* cattle (8.5 mm; Ginther *et al*., 1996). The acquisition of ovulatory capacity of the dominant follicle, measured by the ovulation after LH challenge, occurs at a smaller diameter in *Bos indicus* (7 to 8.4 mm; Gimenes *et al*., 2008) than *Bos taurus* cattle (10 mm; Sartori *et al*., 2001). The maximum diameters of the dominant follicle (10-12 mm *vs*. 14-20 mm) and the CL (17-21 mm *vs*.

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20-30 mm) are also smaller in *Bos indicus* than in *Bos taurus* cattle (reviewed by Bó *et al*., 2003). Regarding the estrous behavior, *Bos indicus* breeds exhibit estrus of shorter duration compared to *Bos taurus*; (Figueiredo *et al*., 1997; Bó *et al*., 2003) or with high producing dairy cows (milk production is inversely proportional to estrus duration; Lopez *et al*., 2004; Wiltbank *et al*., 2006).

These differences have important practical implications when setting protocols for TAI and TET. The selection of embryo recipients may also be influenced by physiological differences between the genetic groups. For example, because the CL is more difficult to palpate (smaller) in *Bos indicus* cattle, recipients suitable to receive an embryo may be rejected on CL size evaluation if the particularities of breed are unknown. Previous studies have also shown that the P4 content of the CL and serum P4 concentrations were lower in *Bos indicus* than in *Bos taurus* cattle (Segerson *et al*., 1984). Therefore, conception rates relative to P4 levels in tropical countries, primarily involving *Bos indicus* recipients on pasture, may be quite different than *Bos taurus* females maintained in cold-temperate environments with more adequate nutrition.

It has also been reported that IVEP is more efficient in *Bos indicus* breeds than in *Bos taurus* breeds (Pontes *et al*., 2010; Guerreiro *et al*., 2014). The greater population of antral follicles found in *Bos indicus* cattle would appear to result in a greater number of suitable oocytes for *in vitro* culture (Batista *et al*., 2014). In this context, *Bos indicus* (Nelore) heifers are reported to have greater number of visualized follicles and to produce greater number of total oocytes per OPU session, cultured COC and blastocyst rates than *Bos taurus* (Holstein) heifers (Gimenes *et al*., 2015; Table 1).

Table 1. Effect of genetic group on oocyte recovery and quality, and developmental competence of *Bos indicus* (Nelore) and *Bos taurus* (Holstein) heifers.

	Genetic group	
	Nelore (n = 9)	Holstein (n = 9)
Number of replicates	6	6
Number of OPU sessions	54	54
Oocyte recovery and quality		
Visualized follicles	41.0 ± 2.1[a]	22.1 ± 1.3[b]
Total oocytes	37.1 ± 2.6[a]	15.4 ± 1.2[b]
Recovery rate (%)	82.3[a]	66.8[b]
Oocytes submitted to IVC	25.6 ± 1.8[a]	9.1 ± 0.9[b]
Developmental competence		
Cleaved structures	21.1 ± 1.6[a]	5.2 ± 0.5[b]
Cleavage rate (%)	82.6[a]	59.9[b]
Blastocysts 7 days after IVF	7.3 ± 0.9[a]	1.1 ± 0.2[b]
Blastocyst rate (%)	28.3[a]	14.1[b]

[a,b]P < 0.05. Adapted from Gimenes *et al*. (2015).

Influence of Anti-Müllerian hormone on ovarian characteristics

The success of SOV and OPU-IVEP is greatly dependent on individual ovarian characteristics that may influence the number and quality of the oocytes that are retrieved (Wise, 1987; Tan and Lu, 1990; Kastrop *et al*., 1991; Pavlok *et al*., 1992; Lonergan *et al*., 1994; Gandolfi *et al*., 1998). It is known, for example, that the number of antral follicles in the early follicular phase directly correlates with ovarian reserve (Frattarelli *et al*., 2000). Indeed, the antral follicular population (AFP) directly represents the follicle cohort in the ovaries, which is associated with the number of oocytes retrieved for IVEP.

A large variability of AFP is reported among different cows, however AFP count is highly repeatable within animal (Burns *et al*., 2005; Ireland *et al*., 2007), and anti-Müllerian hormone (AMH) can be considered a reliable endocrine marker of ovarian reserve (Ireland *et al*., 2007, 2008; Monniaux *et al*., 2012). AMH is a dimeric glycoprotein member of the TGFβ superfamily of growth factors synthesized from granulosa cells of preantral and small antral follicles (growing follicles up to the antral stage or to a diameter of approximately 6 mm) and represents the indirect activity of the follicular pool (Cate *et al*., 1986; Grootegoed *et al*., 1994; Durlinger *et al*., 1999; Weenen *et al*., 2004). In cattle, circulating AMH concentration can help veterinarians to predict AFP in ovaries (Ireland *et al*., 2008; Rico *et al*., 2009; Batista *et al*., 2014), response to SOV treatments (Rico *et al*., 2009; Monniaux *et al*., 2010a, b; Souza *et al*., 2015), and more recently as a marker to predict IVEP performance of *Bos taurus* (Guerreiro *et al*., 2014; Gamarra *et al*., 2015; Vernunft *et al*., 2015) and *Bos indicus* breeds (Guerreiro *et al*., 2014).

Aiming to determine the relation between AMH and AFP in different genetic groups, our group recently conducted a sequence of studies. In the first study (Baldrighi *et al*., 2014), despite the high variability in AFP between individuals within each genetic group, the AFP count was greater in Gir (*Bos indicus*) than in Holstein (*Bos taurus*) and Murrah (*Bubalus bubalis*) heifers (P = 0.01; Fig. 1). Similarly, AMH concentration was lower (P < 0.01) for Holstein and Murrah heifers than for Gir heifers. For the three genetic groups studied, a positive relationship between AFP and AMH concentration was detected.

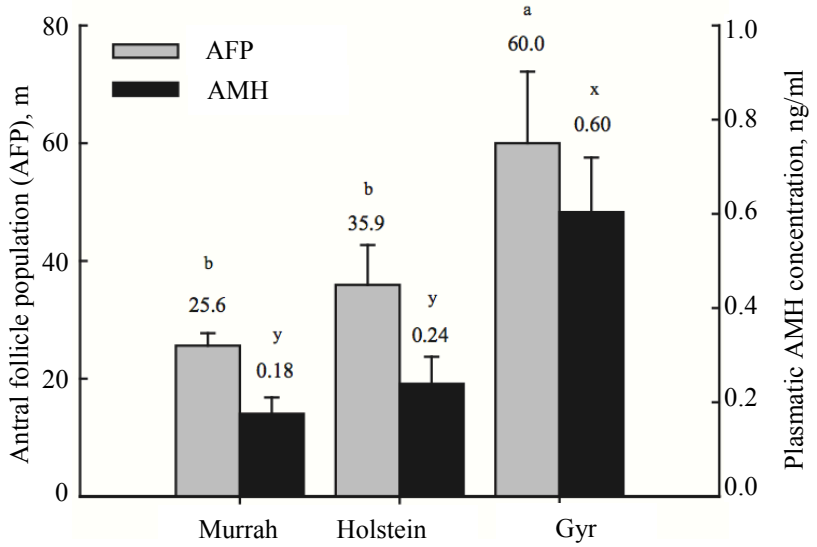

Figure 1. Number of antral follicle population (AFP) and plasma anti-Müllerian hormone (AMH) concentration in Murrah (*Bubalus bubalis*; n = 13), Holstein (*Bos taurus*; n = 15) and Gir (*Bos indicus*; n = 10) heifers. Data are shown as the means ± SEM. Different letters within columns of the same color are different (AFP: a≠b; P = 0.01 and AMH concentration: x≠y; P < 0.001). Adapted from Baldrighi *et al*. (2014).

Similarly, in the second study (Batista *et al*., 2014), the AFP (P < 0.05) and the AMH concentration (P < 0.0001) were higher in Nelore (*Bos indicus*) than in Holstein (*Bos taurus*) heifers, and they were correlated. Furthermore, the number of ovarian follicles observed in all evaluation periods (-120, -60 days and 0 days) was correlated with plasma AMH concentrations in both *Bos taurus* (Holstein) and *Bos indicus* (Nelore) heifers (Fig. 2). These results suggest that AMH could be a possible long-term endocrine marker of ovarian activity. Therefore, a single blood sample taken at a random stage of the oestrous cycle to measure serum AMH concentration could be considered a reliable phenotypic marker to predict the relative number of follicles, regardless of genetic group.

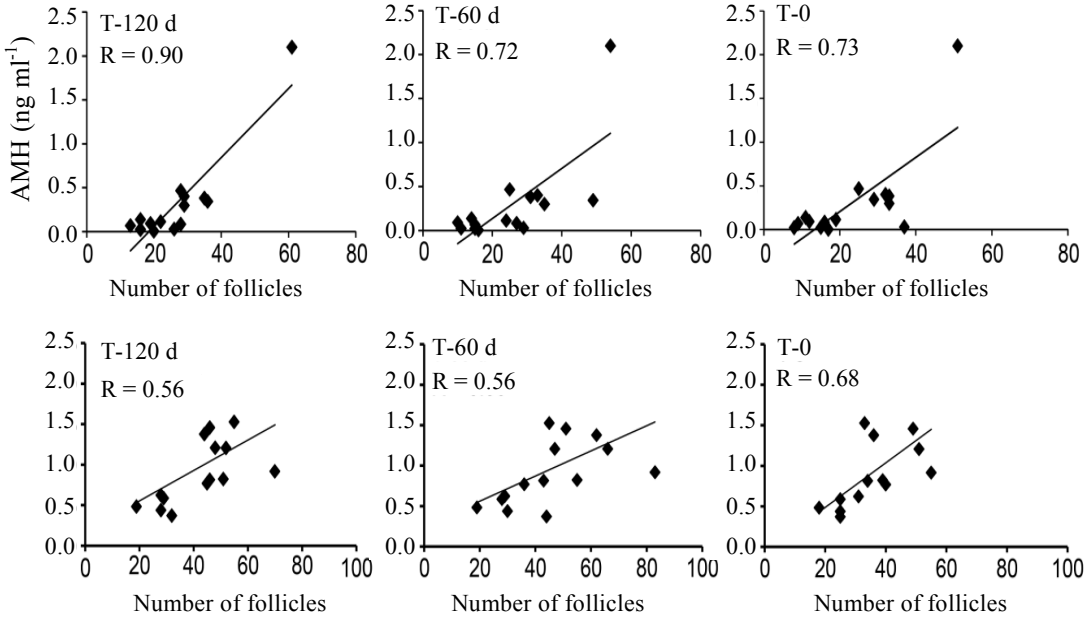

Figure 2**.** Relationship between the numbers of antral follicles counted 120 (T-120) or 60 (T-60) days previous or at (T0) AMH dosage, and plasma AMH concentration in Holstein (n = 16; A) and Nelore (n = 16; B) heifers. Adapted from Batista *et al*. (2014).

The third study was carried out with the same genetic groups. Corroborating the aforementioned findings, plasma AMH in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) heifers had a positive correlation with the number of follicles aspirated, COCs retrieved, COCs cultured, and embryos produced per OPU session (Fig. 3). However, cleavage and blastocyst rates had no correlation with circulating AMH (Fig. 3; Guerreiro *et al*., 2014).

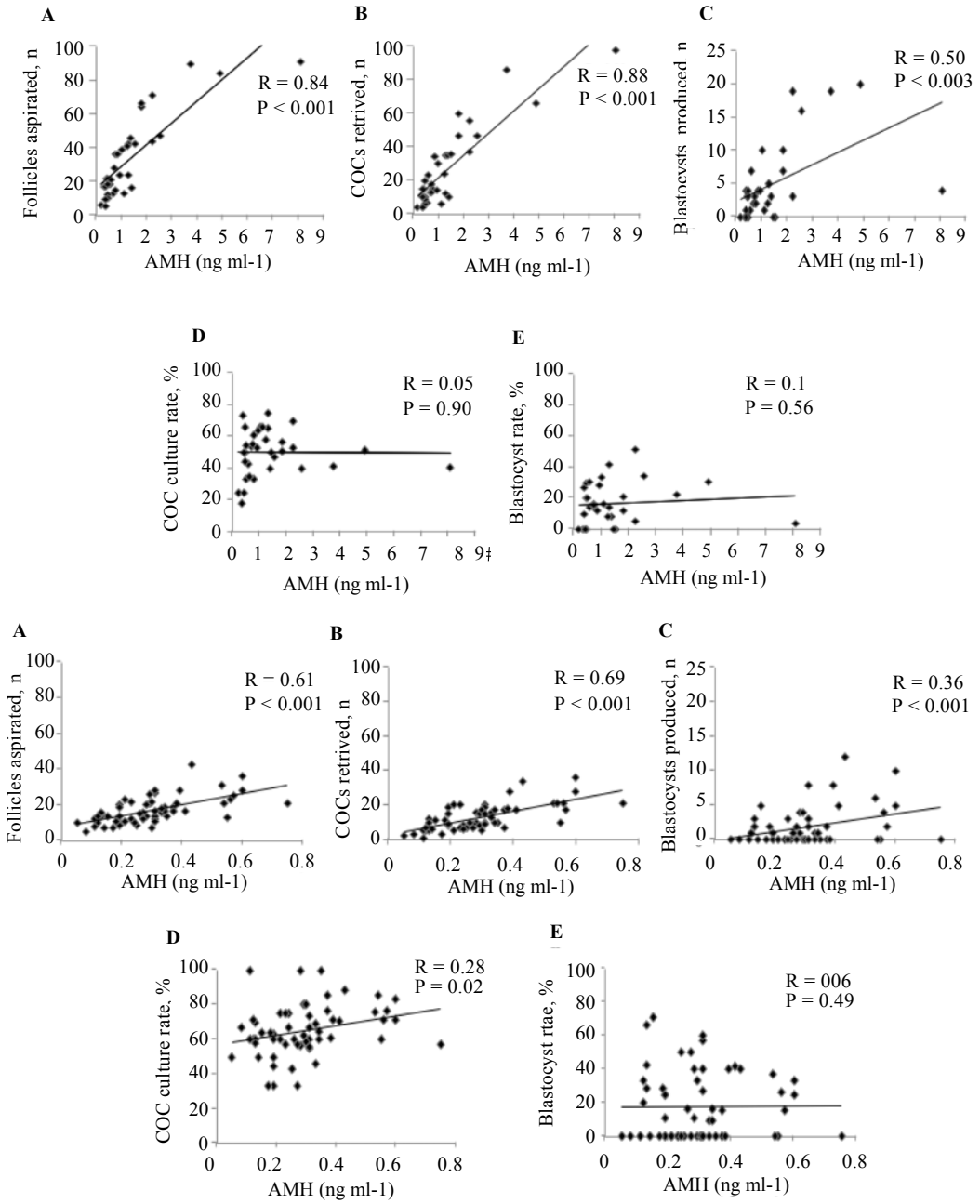

Figure 3. Correlation between plasma anti-Müllerian hormone (AMH) concentrations and variables related to ovum pick-up and *in vitro* embryo production in *Bos indicus* (Nelore; superior figure) and *Bos taurus* (Holstein; inferior figure) donors. Relationship between plasma AMH concentration and the number of follicles aspirated (A), COCs retrieved (B), blastocysts produced (C), COC culture rate (%, D) and blastocyst rate (%, E). Adapted from Guerreiro *et al*. (2014).

Because genomic information allows producers to identify genetic merit of their animals at premature ages, we have recently investigated the possibility of producing embryos from oocytes of young female calves that were only 2-4 months old. We have found greater plasma AMH concentrations in calves compared to cycling heifers in both genetic groups, *Bos indicus* and *Bos taurus* (Fig. 4; Batista *et al*., 2016). Indeed, it was previously shown that AMH concentrations fall in parallel to the number of ovarian follicles as rodents (Kevenaar *et al*., 2006) and women (Piltonen *et al*., 2005) age.

Furthermore, a positive correlation was observed between plasma AMH concentration and the number of follicles ($P < 0.0001$), retrieved COCs ($P < 0.0001$), COCs cultured ($P < 0.0001$), cleaved COCs ($P < 0.0001$ and $P = 0.001$), and produced blastocysts ($P = 0.0003$ and $P = 0.009$) from *Bos indicus* (Nelore) and *Bos taurus* (Holstein; Fig. 5) donor calves. However, there was no correlation between circulating AMH levels and cleavage rate ($P = 0.24$ and $P = 0.36$), COC culture rate ($P = 0.28$ and $P = 0.07$), or blastocyst rate ($P = 0.52$ and $P = 0.08$; Batista *et al*., 2016).

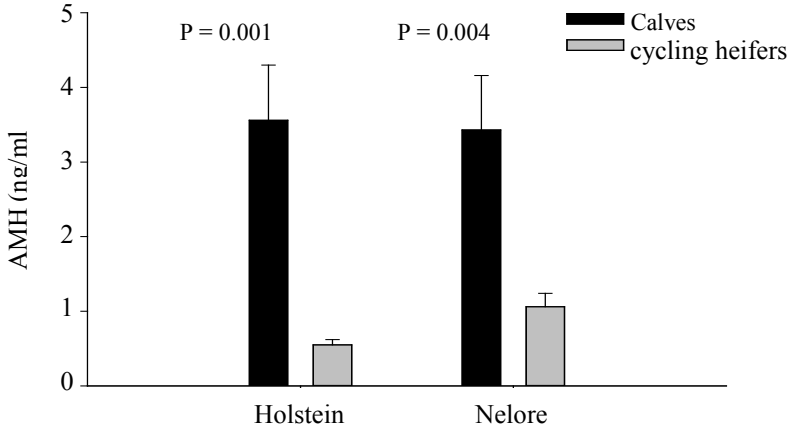

Figure 4. AMH plasma concentration (ng/ml) in calves (aging 2 to 4 months, Holstein: n = 24 and Nelore: n = 30) and cycling heifers (Holstein: n = 10 and Nelore: n = 12). Batista *et al*. (2016).

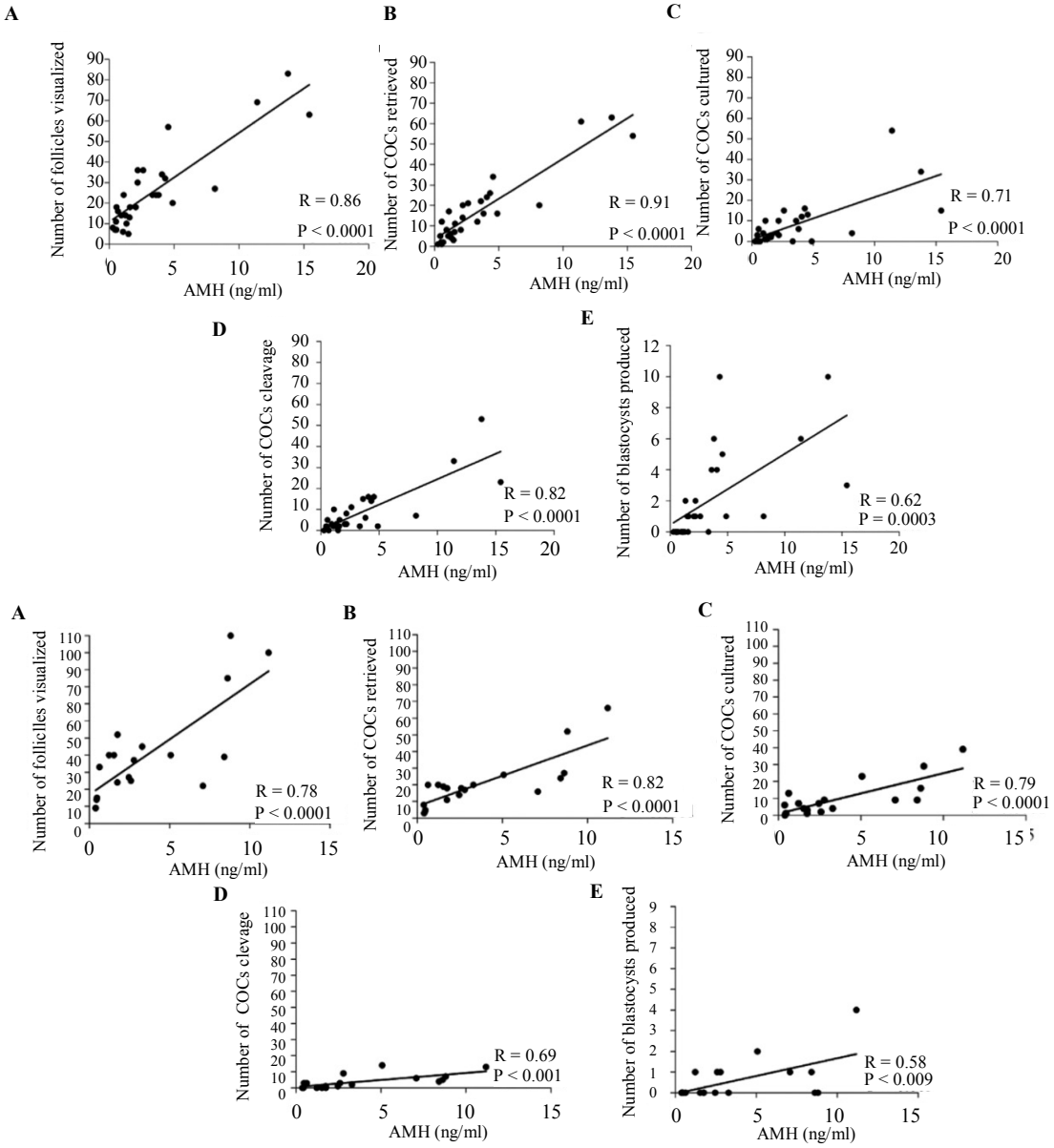

Figure 5. Correlations between plasma anti-Müllerian hormone (AMH) concentrations, the number of follicles and variables related to laparoscopic *ovum pickup*, and *in vitro* embryo production in *Bos indicus* (n = 29; superior figure) and *Bos taurus* (n = 19; inferior figure) donor calves. Relationships between the number of follicles (A), cumulus-oocyte complexes retrieved (B), cultured (C), and cleavage (D), blastocysts produced (E), and AMH concentration (ng/ml). Batista *et al*. (2016).

Metabolic and nutritional factors that influence ovarian characteristics

The nutritional and metabolic status can interfere with follicular growth patterns, secretion of reproductive hormones, and oocyte quality in cattle (Leroy *et al*., 2008; Ashworth *et al*., 2009; Batista *et al*., 2013; Sales *et al*., 2015; Baruselli *et al*., 2016; Ferreira *et al*., 2016a). Thus, metabolic imbalances may cause systemic alterations that can compromise the success of reproductive biotechnologies, such as TAI, SOV and OPU-IVEP (Webb *et al*., 2004; Adamiak *et al*., 2005).

Maternal health and nutritional status during gestation have been reported as important factors that interfere on the number of primordial follicles formed during fetal life (Ireland *et al*., 2011; Evans *et al*., 2012). In this context, the influence of mother's undernutrition on ovarian status of female offspring was previously investigated (Mossa *et al*., 2009). Heifers received diets for maintenance or food restriction (0.6 of energetic needs for maintenance) right before conception until 110 days of pregnancy. The AFP and concentration of AMH of the female calves born from undernourished cows were on average 60% lower than from calves born from cows kept under maintenance diets, when they were 7, 18 and 35 weeks of age. Moreover, studies indicate that disruptions on mother's health during gestation may reduce the ovarian follicular reserve. In this basis, cows with high milk somatic cell count, indicating mammary gland infection, gave birth to female calves with almost 50% less AMH concentration than calves born from healthy cows (low somatic cell count; 0.01 ± 0.08 *vs.* 0.13 ± 0.03 ng/ml; P < 0.05; Ireland *et al*., 2011; Evans *et al*., 2012).

On the other hand, the overfeeding can also have negative aspects on reproduction. A common aspect of commercial SOV and OPU-IVEP programs is the use of non-lactating or late lactation cows as oocyte and embryo donors. In these animal categories, the negative effects of overfeeding (excessive energy intake) can compromise *in vitro* oocyte developmental competence, especially in over-conditioned (high body condition score) females (Adamiak *et al*., 2005). The mechanisms that mediate these negative effects on oocyte competence may be related to endocrine alterations, such as hyperinsulinemia, peripheral resistance of insulin, and increased glucose and IGF-I, which may interfere with glucose transport in embryo cells and increased apoptosis.

Our research group conducted a study to evaluate the impact of different energy intakes on metabolic profiles and oocyte quality of the non-lactating Gir (*Bos indicus*) cows submitted to successive OPU sessions (Sales *et al*., 2015). Diets were formulated to achieve maintenance (M) or 1.7% of maintenance (1.7M) for non-lactating cows. Following 60 days of high energy feeding, cows had reduced *in vitro* oocyte competence (Fig. 6). Cows fed high-energy diets had greater glucose and insulin concentrations and a greater level of insulin resistance as determined by the glucose tolerance test. Furthermore, cows receiving high-energy diet had lower abundance of transcripts *for* GLUT1, IGF1R, IGF2R and HSP70.1 genes in oocytes.

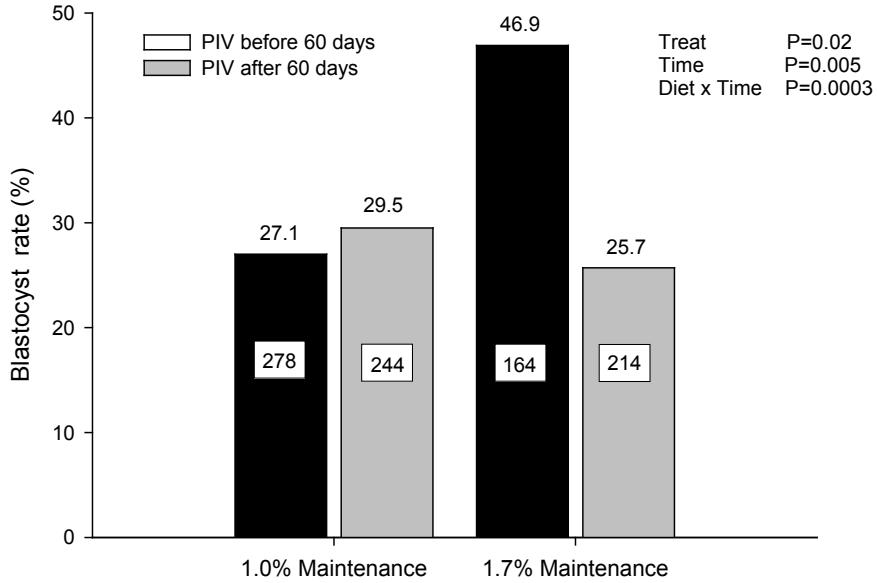

Figure 6. *In vitro* embryo production in non-lactating cows (n = 14) fed diets to meet 100 or 170% of energy of maintenance and submitted to nine OPU session at 14 day intervals. Adapted from Sales *et al*. (2015).

Insulin has an important role in cellular metabolism, however, in excess it may interfere with various metabolic and reproductive processes in dairy cows (De Koster and Opsomer, 2013). During early lactation, low circulating insulin concentrations have been associated with impaired fertility by delaying resumption of cyclicity (Gong *et al*., 2002). Although greater concentrations of insulin are important to restore ovarian cyclicity, it has been shown in heifers that they may also compromise oocyte quality (Adamiak *et al*., 2005) and, therefore, fertility. In that regard, excessive insulin may reduce oocyte quality in heifers (Adamiak *et al*., 2005) and IVEP and gene expression linked to cellular metabolism in nonlactating *Bos indicus* dairy

cows (Sales, 2011). 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; Britt, 1992), 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.

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 2; Ferreira *et al*., 2011 and reviewed by Baruselli *et al*., 2016). In addition to increased number of days not pregnant, late-lactation cows had lower milk yield, greater number of previous inseminations and greater BCS than early-lactation cows (Table 2). 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 blastocyst (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 2; Matthews *et al*., 1985; Hackbart *et al*., 2013). The HOMA-IR was calculated according to a formula presented in the previous studies (Matthews *et al*., 1985; Hackbart *et al*., 2013): [basal insulin (mIU/ml) x 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 2; Ferreira *et al*., 2016b). 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 (Leiva *et al*., 2015). Both relative and absolute numbers of copies of mitochondrial DNA (mtDNA) were reduced in oocytes retrieved from late-lactation cows (Table 2; Ferreira *et al*., 2016a, b), suggesting a disruption of oocyte quality (Ferreira *et al*., 2016a). 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 (Ferreira *et al*., 2016a). Furthermore, there was a greater ratio of BAX/BCL2 in late-lactation cows, indicating an apoptotic phenotype of the oocytes from this category (Ferreira *et al*., 2016a; Table 2). 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.

Environmental factors that influence ovarian characteristics

Mainly in tropical regions, the poor IVEP yields in *Bos taurus* cattle can be partly attributed to the heat stress (Al-Katanani and Hansen, 2002; Al-Katanani *et al*., 2002; Ferreira *et al*., 2011, 2016a). However, previously reports have shown that heat stress also can exert a deleterious effect on ovarian follicular dynamics and oocyte competence in *Bos indicus* cattle (Torres-Júnior *et al*., 2008).

A previous seasonal experiment demonstrated that once the pool of ovarian oocytes is damaged by heat stress, two or three estrous cycles are required (after the end of heat stress) to restore the follicular pool and oocyte quality (Roth *et al*., 2001). However, the study with *Bos indicus* cows (Torres-Júnior *et al*., 2008) showed a carry-over effect of heat stress on blastocyst production up to 105 days after the end of the heat stress (Fig. 7). Therefore, it seems that follicles and oocytes are damaged by heat stress during early stages of folliculogenesis, with a delayed deleterious effect on ovarian function. Nevertheless, *Bos indicus* breeds have been shown to be more resistant to tropical conditions (i.e. elevated temperature and humidity) than breeds that evolved in temperate climates (i.e, *Bos taurus,* as Holstein). Essentially, the adaptation of certain breeds to elevated heat and humidity is related to their ability to thermoregulate their body temperature (Bennett *et al*., 1985; Hammond *et al*., 1996; Gaughan *et al*., 1999).

Heat stress also has a deleterious effect on superovulatory response in Holstein donors. In a recent retrospective analysis, (Vieira *et al*., 2014) reported a negative effect of the warm season in Brazil on the number of IVEP (2.8 ± 0.3 *vs*. 4.4 ± 0.4; P = 0.03) and percentage of embryos classified as grade I and II (21.4 *vs*. 32.8%, P < 0.0001) in Holstein donors. In addition, Ferreira *et al*. (2011) reported decreased COC numbers in Holstein cows when OPU was performed during the summer months. Yet, when blastocyst rates were evaluated, an interaction between group and season indicated that the effect of season was dependent on animal category. Heat stress decreased blastocyst rate for heifers, peak lactation and repeat breeder cows, however this drop compared to winter was more intense for repeat breeders (Fig. 8; Ferreira *et al*., 2011). Regardless of season, blastocyst rates were lower in repeat breeder cow than in heifers. Additionally, repeat breeder blastocyst quality was compromised in comparison to heifers and cows at peak lactation during the summer (Ferreira *et al*., 2011).

Table 2. Ovum pick up, *in vitro* embryo production and metabolic profile of high production Holstein cows during early or late in lactation.

	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 to 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
BHB 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

Adapted from Ferreira *et al*. (2016a, b).

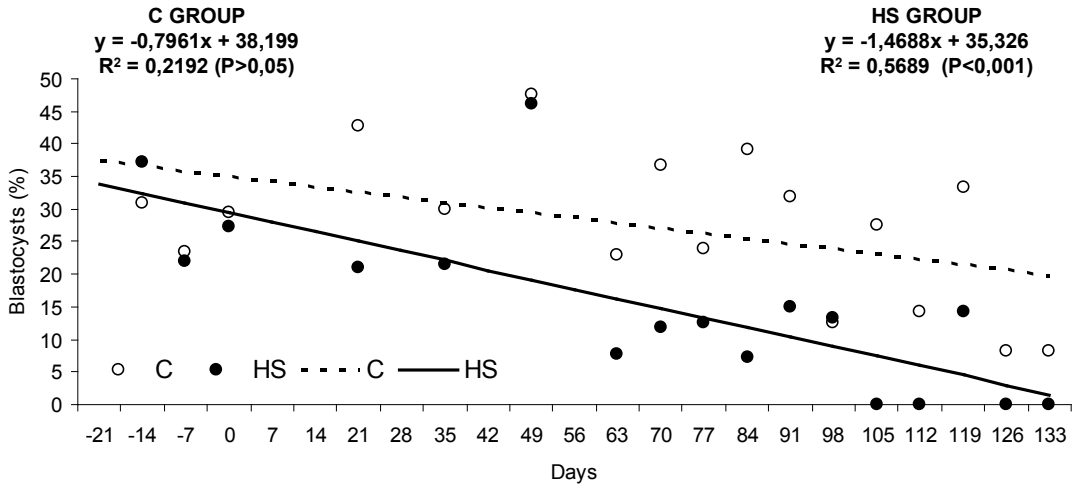

Figure 7. Percentage of blastocysts and regression equation's adjusted lines of oocytes recovered from Gyr (*Bos indicus*) cows exposed to thermoneutral (C) or heat-stress (HS) treatments. Adapted from Torres-Júnior *et al*. (2008).

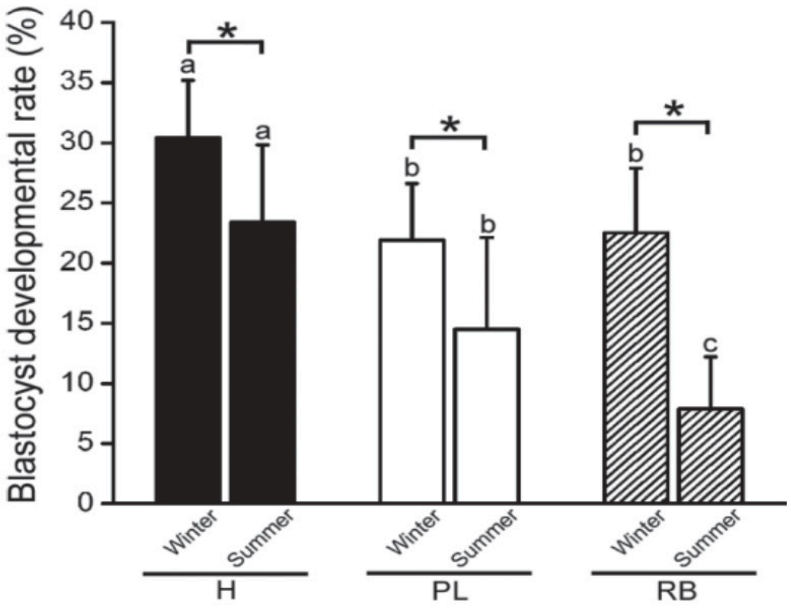

Figure 8. Blastocyst rate 7 d post-*in vitro* insemination of Holstein cattle oocytes of different groups during summer and winter [heifers (H; n = 150 and 244, respectively), high-producing cows in peak lactation (PL; n = 103 and 191, respectively), and repeat-breeder cows (RB; n = 177 and 413, respectively)]. Interaction season-group ($P < 0.0001$); mean (± SEM) values within season (a≠ b ≠ c) and within group (*) differ ($P < 0.0001$). Adapted from Ferreira *et al*. (2011).

In a subsequent study, the same patter previously described for blastocyst rate (Ferreira *et al*., 2011) was observed for pregnancy per AI (P/AI) after TAI of females of the same three categories during the summer and winter (Fig. 9; Ferreira *et al*., 2013). As expected, heat stress reduced P/AI of all categories of Holstein females studied (heifers, peak lactation and repeat breeder cows), probably because of heat-disruption of oocyte quality (Al-Katanani *et al*., 2002; Torres-Júnior *et al*., 2008; Ferreira *et al*., 2011, 2016a, b).

Thus, heat stress has a deleterious effect on oocyte quality of both *Bos indicus* and *Bos taurus* dairy females, potentially decreasing the results of TAI, SOV and OPU-IVEP procedures.

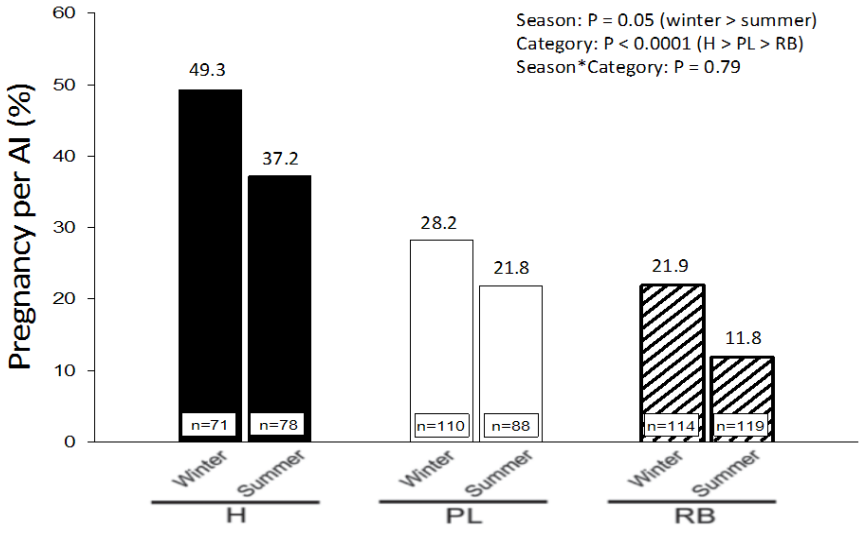

Figure 9. Pregnancy per artificial insemination (AI) of Holstein cattle of different categories during summer and winter (heifers = H, high-producing cows in peak lactation = PL and repeat-breeder cows = RB). Adapted from Ferreira *et al*. (2013).

Conclusion

The success of the application of reproductive biotechniques is closely dependent on individual ovarian characteristics, genetic particularities, nutritional and metabolic status, and environmental factors that may influence the number and quality of the oocytes and embryos. Therefore, factors related to breed, follicular count (AMH), heat stress and nutrition should be considered when applying TAI, SOV, OPU-IVEP and TET in the field. Adequate control of environmental and nutritional conditions should be one of the requisites to be accomplished before implementing any reproduction biotechnology. On the other hand, the knowledge of physiological differences between *Bos indicus* and *Bos taurus* cattle is crucial to determine he correct strategies to manipulate follicular wave dynamics for TAI, SOV, OPU-IVEP and TET programs. Additionally, the selection of oocyte and embryo donors with greater follicular population can optimize the efficiency of embryo production techniques. Once these biotechnologies can be efficiently applied on a large scale in the field, significant enhancements in livestock genetic gain can be accomplished with great productivity and economic return for the activity.

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