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Factors that interfere with oocyte quality for *in vitro* production of cattle embryos: effects of different developmental & reproductive stages

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Abstract

The success of IVP is ultimately dependent on the number and quality of the cumulus-oocyte complexes (COC) harvested during the OPU procedure. Several factors appear to be critical to oocyte quality including follicle size, environment factors such as heat-stress, genetic background, age and lactation status of donor animals, all having a remarkable influence on the results of IVP. The aim of this review is to highlight some critical areas that can help veterinary practitioners to enhance OPU efficiency and successfully implement IVP into their routine practice. Focus will be given to recent findings in the literature and underlying physiological aspects that may be interfering with the quality of oocytes addressed to IVP in cattle at younger ages (calves and prepubertal heifers), pregnant vs non-pregnant status, and possible interactions with lactation and days postpartum during OPU.

Keywords: bovine, embryo, IVF, oocyte, pregnancy.

Introduction

Oocyte collection by ovum pick-up (OPU) associated with *in vitro* production of embryos (IVP) are important technologies that can improve efficiency of both dairy and beef herds (Merton *et al*., 2003; Pontes *et al*., 2010). The IVP industry is evolving fast in the last decade, and some parallel technologies such as genomics and the discovery of embryo production markers (i.e. AMH assay) are driving a more targeted and efficient genetic progress through IVP. For example, the use of genomic technology allows the identification of genetic superior animals at much earlier ages. As a result, producers are pushing both the industry and the scientific community to develop techniques that are more suited to younger animals sent to OPU-IVP routines, accelerating the genetic gain by decreasing generation intervals.

Despite of animal age in which OPU is performed, the overall success of IVP is ultimately dependent on the number and quality of the cumulus-oocyte complexes (COC) harvested during the OPU procedure. Evidently, several factors appear to be critical to oocyte quality including follicle size (Lonergan *et al*., 1994; Seneda *et al*., 2001), environment factors such as heat-stress (Torres-Júnior *et al*., 2008; Ferreira *et al*., 2011; Ferreira *et al*., 2016), genetic background (Gimenes *et al*., 2015; Sales *et al*., 2015), age (Batista *et al*., 2016a) and lactation status of donor animals (Baruselli *et al*., 2016), all having a remarkable influence on the results of IVP. Additionally, there is a growing body of scientific literature demonstrating the direct role of hormonal milieu during follicle development on oocyte quality. Focus has been given to the possible positive effects of circulating levels of P4 in determining oocyte quality in IVP (Lonergan, 2011). However, a recent study from our research group has demonstrated that neither exposure to lower levels of LH, nor cycles of P4, are limiting to oocyte viability and development to blastocyst stage (Batista *et al*., 2016b). Further research is needed to elucidate some of these rather complex interactions between reproductive hormones and oocyte quality.

Cattle breed is also one of the key factors that influence the efficiency of OPU-IVP. Interestingly, *Bos indicus* (also known as zebu breeds) and *Bos taurus* donors seem to have significant differences regarding the results of IVP (Pontes *et al*., 2010; Guerreiro *et al*., 2014a; Gimenes *et al*., 2015). It appears quite noticeable in most of the published literature that *Bos indicus* females have much greater numbers of oocytes retrieved during OPU; presumably due to greater numbers of ovarian follicle population and plasma anti-Müllerian concentration (Batista *et al*., 2014). Furthermore, zebu donors also have been shown to yield greater amounts of viable oocytes compared to *Bos taurus* donors (Guerreiro *et al*., 2014a; Gimenes *et al*., 2015).

Some management issues such as the type of diet and level of feed intake, as well as heat stress are widely known to have a great impact on oocyte quality and IVP. However, the interaction of these key factors may differ in *Bos taurus* and *Bos indicus* cattle. The level of energy intake for example, can affect circulating levels of insulin and IGF-1 (reviewed by *Sartori et al*., 2016). As a result, excessive increase in insulin concentration in blood, mainly in dairy cows later in lactation, are negatively associated with oocyte quality (reviewed by Baruselli *et al*., 2016). Exposure to heat stress is widely known to be deleterious to oocyte competence in *Bos taurus* cattle (Ferreira *et al*., 2011; Ferreira *et al*., 2016). Surprisingly, *Bos indicus* donors although more resistant to environmental heat stress conditions, are also affected by heat stress and may require nearly four months to recover oocyte quality to pre-heat stress levels, even after a short period of heat exposure (Torres-Júnior *et al*., 2008).

Altogether, several factors can be detrimental to

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oocyte quality and must be taken into account during IVP in cattle. Thus, the aim of this review is to highlight some critical areas that can help veterinary practitioners to enhance OPU efficiency and successfully implement IVP into their routine practice. Focus will be given to recent findings in the literature and underlying physiological aspects that may be interfering with the quality of oocytes addressed to IVP in cattle at younger ages (calves and prepubertal heifers), pregnant vs non-pregnant status, and possible interactions with lactation and days postpartum during OPU.

Effect of different developmental & reproductive stages on oocyte quality and *in vitro* embryo production

Oocyte quality and IVP in donor calves

In cattle the population of ovarian follicles at birth is estimated at 235,000 (Ericksson, 1966; Betteridge *et al*., 1989). However, as in all mammals, this follicle population quickly decreases with aging (Ericksson, 1966). Therefore, young heifers have more antral follicles in their ovaries (Desjardins and Hafs, 1969), which could be associated with more efficient IVP. Previous studies have shown that calves require gonadotropin stimulation before oocyte collection and IVP to achieve acceptable results (Presicce *et al*., 1997; Maclellan *et al*., 1998; Taneja *et al*., 2000). Among the hormonal protocols employed in calves selected for oocyte recovery, most use follicle-stimulating hormone (FSH) treatment before using laparoscopic OPU (LOPU) and IVP technologies (Armstrong *et al*., 1992; Fry *et al*., 1998).

Recently, our research group conducted a series of studies to evaluate different hormonal protocols for collection of oocytes followed by IVP in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) calves (Batista *et al*., 2016a). An experiment was conducted at a commercial beef farm near Paulínia city (São Paulo, Brazil). A total of 45 Nelore donors were used: 30 calves (3 to 4 month) and 15 cyclic heifers (18 to 24 month). In a second experiment conducted at the São Paulo University Campus (USP, Pirassununga Campus, SP, Brazil), a total of 34 Holstein donors were used, including 24 calves (3 to 4 month) and 10 cyclic heifers aged 14 to 16 month. All calves were randomly assigned to receive a superstimulatory treatment with pFSH (calves with pFSH, n = 15) or not (calves without FSH, n = 15). Cycling heifers were subjected to a transvaginal ultrasound - guided OPU followed by IVP procedure at random stages of the estrous cycle.

All the calves underwent to LOPU. Calves without FSH were also subjected to LOPU at random stages of the estrous cycle. Superistimulated calves were treated before LOPU with an intravaginal progesterone device (day 0, Eazi-Breed CIDR, 0.33 g; Zoetis, São Paulo, SP, Brazil). After 5 days, calves received 4 treatments of porcine FSH (pFSH; 140 mg of pFSH; Folltropin, Agener, SP, Brazil) administered twice daily in decreasing doses (40 mg [day 5, AM], 40 mg [day 5, PM], 30 mg [day 6, AM]) over a 2-day period. The LOPU was performed 12 h after the last treatment with pFSH (day 7).

In *Bos indicus* donors, the number of retrieved COCs was greater in calves with FSH and in cycling heifers, compared to calves without FSH ($P = 0.04$). Furthermore, COC culture rate was greater in calves treated with FSH and in cycling heifers, compared to calves without FSH ($P = 0.01$). However, cleavage rate was similar for all 3 groups (calves without FSH, calves with FSH, and cycling heifers; $P = 0.41$; data shown in Table 1). Despite these positive effects of FSH treatment, the number of blastocysts produced was similar in calves with and without FSH, and this number was lower than in cycling heifers ($P < 0.0001$; Table 1).

In *Bos taurus* donors, the number of visualized follicles ($P = 0.01$) and recovered oocytes ($P < 0.0001$) was greater in calves with FSH compared to calves without FSH and to cycling heifers. The number of cultured COCs was similar in calves without FSH and in cycling heifers, and both groups had fewer cultured COCs than did calves with FSH ($P < 0.0001$). Despite these positive effects of FSH treatment, the number of blastocysts produced was similar in calves with and without FSH, and this number was lower than in cycling heifers (data summarized in Table 2).

The results presented herein demonstrate that it is possible to produce embryos for calves using LOPU/IVP. The FSH treatment could be used as a superstimulation treatment to improve the LOPU/IVP efficiency in young calves. However, further studies are needed to improve embryo production in calves compared to mature animals.

Table 1. Number of visualized follicles, COCs and blastocysts (mean ± SEM) after LOPU-IVP in *Bos indicus* (Nelore) donor calves and after OPU - IVP in *Bos indicus* (Nelore) cycling heifers.

Item	*Bos indicus*			
	Calves without FSH	Calves with FSH	Cycling heifers	P value[d]
Total follicles visualized	19.7 ± 4^{z}	32.3 ± 5.9^{y}	47.1 ± 6.3^{x}	0.003
Total COCs retrieved	13.5 ± 3.6^{b}	20.9 ± 5.1^{ab}	29.9 ± 5.3^{a}	0.04
Recovery rate (%)[e]	68.5^{a}	64.7^{b}	63.6^{b}	0.02
COCs cultured	4.7 ± 1.4^{c}	11.3 ± 4.0^{b}	18.1 ± 4.0^{a}	<0.0001
COCs cultured rate (%)[f]	35.1^{b}	54.3^{a}	60.6^{a}	0.01
Cleavage rate (%)[g]	47.0	52.2	50.3	0.41
Blastocysts produced	1.7 ± 0.7^{b}	2.3 ± 0.8^{b}	9.3 ± 2.0^{a}	<0.0001
Blastocyst rate (%)[h]	12.9^{b}	11.3^{b}	30.9^{a}	<0.0001

[d]Data with different superscripts in the same line differ with $P \leq 0.05$ ($a \neq b \neq c$) or $P \leq 0.06$ ($x \neq y \neq z$). [e]Total number of COCs/number of follicles aspirated. [f]Number of COCs cultured/number of follicles aspirated. [g]Number of cleaved zygotes/ number of COCs. [h]Number of blastocyst/number of COCs.

Table 2. Number of visualized follicles, COCs and blastocysts (mean ± SEM) after LOPU-IVP in *Bos taurus* (Holstein) donor calves and after OPU - IVP in *Bos taurus* (Holstein) cycling heifers.

	Bos taurus			
	Calves without FSH	Calves with FSH	Cycling heifers	P-values
Total follicles visualized	22.7 ± 4.2[b]	54.3 ± 9.5[a]	24.9 ± 3.6[b]	0.01
Total COCs retrieved	11.7 ± 2.4[bx]	22.4 ± 5.4[a]	9.2 ± 1.7[cy]	<0.0001
Recovery rate (%)[1]	51.3[a]	41.3[a]	36.9[b]	0.01
COCs cultured	3.6 ± 1.0[b]	12.3 ± 3.5[a]	4.7 ± 1.3[b]	<0.0001
COCs cultured rate (%)[2]	30.7[b]	37.7[a]	51.1[a]	0.02
Cleavage rate (%)[3]	17.8	30.5	26.1	0.47
Blastocyst produced	0.4 ± 0.2	0.7 ± 0.4	0.5 ± 0.3	0.78
Blastocyst rate (%)[4]	2.9	2.0	4.3	0.60

[d]Data with different superscripts in the same line differ with P ≤ 0.05 (a ≠ b ≠ c) or P ≤ 0.06 (x ≠ y ≠ z). [e]Total number of COCs/number of follicles aspirated. [f]Number of COCs cultured/number of follicles aspirated. [g]Number of cleaved zygotes/ number of COCs. [h]Number of blastocyst/number of COCs.

Oocyte quality and IVP in prepubertal and pubertal heifers

Several research labs have successfully produced viable embryos from prepubertal heifers (Armstrong *et al*., 1992; Fry *et al*., 1998; Taneja *et al*., 2000). However, there are some concerns that oocytes from young females have a lower developmental capacity than those from adult donors (Khatir *et al*., 1996; Presicce *et al*., 1997; Majerus *et al*., 1999)

Recently, our research group performed a study at Santa Rita farm located near Descalvado city in São Paulo state (Guerreiro *et al*., 2014b), where a total of 120 donors of four animal categories were used, as follows: prepubertal heifers (n = 30), pubertal heifers (n = 30), lactating cows (n = 30) and non-lactating cows (n = 30). Donors were submitted to OPU without previous synchronization of the follicular wave. Six OPU sessions were performed with five animals of each category, 20 donors *per* session.

Immediately before the OPU, all follicles were quantified and all visible follicles (≥2 mm) were punctured and total recovered structures, quantity and quality of viable oocytes were registered. All viable oocytes were submitted to IVP and their development (cleavage and blastocyst rate) was evaluated. Sex-sorted sperm from the same bull and semen batch were used to fertilize oocytes from all donor categories in all OPU sessions. Produced embryos (n = 206) were transferred into crossbred recipients (*Bos taurus* x *Bos indicus*).

No difference was observed between experimental groups, regarding total number of aspirated follicles (P = 0.08). Despite similar number of total recovered oocytes (P = 0.12), prepubertal heifers had an intermediate quantity of viable oocytes, and non-lactating cows produced more viable oocytes (P = 0.03), when compared to lactating cows. Still, prepubertal donors had lower cleavage rate (P < 0.0001) and lower blastocyst rate (P < 0.0001) compared to other categories (Table 3).

Thus, it is concluded that prepubertal Holstein donors have low competence for *in vitro* embryo production, being non-lactating cows the most efficient category for IVP. Embryos originated from prepuberal animals resulted in inferior conception rate in comparison to embryos produced from lactating cows and non-lactating cows [prepubertal: 0.0% (0/15)[b]; pubertal: 9.7% (3/28)[b]; lactating cows: 28.6% (10/25)[a]; non lactating cows: 32.7% (36/74)[a]; P < 0.05; Fig. 1]. However, similar conception rate was verified for embryos produced from pre-pubertal and pubertal donors.

Table 3. Number of aspirated follicles, oocytes and embryos produced after OPU-IVP in prepubertal and pubertal heifers, and in lactating and non-lactating cows from the Holstein breed. Data is presented as mean ± standard error mean.

Item	Heifer		Cows		
	Prepubertal	Pubertal	Lactating	Non-Lactating	P value
N	30	30	30	30	
Total follicles aspirated	18.3 ± 2.1	17.3 ± 1.2	14.0 ± 1.0	17.7 ± 1.7	0.08
Total COC retrieved[1]	14.2 ± 2.2	13.1 ± 1.1	9.8 ± 1.1	14.6 ± 1.7	0.12
COCs cultured	10.5 ± 1.8[ab]	8.3 ± 0.8[ab]	6.5 ± 0.9[b]	11.5 ± 1.4[a]	0.03
Cleavage rate (%)[2]	68.6[b]	98.8[a]	87.6[a]	10.1[a]	<0.0001
Blastocysts produced	0.5 ± 0.2[b]	1.1 ± 0.2[b]	1.2 ± 0.4[b]	4.2 ± 0.6[a]	<0.0001
Blastocysts rate (%)[3]	4.8[c]	12.7[b]	18.0[b]	36.5[a]	<0.0001

[1]COC - cumulus oocyte complex. [2]Number of cleaved embryos/viable COCs. [3]Number of blastocysts/viable COCs. [a,b,c]Different letters within rows indicate statistical difference (P < 0.05).

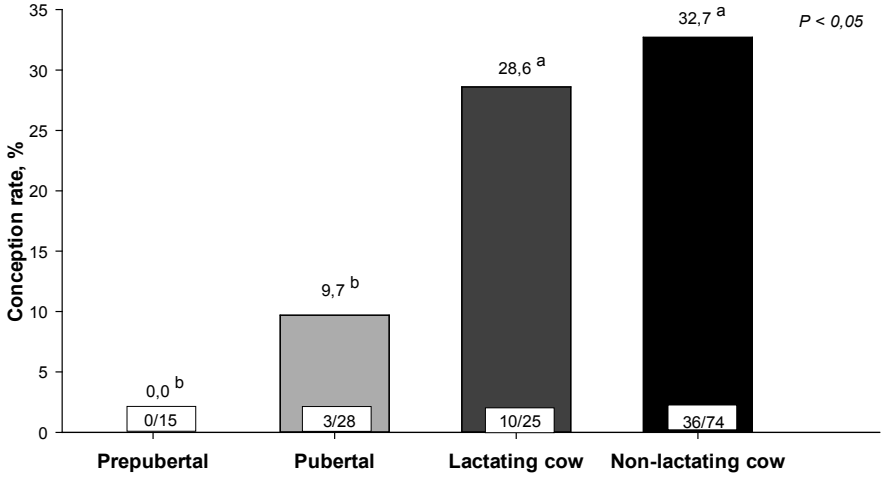

Figure 1. Conception rate after embryo transfer. Donors were prepubertal and pubertal heifers, and lactating and non-lactating cows from the Holstein breed.

We performed another trial with prepubertal Nelore heifer (*Bos indicus*) housed at Instituto de Zootecnia in Sertãozinho, São Paulo state (Batista *et al*., 2016; FMVZ/USP, São Paulo, Brazil; unpublished data). In this experiment we evaluated OPU-IVP production at different ages and cyclicity status. The experimental design included: prepubertal heifers aged 8 to 12 month (n = 24), prepubertal heifers aged 18 to 22 month (n = 20) and cycling heifers aged 22 to 26 month (n = 25). Data is summarized in Table 4.

Briefly, prepubertal heifers aged 8 to 12 month had lower numbers of visualized follicles, lower numbers of recovered oocytes than older heifers, despite cyclicity status. There were no differences across experimental groups in terms of the rate of COCs cultured or their cleavage rate. However, number of blastocysts produced, as well as blastocyst rate increased both with increasing age and after animals became cyclic (Table 4).

The results of OPU/IVP in prepubertal heifers demonstrate reduced efficiency compared to cycling heifers and adult animals. Further studies should be conducted to try to improve the efficiency of production in this age category.

Table 4. Number of visualized follicles, COCs and blastocysts (mean ± SEM) after OPU - IVP in *Bos indicus* (Nelore) prepubertal and pubertal and heifers.

Item	Prepubertal heifers (8 - 12 month) (n = 24)	Prepubertal heifers (18 - 22 month) (n = 20)	Pubertal heifers (22 - 26 month) (n = 25)	P value[a]	
				Age[b]	Ciclicality[c]
Total follicles visualized	19.7 ± 2.1	41.3 ± 5.28	34.0 ± 3.3	<0.0001	0.0002
Total COCs retrieved	13.4 ± 1.7	30.8 ± 5.8	22.6 ± 3.2	<0.0001	<0.0001
Total COCs cleaved	5.6 ± 0.8	14.8 ± 2.5	13.3 ± 1.9	<0.0001	<0.0001
COCs cultured	7.6 ± 1.0	16.8 ± 2.7	15.1 ± 2.2	<0.0001	<0.0001
COCs cultured rate (%)[d]	57.0	54.0	60.0	0.13	0.45
Cleavage rate (%)[e]	73.0	88.0	84.0	<0.0001	0.25
Blastocysts produced	1.5 ± 0.3	4.7 ± 0.9	7.2 ± 1.2	<0.0001	<0.0001
Blastocyst rate (%)[f]	20.2	28.1	47.0	0.05	<0.0001

[a]Effect of evaluated group. [b]Effect of age in the prepubertal group (8-12 month *vs*. 18-24 month). [c]Effect of cyclicity (cyclic *vs*. non cyclic). [d]Number of viable oocytes/number of total oocytes. [e]Number of cleaved oocytes/number of cultured oocytes. [f]Number of blastocysts/number of cultured oocytes.

Oocyte quality and IVP in pregnant donors

Recently, our research group studied the effect of pregnancy on oocyte quality and IVP of Holstein heifers (Bayeux *et al*., 2016). We evaluated 179 Holstein donors (*Bos taurus*) of 3 categories: prepubertal heifers (8 to 10 month; n = 60); pubertal heifers (10 to 12 month; n = 60) and pregnant heifers (14 to 18 month; n = 59). All animals underwent ovum pickup (OPU) at random stages of the estrous cycle. Sex-sorted sperm from the same bull and semen batch were used to fertilize oocytes from all donor categories in all OPU sessions. Pubertal heifers had a greater number of recovered oocytes as well as COCs cultured compared to other categories. In contrast, cleavage rate was similar between pubertal and pregnant heifers. Interestingly, pregnant heifers had a greater number of embryos produced per OPU and ultimately greater blastocyst rate when compared to other heifer-categories (Table 5). These results indicate that pregnant heifers were more efficient in terms of IVP compared to prepubertal and pubertal Holstein (*Bos taurus*) heifers.

Table 5. Number of recovered oocytes, COCs cultured, blastocysts, cleavage and blastocyst rate after OPU - IVP in *Bos taurus* (Holstein) donors in different categories.

Item	Heifers			P value
	Prepubertal (n = 60)	Pubertal (n = 60)	Pregnant (n = 59)	
Number of COCs retrieved	9.8 ± 1.3^{b}	15.6 ± 1.4^{a}	9.8 ± 1.6^{b}	0.001
COCs cultured	4.6 ± 0.6^{b}	9.1 ± 0.9^{a}	5.6 ± 1.1^{b}	0.001
Cleavage rate (%)[d]	31^{b}	56^{a}	78^{a}	0.001
Blastocysts produced	0.13 ± 0.1^{c}	0.9 ± 0.2^{b}	1.8 ± 0.3^{a}	<0.001
Blastocyst rate (%)[e]	2^{c}	14^{b}	37^{a}	<0.001

[d]Number of cleaved oocytes/number of cultured oocytes. [e]Number of blastocysts/number of cultured oocytes. Abbreviations: COC, cumulus - oocyte complex; IVP, *in vitro* embryo production; OPU, ovum pickup. Data with different superscripts in the same row differ at $P < 0.001$.

Oocyte quality and IVP during the early postpartum in donors

To assess the impact of early postpartum period on IVP in beef and dairy cattle, we have recently performed a study utilizing *Bos indicus* (Nelore; Pierucci *et al*., 2015) and *Bos taurus* (Holstein; Sala, 2013) cows. Ultrasound-guided follicular aspirations were performed every 14 days, from 30 to 86 days postpartum. Then, within breed, cows were blocked when 50% of the animals were cycling, which occurred at 30 days postpartum for *Bos taurus* and at 44 days postpartum in *Bos indicus* cows. Thus, we ended up with 2 experiments: experiment 1, *Bos taurus* cows with CL present (n = 14) and without CL present (n = 11) at 30 days with postpartum, and experiment 2, *Bos indicus* cows without CL present (n = 7) and without CL present (n = 8) at 44 days postpartum.

There was a significant effect of postpartum period, only for Nelore cows, in the number of aspirated follicles, recovered oocytes, and number of viable oocytes. In contrast, number of blastocysts (Fig. 2) as well as blastocyst rate did not differ with increasing days postpartum and cyclicity, both in *Bos indicus* and *Bos taurus* cows (Fig. 3).

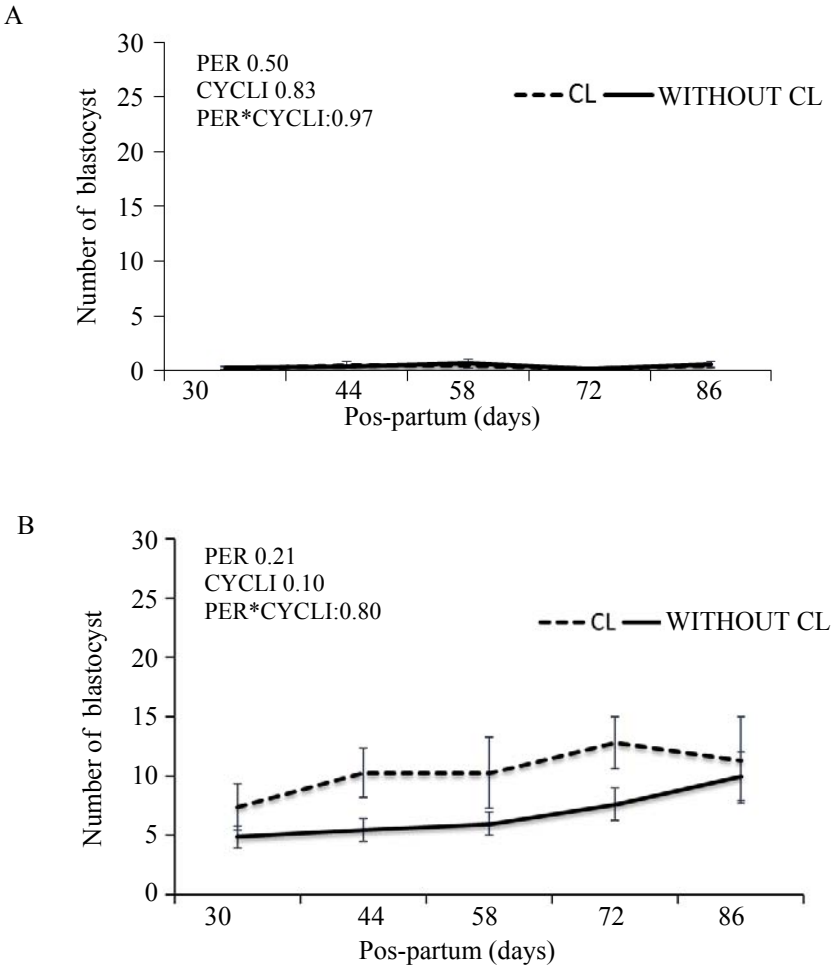

Figure 2. Number of blastocysts according to days postpartum in Holstein *Bos taurus* (A) and Nelore *Bos indicus* cows (B).

As observed in a previous study (Matoba *et al*., 2012), these results do not provide evidence of an effect of lactation-induced metabolic stress on oocyte developmental competence, in the early postpartum in dairy and beef cows, in terms of morphological ability to develop following *in vitro* fertilization (IVF). Previous research has shown impairment in fertility during early post partum due to metabolic disorder, mainly related to negative energy balance (Leroy *et al*., 2012). In these studies, fertility was evaluated after artificial insemination, with significant endocrine and metabolic alterations in the microenvironments of the dominant follicle. In studies with OPU/IVP, follicles are aspirated with approximately 2 to 3 mm, before the growth phase of the dominant follicle. This may partly explain the difference in results between experiments.

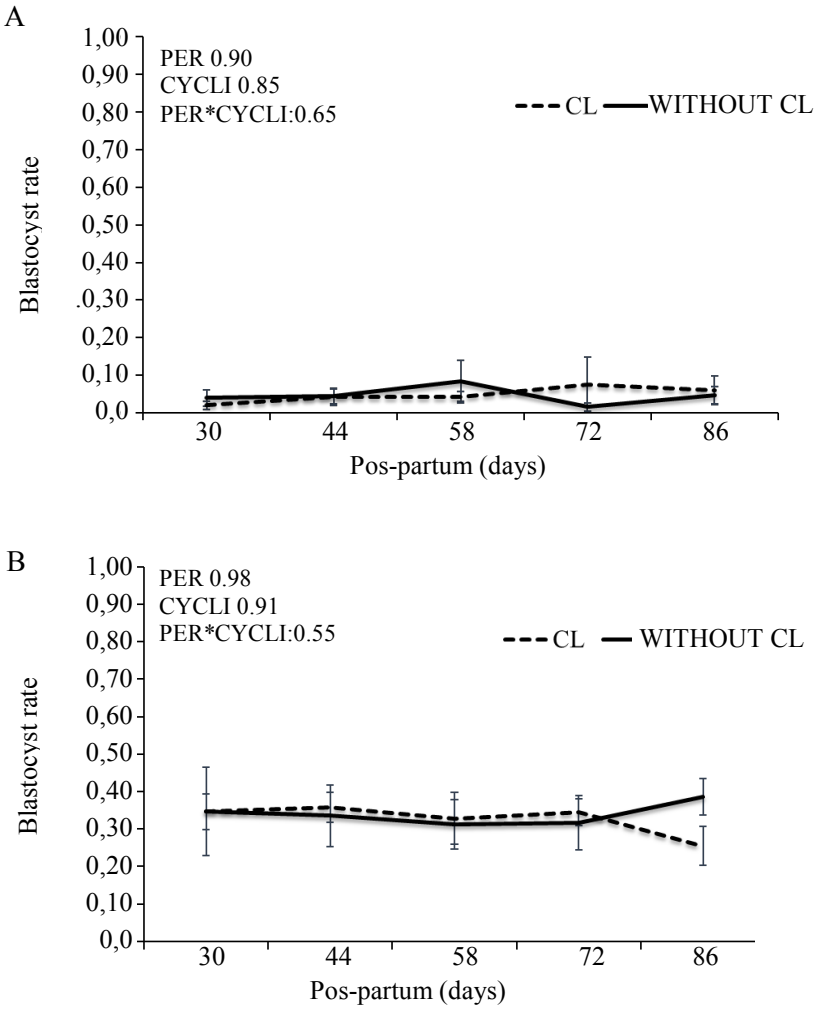

Figure 3. Blastocyst rate according to days postpartum in Holstein *Bos taurus* (A) and Nelore *Bos indicus* cows (B).

Oocyte quality and IVP during the during early or late lactation period

Clearly evaluating the effects of lactation on oocyte quality can be rather complex. For example, testing the isolated effects of lactation status per se on oocyte quality might be confounded by interactions with heat-stress for example, since lactating cows are generally more susceptible to heat stress. Also, lactating cows may suffer from different metabolic problems as lactation progresses until the dry-period. In addition, the type of diet and level of dry matter intake between lactating and non-lactating cows may also influence results of OPU-IVP. Despite of that, the negative effects of excessive energy intake was shown to compromise *in vitro* oocyte developmental competence, especially in over-conditioned (high body condition score) females (Adamiak, 2005). The mechanisms mediating these negative effects on oocyte competence may be related to endocrine alterations, such as hyperinsulinemia, peripheral insulin resistance, and increased glucose, NEFA and IGF-I, which may interfere with glucose transport in embryo cells and induce increased rates of apoptosis.

Lactating cows have been selected for milk production, making the modern dairy cow prone to peripheral insulin resistance to maintain milk production. Several factors have been involved to induce insulin resistance in lactating cows including excessive negative energy balance in early postpartum. Intriguingly, lactating dairy cows are again prone to insulin resistance that appears to happen more evidently in animals with greater body condition scores and having an excessive intake of diets with a high energy content. This is a rather common issue particularly in commercial dairies utilizing a single diet throughout the entire lactation. To

test whether stage of lactation and possible interactions with insulin resistance might influence oocyte quality, our research group (Baruselli *et al*. 2016), utilized Holstein cows that were either at early or late days in milk production at the moment of OPU-IVP. Results of this study clearly showed that insulin resistance associated with late lactation period (later lactation cows had greater circulating insulin levels) can disrupt oocyte quality and lower the efficiency of IVP. For example, we observed that cows at later lactation had a greater number of recovered oocytes per OPU session. In contrast, number of blastocysts, as well as blastocyst rates, were greatly reduced in cows at later lactation. In addition, a number of apoptotic genes were upregulated in cows with greater days in milk. These findings corroborate previous studies showing that lactating cows at later lactation are prone to insulin resistance, which clearly seem to lower oocyte viability during IVP procedures.

Table 6. Ovum pick-up and *in vitro* embryo production results from high producing Holstein cows during early or late lactation periods.

Item	Stage of lactation		P value
	Early	Late	
No. of animals	70	67	
DIM, days	110.5 ± 20.8	425.6 ± 21.0	N/A
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
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
No. of cleaved oocytes (day 3)	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 (day 7)	2.2 ± 0.4	1.4 ± 0.3	0.06
Blastocyst rate (%)	23.0 ± 0.1	13.3 ± 0.1	0.0005

Adapted from Baruselli *et al*., 2016.

Oocyte quality and IVP in non-lactating donors

Oocyte quality has been considered an important factor contributing to the low fertility reported for high producing lactating dairy cattle (Walsh *et al*., 2011). Thus, we hypothesized that OPU-IVP procedures would result in a higher number of blastocysts per OPU session in non-lactating than in lactating donors. Our data showed higher number of blastocysts per OPU session in non-lactating than in lactating donors (Vieira *et al*., 2014). Non-lactating cows produced a higher blastocyst rate (41.9 *vs*. 13.4%; P = 0.001) and a higher number of transferable embryos per OPU (3.5 ± 0.5 *vs*. 1.3 ± 0.3; P = 0.003) than lactating Holsteins cows. Similar results were observed in a previously mentioned trial performed by our group (Table 3).

Dairy cows have a peculiar metabolic system, linked to nutrition and disruption of endocrine profiles. The metabolic profile of lactating dairy cows is commonly characterized by lower concentrations of progesterone and estradiol (Wiltbank *et al*., 2006) and increased concentrations of NEFA (nonesterified fatty acids) and BHBA (b-hydroxybutyrate; Leroy *et al*., 2005); and this peculiar metabolism has been associated with a suboptimal follicle microenvironment, compromising oocyte quality and resulting in a failure to conceive (Sartori *et al*., 2002, 2004; Wiltbank *et al*., 2006; Leroy *et al*., 2008a, b; Walsh *et al*., 2011). Therefore, the greater challenge of lactating cows to maintain an optimal reproductive efficiency may help explain the lower results observed in IVP. Thus, non-lactating donors may be considered the preferred donor in OPU-IVP programs, due to the higher yield of embryos per OPU session.

In another experiment (Sales *et al*., 2015) we studied the effects of different dietary energy levels [100 and 170% for maintenance (M) and high energy (1.7M), respectively] on metabolic, endocrine, and reproductive parameters in non-lactating *Bos indicus* (Gir; n = 14) and *Bos taurus* (Holstein; n = 14) cows submitted to OPU and IVP each 14 days. We measured glucose and insulin concentrations and performed glucose tolerance tests and the relative quantification of transcripts (PRDX1, HSP70.1, GLUT1, GLUT5, IGF1R, and IGF2R) from oocytes recovered at the end of the experimental period. No interactions were observed between the effects of breed and dietary energy level on the qualitative (viable oocytes, quality grade, and oocyte quality index) and quantitative (oocytes recovered) variables. There were no effects of dietary energy level on the qualitative and quantitative oocyte variables. *In vitro* embryo production (cleavage and blastocyst rates and number of embryos) was similar between diets, but the 1.7M diet reduced *in vitro* embryo production in *Bos indicus* cows after 60 days of treatment. Moreover, *Bos indicus* cows on the 1.7M diet showed lower transcript abundance for the HSP70.1, GLUT1, IGF1R, and IGF2R genes. All cows fed 1.7M diets had greater glucose and insulin concentrations and greater insulin resistance according to the glucose tolerance test. These results suggest that intake of a high energy diet for a long period reduces *in vitro* embryo production in non-lactating *Bos indicus* cows by causing

a hyperinsulinemic state, and promoting down-regulation of genes involved in cellular metabolism.

Conclusions and future directions

In conclusion, the IVP from younger beef or dairy cattle seem quite possible, although improvements are still needed to further improve this technology that came as a complement for genomic testing. More importantly, veterinarians working with OPU-IVP need to account for varying physiological aspects when working with specific cattle breeds (*Bos indicus vs*. *Bos taurus*). For example, avoiding working with cows submitted to a high energy diet for a long period and/or under heat stress, both factors that may induce poor oocyte quality, is highly advisable.

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