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Number of oocytes retrieved per donor during OPU and its relationship with *in vitro* embryo production and field fertility following embryo transfer

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Abstract

The association of OPU-IVEP is an important instrument to drive genetic progress. *In vitro* embryo production (IVEP) has remarkably expanded in the last decade compared to *in vivo* embryo production. Because of the high repeatability of oocyte retrieval within oocyte-donors, studies exploring the relationship between the number of oocytes recovered per OPU section with IVEP efficiency, as well as with field fertility (pregnancy results following embryo transfer; P/ET) are extremely important to guide cow-donor selection and optimize field reproduction efficiency and the herd's genetic gain. Based on this rationale, our group conducted a retrospective analysis of a large database comprising IVEP records from several cattle breeds, including *Bos indicus* and *Bos taurus* for either beef or dairy purposes. A total of 205,140 oocytes recovered from 7,906 OPU procedures of 6,902 donors (5,227 beef and 1,675 dairy) of Brazilian farms were analyzed. Beef breeds analyzed were Nelore (*Bos indicus*) and Senepol (*Bos taurus*) and dairy breeds were Gyr (*Bos indicus*) and Holstein (*Bos taurus*). According to our analysis, the IVEP in beef cattle had a great improvement throughout the last years, with a remarkable increase in numbers of pregnancies per OPU compared to late 90's (averaging only 1 pregnancy per OPU in 1998 vs 2,4 in 2014). As for the distribution of oocytes retrieved, both *Bos indicus* beef (Nelore = 27.2) and dairy (Gyr = 23.8) breeds seem to yield greater average numbers of oocytes per OPU compared to *Bos taurus* (Senepol = 21.8; Holstein = 19.3). Despite these differences across genetic groups, outstanding donors can be found in all breeds and the number of oocytes retrieved per donor seems consistent across time. For both beef cattle breeds studied, it appears that number of oocytes retrieved at OPU had a negative but minor effect on both cleavage and blastocyst rates, especially for Senepol breed. Conversely, in dairy breeds the number of oocytes recovered per OPU had essentially no effect on cleavage rates, but we captured a trend for lower blastocyst rates with greater numbers of oocytes per OPU. For both, beef and dairy breeds the number of blastocyst per OPU was greater when higher number of oocytes were recovered per OPU, regardless of genetic group. Pregnancy rate following ET in Nelore breeds was lower in donors with greater amounts of oocytes retrieved per OPU. In contrast, in the Senepol breed and both dairy breeds (Gyr and Holstein) pregnancy rates after ET seems to increase when the number oocytes recovered per OPU increases. In addition, the semen utilized had a major impact of IVEP efficiency: top ranking sires yielded outstanding blastocyst rates, while poor performers produced very low blastocyst rates. The season of the year also had effect on IVEP, with *Bos indicus* breeds showing less variation in IVEP results throughout the year. In conclusion, despite the evolution of IVEP in the last two decades, the number of oocytes recovered per OPU had a minor effect both on blastocyst rate and pregnancy rates after ET. However as more oocytes are collected, the number of produced blastocysts improves. Thus, it seems important to identify donors with greater oocyte recovery-per-OPU potential, especially in cattle breeds yielding fewer oocytes per OPU, such as Holstein, to assure greater IVEP efficiency. It is also clear that cattle breed, semen used during IVEP and season of the year can potentially influence IVEP and field fertility results. A holistic approach controlling the quality of the performed OPU, consistency in lab routines, as well as selecting donors with high genetic value (through genomics) and greater oocyte population (through AMH assays or ultrasound) are highly advisable.

Keywords: antral follicle population, pregnancy rate, cattle, bovine.

Introduction

The association of reproductive efficiency and genetic selection is strategic for the success of dairy and beef industries. Reproductive technologies, such as ovum-pick-up (OPU) and *in vitro* embryo production (IVEP) can rapidly enhance genetics of cattle through both the female and male linage. However, the outcomes of these techniques are highly impacted by individual physiological characteristics of the animal, such as the high variability in ovarian antral follicle population (AFP; Ireland *et al.*, 2011; Pontes *et al.*, 2011). For example, a number of recent studies have

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shown the strong correlation between blood circulating concentrations of anti-Müllerian hormone (AMH), AFP and IVEP in cattle of different genetic groups (Baldrighi *et al*., 2014; Batista *et al*., 2014; Guerreiro *et al*., 2014). In addition, a large variability of AFP among oocyte donors has also been reported. Despite of that, the high repeatability of oocyte retrieval within oocyte-donors allows identification of donors with enhanced potential for oocyte production (Boni *et al*., 1997; Burns *et al*., 2005; Ireland *et al*., 2007, 2008; Baruselli *et al*., 2015; Monteiro *et al*., 2017). Thus, studies further exploring the relationship between the number of oocytes recovered per OPU section (highly correlated with AFP) and with IVEP efficiency, as well as with field fertility (pregnancy results following embryo transfer; P/ET), are extremely important to guide decision making in terms of donors selection to ultimately fasten evolution on embryo production technology.

Based on this rationale, our group has conducted a retrospective analysis of a large database from a Brazilian commercial IVEP enterprise in order to elucidate the effects of the number of oocytes recovered per OPU on IVEP efficiency and pregnancy results following ET. The database encompassed several cattle breeds, including *Bos indicus* and *Bos taurus* of either beef or dairy purposes. This database comprised a total of 205,140 oocytes recovered from 7,906 OPU procedures of 6,902 donors (5,227 beef and 1,675 dairy) from 2001 to 2017, in which all OPU/IVEP/ET were conducted in Brazil. Briefly, the OPUs were performed by 97 veterinarians and IVEP was done by the same IVEP enterprise. Beef breeds analyzed included Nelore (*Bos indicus*) and Senepol (*Bos taurus*) breeds and for the dairy breeds were utilized data from Gyr (*Bos indicus*) and Holstein (*Bos taurus*) breeds. The beef cattle database included 163,549 oocytes (154,386 from 5612 OPU of 5,048 Nelore donors and 9,163 from 421 OPU of 179 Senepol donors). For dairy breeds it included 41,591 oocytes, in which 28,584 were from 1,200 OPU performed in 956 Gyr cows and 13,007 from 673 OPU done in 719 Holstein cows.

Statistical analyses and data editing

The total amount of oocytes recovered per OPU was divided in quartiles within each cattle breed. Then, the dataset was edited employing two criteria. The first criterion was to restrict the cleavage to eliminate biologically abnormal results. Thus, the final dataset for cleavage rates that were lower than 10% and greater than 90% were excluded from final analysis. Similarly, the second criterion was to restrict the blastocyst rate, eliminating rates that were lower than 10% and greater than 90%.

Statistical analyses were performed using the GLIMMIX procedure utilizing the Statistical Analysis System (SAS, Version 9.4 for Windows; SAS Inst., Cary, NC). The variables evaluated were the total number of COCs recovered, cleavage rate (number of cleaved zygotes per total number of COCs recovered), blastocyst rate (number of blastocysts produced per total number of COCs recovered), pregnancy rate (number of pregnancies per total number of blastocysts transferred). The binomial distribution was assumed for categorical response variables. Continuous data were tested for normality of the residues using the Guided Data Analysis, and transformed when necessary. The fixed effects included in the model were quartile of total amount of oocytes (Q1, Q2, Q3 and Q4), year of OPU (2001 to 2017), season of OPU, technician who performed the OPU and meaningful two-way interactions. The number of COCs cultured (CIV) was considered as a covariate when required. The interaction of semen and donor was included as a random effect. Statistical differences with $P < 0.05$ were considered significant.

Correlations between cleavage rate, blastocyst rate and pregnancy rate with total oocytes recovered were determined using Proc CORR of SAS. Logistic regression curves were created using the coefficients provided by the interactive data analysis from SAS and the formula $y = \exp(\alpha \times x + b) / [1 + \exp(\alpha \times x + b)]$, where y = success probability of pregnancy, cleavage or blastocyst; exp = exponential; α = slope of the logistic equation; b = intercept of the logistic equation; and x = total oocytes recovered.

The HPMIXED procedure of SAS through the best linear unbiased prediction (BLUP) analysis was utilized to rank sires from all breeds in terms of blastocyst success as well as field fertility following ET.

OPU/IVEP procedure: a remarkable progress over the last decades

The OPU-IVEP combined is an important tool to drive genetic progress. *In vitro* embryo production has expanded remarkably in the last decade when compared to *in vivo* embryo production; and lately accounts for 40.6% of the total embryo production in the world (1,275,874 embryos; Perry, 2014). The outstanding growth of IVEP seen in the last decade is a consequence of a number of factors, such as the significant improvement of *in vitro* culture procedures and the successful use of sex-sorted semen in the IVEP programs enabling the manipulation of the proportion of male and female embryos produced (Blondin, 2015). The evolution of IVEP through the last decades is clearly shown in Table 1 (data analyzed for all breeds). It is obvious the great improvement IVEP, with the remarkable increase in numbers of pregnancies per OPU, improving nearly 2 times in later years compared to late 90's.

Table 1. Evolution of OPU/IVEP from 1998 to 2014.

Year	OPU (n)	MIV	% CLIVED	% BLAST	% P/ET	PREG/OPU
1998	56	11.2	55.2%	22.5%	40.4%	1.0
1999	510	8.7	71.3%	25.9%	39.9%	0.9
2000	1182	13.7	68.4%	30.2%	39.6%	1.6
2001	2556	14.9	87.9%	30.2%	39.7%	1.8
2002	4116	20.3	72.1%	24.5%	39.9%	2.0
2003	5430	20.5	72.8%	26.6%	39.8%	2.2
2004	3731	21.7	69.1%	25.6%	42.3%	2.3
2005	995	20.0	70.9%	32.6%	41.0%	2.7
2006	968	20.4	71.2%	32.9%	35.5%	2.4
2007	1025	24.6	69.1%	32.6%	37.2%	3.0
2008	1171	20.3	66.7%	32.2%	38.6%	2.5
2009	1580	15.4	69.4%	28.8%	41.5%	1.8
2010	703	20.0	70.0%	27.3%	41.8%	2.3
2011	378	18.9	65.6%	26.9%	41.7%	2.1
2012	320	21.1	61.8%	20.7%	44.4%	1.9
2013	157	20.6	66.4%	27.3%	42.0%	2.4
2014	485	16.0	69.1%	32.0%	47.3%	2.4
Total	25,363	19.2	72.2%	27.8%	40.0%	2.1

Variation in oocyte retrieval during OPU procedures

Several research groups have reported a great variation in oocyte retrieval per OPU across breeds (Pontes *et al*., 2010; Gimenes *et al*., 2015; Sales *et al*., 2015). Data shown in Fig. 1 corroborate with such findings and shows the wide distribution of oocytes retrieved across main beef and dairy cattle breeds receiving OPU/IVEP in Brazil in the last two decades. Altogether, both beef and dairy breeds having *Bos indicus* blood seem to yield greater average numbers of oocytes per OPU (Beef: Nelore = 27.5 ± 0.3 *vs*. Senepol = 21.8 ± 0.7; Dairy: Gyr = 23.8 ± 0.5 *vs*. Holstein = 19.3 ± 0.6). This information is very critical to IVEP routine, since the average number of viable embryos produced during IVEP is highly correlated with initial numbers of oocytes sent to *in vitro* embryo production. As a result, it seems far more important to identify donors with greater oocyte recovery-per-OPU potential in some breeds such as Holstein for example though ultrasound selection or AMH assay. This may determine IVEP success in some cattle breeds yielding fewer oocytes per OPU.

Despite the great differences in total of oocytes retrieved per OPU procedure across breeds, outstanding donors can be found in all breeds and the number of oocytes retrieved per individual donor seems consistent across time. In fact, it was previously shown the occurrence of high within-cow repeatability in oocyte production over time (Boni *et al*., 1997; Ireland *et al*., 2007, 2008; Baruselli *et al*., 2015; Monteiro *et al*., 2017). However, contrasting results were shown across genetic groups. For *Bos taurus*, the number of recovered oocytes seemed fairly constant, even up to 32 consecutive OPU sessions (Petyim *et al*., 2003). Conversely, in *Bos indicus* cattle, decreased numbers of recovered oocytes following consecutive OPU sessions has been reported (Gimenes *et al*., 2015); it appears to be an issue particularly within donors with high numbers of cumulus-oocyte complex (COC) retrieved at the beginning of the program (Monteiro *et al*., 2017). Despite that, donors classified as high COC resulted in increased blastocyst production per OPU. More importantly, the high-repeatability efficiency in terms of COC retrieved (shown in Table 2 below) is a remarkable finding that has major implications to IVEP labs worldwide (Monteiro *et al*., 2017).

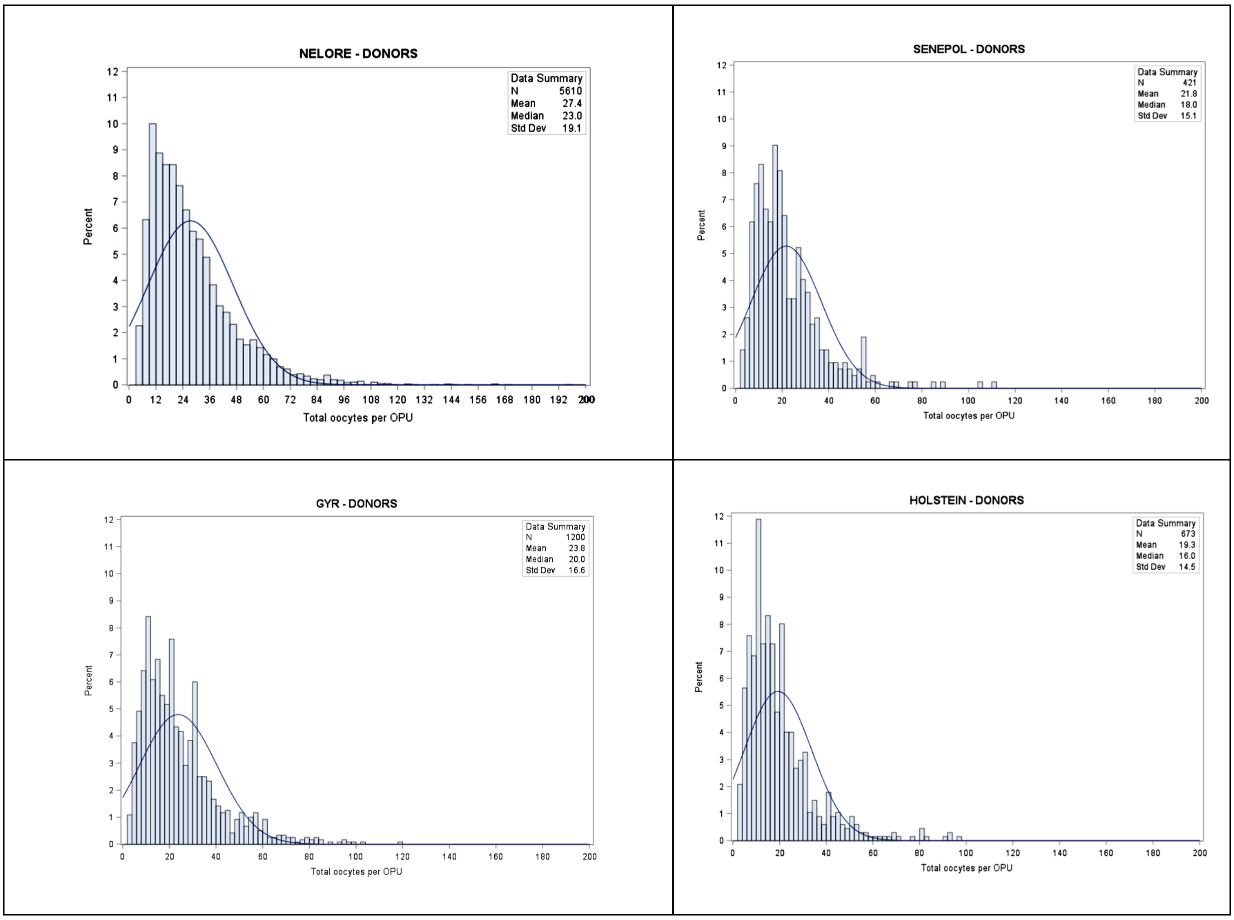

Figure 1. Distribution of oocytes retrieved per OPU in beef (*Bos indicus* = Nelore; and *Bos taurus* = Senepol – upper panels) and in dairy breeds (*Bos indicus* = Gyr; and *Bos taurus* = Holstein – lower panels).

Table 2. Repeatability of OPU/IVEP in *Bos indicus* (Nelore) oocyte donors.

Variable	Repeatability
Recovered COC	0.81
Cultured COC	0.81
Blastocyst	0.79
Cultured COC rate	0.55
Blastocyst rate	0.69

Adapted from Monteiro *et al*., 2017.

Number of oocytes retrieved at OPU: impact on *in vitro* embryo production

In the following sessions, we have described the impact of the total number of oocytes retrieved per OPU on embryo viability during the IVEP, as well as pregnancy results following ET. This is an important topic for field vets, mainly because studies utilizing a limited number of donor-cows yielded mixed findings in the currently available scientific literature. Thus, this topic warrants further investigations utilizing large number of records to more clearly draw final conclusions in this subject, as we describe below.

Beef breeds – impact of number of oocytes per OPU on embryo viability

Tables 3 and 4, as well Fig. 2 and 3, are summarizing our findings for beef breeds (*Bos indicus* represented by Nelore breed and *Bos taurus* represented by Senepol breed) in terms of the relationship between number of oocytes recovered during OPU and its relationship to IVEP efficiency. Altogether, it appears that number of oocytes retrieved at OPU had a minor effect on both cleavage and blastocyst rates (Table 3 and 4), except by a minor negative trend for blastocyst rate in the Senepol breed (Table 4 and Fig. 3B). In Nelore, only a tendency ($P = 0.08$; Table 3) was observed for reduction of the cleavage rate as the number of recovered oocytes increased.

As expected, the number of blastocyst per OPU is greater for beef donors with higher number of oocytes recovered per OPU, regardless of genetic group (Table 3 and 4).

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Table 3. Effect of retrieved numbers of oocytes per OPU from Nelore (*Bos indicus*) donors on IVEP.

Variable	Quartile (Lower)	Intermediate Quartile (Lower)	Intermediate Quartile (Superior)	Quartile (Superior)	P-value
Quartile, n	1403	1403	1403	1403	-
Oocytes recovered per OPU	9.4^{d} (13246/1403)	18.1^{c} (25376/1403)	28.6^{b} (40119/1403)	53.9^{a} (75645/1403)	< 0.001
Cleavage rate, %	68.80% (8089/11756)	66.70% (14900/22337)	66.20% (23582/35634)	65.10% (42714/66860)	0.08
Blastocyst rate, %	45% (3634/8089)	45.70% (6841/14900)	46.10% (10948/23582)	44.40% (19001/42714)	0.23
Blastocyst per OPU	2.6^{d} (3604/11756)	4.9^{c} (6841/22337)	7.8^{b} (10948/35634)	13.5^{a} (19001/66860)	< 0.001

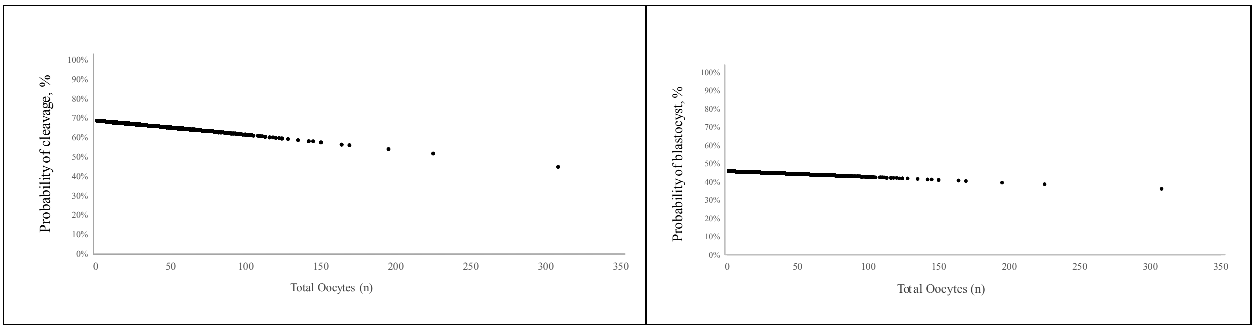

Figure 2. Probability of cleavage rate (A) and blastocyst rate (B) as a function of numbers of retrieved oocytes per OPU in Nelore (*Bos indicus*) donors (from 154,386 fertilized oocytes). [Logit(PROB_CLEAVAGE) = 0.7833-0.0032*Total oocytes; P < 0.001]; [Logit (PROB_BLASTOCYST= -0.1539-0.0013*Total oocytes; P = 0.01].

Table 4. Effect of retrieved numbers of oocytes per OPU from Senepol (*Bos taurus*) donors on IVEP.

Variable	Quartile (Lower)	Intermediate Quartile (Lower)	Intermediate Quartile (Superior)	Quartile (Superior)	P-value
Quartile, n	105	105	105	106	-
Oocytes recovered per OPU	7.9^{d} (827/105)	14.8^{c} (1552/105)	22.3^{b} (2339/105)	41.9^{a} (4445/106)	<.0001
Cleavage rate, %	58.2%^{b} (481/827)	61.5%^{ab} (955/1552)	66.6%^{a} (1558/2339)	61%^{ab} (2710/4445)	0.001
Blastocyst rate, %	33%^{a} (273/827)	27.2%^{b} (422/1552)	28.1%^{b} (658/2339)	26.6%^{c} (1181/4445)	<.0001
Blastocyst per OPU	2.6^{d} (273/105)	4.1^{c} (422/105)	6.3^{b} (658/105)	11.2^{a} (1181/106)	<.0001

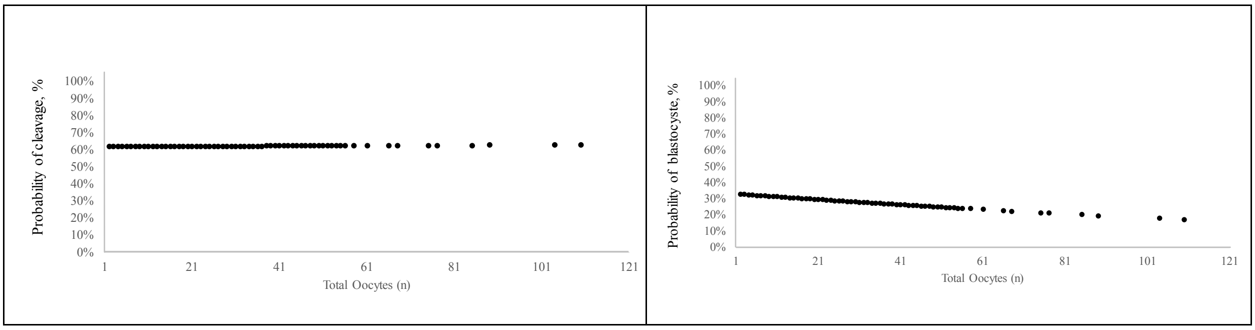

Figure 3. Probability of cleavage rate (A) and blastocyst rate (B) as a function of the number of retrieved oocyte per OPU in Senepol (*Bos taurus*) donors (from 9,163 fertilized oocytes). [Logit(PROB_CLEAVAGE) = 0.486+0.0004*Total oocytes; P = 0.8598]; [Logit(PROB_BLASTOCYST)= -0.7093-0.008*Total oocytes; P = 0.0011].

Dairy breeds – impact of number of oocytes per OPU on embryo viability

In dairy breeds, we considered that the number of oocytes recovered per OPU had essentially no effect on cleavage rates, and that differences observed might possibly be explained by lab artifacts such as greater number of oocytes to be handled at the time. However, we did capture lower blastocyst rates with increasing numbers of oocytes per OPU for both genetic groups (Fig. 4 and 5), although still minor and with debatable biological importance to field veterinarians.

As similarly found in beef breeds, the number of blastocyst per OPU is greater for dairy donors with higher number of oocytes recovered per OPU, regardless of genetic group (Table 4 and 5).

Table 5. Effect of retrieved numbers of oocytes per OPU from Gyr (*Bos indicus*) donors on IVEP.

Variable	Quartile (Lower)	Intermediate Quartile (Lower)	Intermediate Quartile (Superior)	Quartile (Superior)	P-value
Quartile, n	300	300	300	300	-
Oocytes retrieved per OPU	8.0[d] (2399/300)	15.7[c] (4696/299)	24.9[b] (7487/301)	46.7[a] (14002/300)	<.0001
Cleavage rate, %	57.4%[b] (1376/2399)	57.3%[b] (2704/4717)	59.2%[a] (4420/6033)	58.1%[b] (8136/14002)	<.0001
Blastocyst rate, %	30.56%[a] (734/2399)	29.5%[b] (1392/4717)	28.5%[c] (2127/7466)	28.2%[d] (3951/14002)	<.0001
Blastocyst per OPU	2.5[d] (734/300)	4.6[c] (1392/300)	7.1[b] (2127/300)	13.2[a] (3951/300)	<.0001

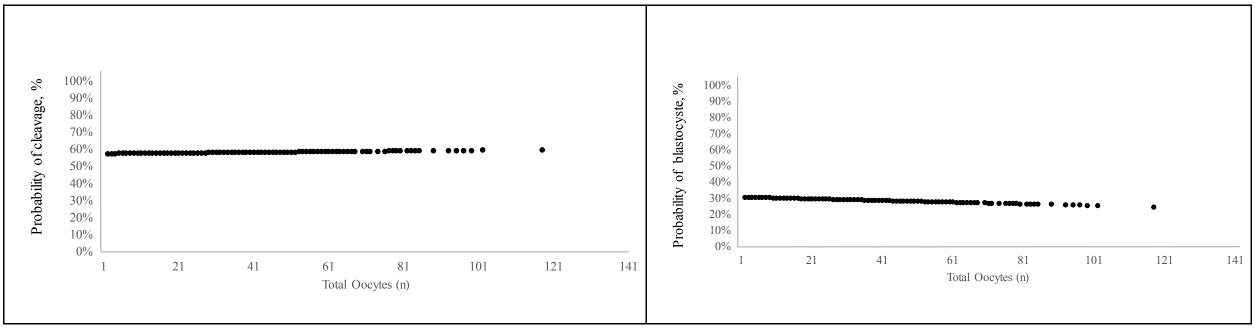

Figure 4. Probability of cleavage rate (A) and blastocyst rate (B) as a function of the number of retrieved oocytes per OPU in Gyr (*Bos indicus*) donors (from 28,584 fertilized oocytes). [Logit(PROB_CLEAVAGE) = 0.304+0.0008*Total oocytes; P = 0.5203]; [Logit(PROB_BLASTOCYSTE) = -0.8177-0.0026 *Total oocytes; P = 0.0305].

Table 6. Effect of retrieved numbers of oocytes per OPU from Holstein (*Bos taurus*) donors on IVEP.

Variable	Quartile (Lower)	Intermediate Quartile (Lower)	Intermediate Quartile (Superior)	Quartile (Superior)	P-value
Quartile, n	168	168	168	169	-
Oocytes recovered per OPU	6.7[d] (1120/168)	12.5[c] (2098/168)	19.4[b] (3256/168)	38.7[a] (6533/169)	<.0001
Cleavage rate, %	50.9%[b] (570/1120)	53.9%[ab] (1130/2098)	57.8%[a] (1883/3256)	52.7%[ab] (3441/6533)	<.0001
Blastocyst rate, %	26.1%[a] (292/1120)	26.2%[a] (550/2098)	24.8%[b] (806/3256)	20.3%[c] (1324/6533)	<.0001
Blastocyst per OPU	1.7[d] (292/168)	3.3[c] (550/168)	4.8[b] (806/168)	7.8[a] (1324/168)	<.0001

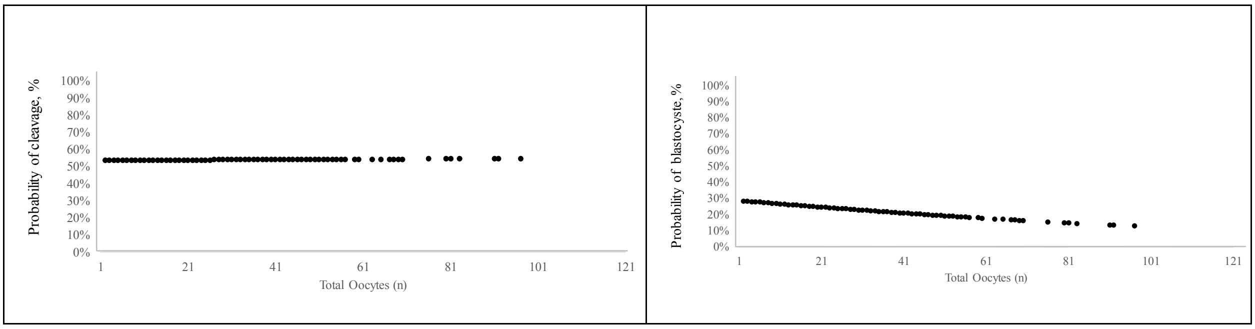

Figure 5. Probability of cleavage rate (A) and blastocyst rate (B) as a function of the number of retrieved oocyte of Hostein (*Bos taurus*) donor (from 13,007 fertilized oocytes). [Logit(PROB_CLEAVAGE) = 0.147+0.0004*Total oocytes; P = 0.7946]; [Logit(PROB_BLASTOCYSTE) = -0.9127-0.0106*Total oocytes; P < 0.0001].

Number of oocytes retrieved at OPU: impact on field conception results

Beef breeds – impact of number of oocytes per OPU on pregnancy results following ET.

Pregnancy results following ET in Nelore breeds was lower in donors with greater amounts of oocytes retrieved per OPU (lower quartile versus the others; Table 8 and Fig. 6). These differences were captured due to the large amount of recorded OPUs in the Nelore breed, and its biological significance is debatable, since it might be related to constrains in trying to transfer larger numbers of embryos on the same day. For example, in terms of utilizing recipients in the field, it is likely that a greater percentage of recipients are used when greater amounts of oocytes need to be transferred. Field vets tend to utilize recipients more aggressively in these cases even perhaps with less than ideal CL quality or synchronization with embryo age and days post-ovulation been less than ideal in some recipients. Still, these constrains are common in the field and the data reported herein should represent more realistically expected results in the field.

In contrast, in the Senepol breed, pregnancy increased in a positive fashion in relation to oocytes recovered per OPU (Table 7 and Fig. 6). This could possibly be related to the smaller sample size in the lower quartile (n = 215 transfers), and we do not have a clear explanation for these results otherwise.

Table **7**. Effect of retrieved numbers of oocytes per OPU from Nelore and Senepol donors on pregnancy results following embryo transfer.

Variable	Quartile (Lower)	Intermediate Quartile (Lower)	Intermediate Quartile (Superior)	Quartile (Superior)	P-value
Nelore - Pregnancy rate, %	44.2%[a] (187/424)	40.3%[b] (302/750)	39.9%[b] (414/899)	38.6%[b] (387/1003)	0.0010
Senepol - Pregnancy rate, %	10.0%[b] (21/215)	17.6%[a] (59/337)	19.8%[a] (101/508)	19.5%[a] (164/841)	0.0015

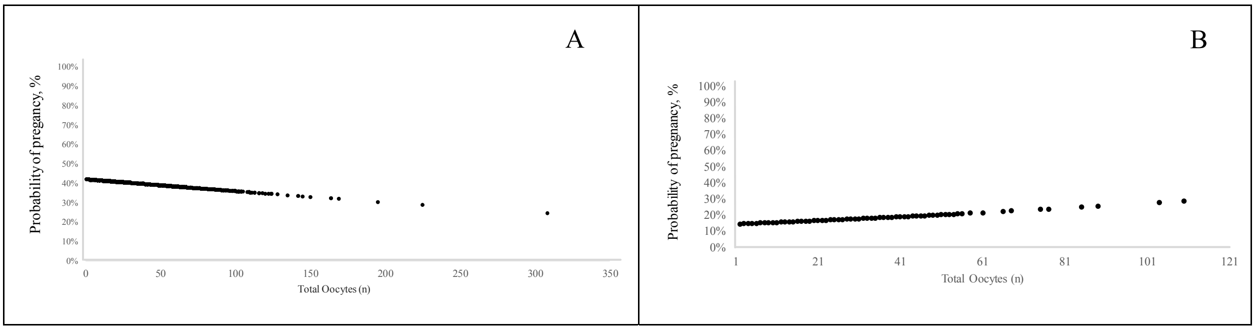

Figure 6. Probability of recipient pregnancy rate as a function of the number of retrieved oocyte per OPU in Nelore (*Bos indicus*; n = 3,076; A) [Logit(PROB_PREGNANCY) = -0.3163-0.0026*Total Oocytes; P < 0.0001] and (B) Senepol (*Bos taurus;* n = 1,901) donors [Logit(PROB_PREGNANCY) = -1.7818+0.008*Total oocytes; P = 0.0793].

Dairy breeds - impact of number of oocytes per OPU on pregnancy results following ET.

For dairy breeds, the overall pregnancy results after ET followed a slight positive trend as the number of oocytes recovered per OPU increased (Table 8). The only difference observed was for Holstein donors, when an increase on pregnancy after ET was observed only when comparing the lower quartile to upper quartiles, similarly as for Senepol breed (Table 7 and 8).

Additionally, the analysis of probability indicated improved pregnancy results after ET when the number of oocytes recovered per OPU increased in Gyr donors and a small trend for the same behavior for Holstein donors (Fig. 7).

Table 8. Effect of retrieved numbers of oocytes per OPU from Gyr (*Bos indicus*) and Holstein (*Bos taurus*) donors on pregnancy results following embryo transfer.

Variable	Quartile (Lower)	Intermediate Quartile (Lower)	Intermediate Quartile (Superior)	Quartile (Superior)	P-value
Gyr - Pregnancy rate, %	25.5% (139/543)	26.6% (267/1001)	27.1% (420/1549)	29.9% (893/2982)	0.29
Holstein - Pregnancy rate, %	16.9%^{b} (32/188)	27.2%^{a} (113/418)	25.9%^{ab} (171/660)	25.9%^{ab} (252/971)	0.0002

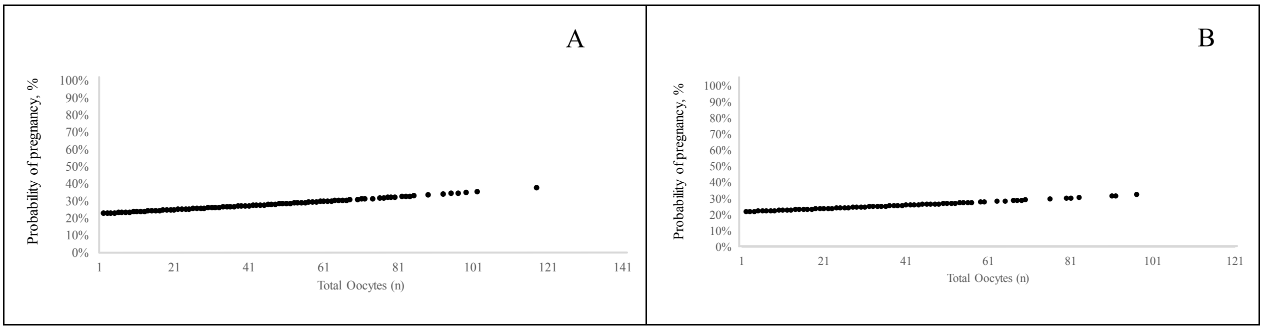

Figure 7. Probability of recipient pregnancy rate as a function of the number of retrieved oocyte per OPU in Gyr (*Bos indicus*; n = 6,075; A) [Logit(PROB_PREGNANCY) = -1.2181+0.0061*Total oocytes; P = 0.0133] and (B) Holstein (*Bos taurus*; n = 2,237) donors [Logit(PROB_PREGNANCY) = -1.2637+0.0056*Total oocytes; P = 0.1140].

Other nuisance factors altering IVEP efficiency

Effect of IVF-sire used during IVEP

Despite of cattle breed, as expected, the semen utilized also had a major impact of IVEP efficiency. As shown in Fig. 8, top ranking sires used in Nelore (*Bos indicus*) donors yielded outstanding blastocyst rates consistently averaging over 30% (18 sires out of 48). In contrast, poor performers (5 out of 48 sires) produced very low blastocyst rates that were lower than 20%. These results highlight the importance of pruning out sires with inadequate results in blastocyst formation during IVEP procedures.

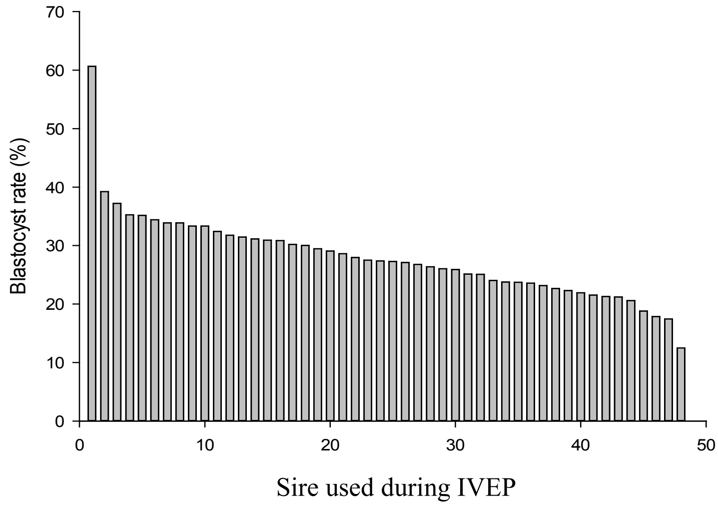

Figure 8. Blastocyst rate (%) for 48 Nelore sires used in at least 50 OPU sessions during IVEP from Nelore (*Bos indicus*) donors.

Effect of season on IVEP efficiency

In general, season also had effect on IVEP, with *Bos indicus* breeds showing less variation in IVEP results throughout the year. In other hand, *Bos taurus* breeds had greater variation in blastocyst rates throughout the differing seasons, which might be accounted by heat stress and possible interactions with feed/pasture quality (Fig. 9). The great susceptibility of *Bos taurus* oocytes to heat stress was previously demonstrated by several groups including ours (Al-Katanani *et al*., 2002; Ferreira *et al*., 2011, 2013, 2016; Paula-Lopes *et al*., 2003; Roth, 2008; Roth *et al*., 2000). Although less intense, the heat stress was also shown to negatively affects oocyte competence in *Bos indicus* cattle (Paula-Lopes *et al*., 2003; Hansen, 2004; Torres-Júnior *et al*., 2008). Thus, variation in temperature-humidity index in which cattle are exposed to, as well as feed quality to avoid, for example, issues with subclinical acidosis or postpartum ketosis or even high blood-urea levels need to be considered during IVEP.

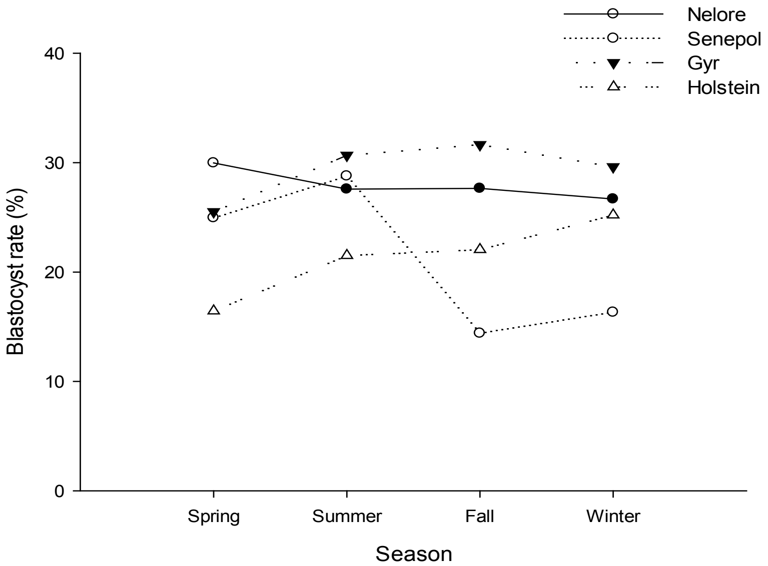

Figure 9. Blastocyst rate (%) throughout the year for beef donors [Nelore (*Bos indicus*), Senepol (*Bos taurus*)], and dairy donors [Gyr (*Bos indicus*) and Holstein (*Bos taurus*)].

Closing remarks & current barriers to OPU/IVF in cattle

In conclusion, IVEP has improved a lot in the last two decades, with greater progress attained particularly in beef breeds. Despite of that, the number of oocytes recovered per OPU had a minor effect both on embryo viability (measured by blastocyst rate success) and on pregnancy rates following ET. It is clear though that other factors such as cattle breed, semen used during IVEP, season, as well as other cow-nuisance variables not considered in the current study such as body condition score or level of negative energy balance experienced by the donor will undeniably alter IVEP and field fertility results. A holistic approach controlling OPU and lab variation, as well as selecting donors with greater genetic value (through genomics) and oocyte population (through AMH assays or ultrasound) are highly advisable.

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