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Effect of follicle wave synchronization and gonadotropin treatments on the number and quality of cumulus-oocyte complex obtained by ultrasound-guided ovum pick-up in beef cattle

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Abstract

Four experiments were designed to evaluate the effect of different hormonal treatments on the number and quality of cumulus oocyte complexes (COCs) recovered by ovum pick-up (OPU) in beef cattle. Experiment 1 compared the synchronization of follicle wave emergence with 2 mg estradiol benzoate (EB) and 50 mg progesterone (P4) given intramuscularly (i.m.) 6 days before OPU versus a control group in which donors did not receive any treatment. Experiment 2 evaluated the use of equine chorionic gonadotropin (eCG) and prostaglandin F2α prior to OPU. Experiment 3 compared the effect of dominant follicle removal (DFR) by ultrasound guided follicle aspiration or EB+P4 to control follicular wave emergence, and treatment with eCG or FSH to superstimulate follicle growth. Experiment 4 evaluated the effect of inserting a progesterone releasing device (CIDR) during the superstimulation treatment. In experiment 1, treatment with EB+P4 resulted in more ($P < 0.05$) viable COCs (5.2 ± 0.9 *vs*. 2.1 ± 0.4) than the controls. In experiment 2, prostaglandin F2α prior to OPU increased ($P < 0.05$) the number of viable COCs (7.9 ± 1.1), but treatment with eCG did not affect ($P > 0.1$) the number of COCs (4.5 ± 1.0) recovered compared to the controls (4.7 ± 0.7). In experiment 3, there were no differences ($P > 0.1$) between DFR and EB+P4; however, treatment with FSH resulted in more ($P < 0.05$) COCs recovered than eCG (6.8 ± 0.6 *vs*. 3.7 ± 0.6). In experiment 4, insertion of a CIDR device did not affect the number of COCs recovered compared to the controls (6.3 ± 0.7 *vs*. 5.8 ± 0.7, respectively). In conclusion, the use of treatments that synchronize follicle wave emergence, prostaglandin F2α to avoid the presence CL at the time of OPU and superstimulation with FSH were useful to improve the number of COCs recovered in beef cattle. Conversely, the insertion of a CIDR device during the superstimulation treatment prior to OPU did not improve the number of COCs recovered nor their quality.

Keywords: dominant follicle removal, eCG, estradiol, FSH, oocyte quality.

Introduction

The ultrasound-guided follicular aspiration technique for collection of cumulus oocyte complexes (COCs), also known as ovum pick-up (OPU) was developed in the late 1980's and has proved to be a useful tool in the production of cattle embryos *in-vitro* (IVP: Pieterse *et al*., 1991; Pontes *et al*., 2009). It has been proposed that the OPU/IVP technique may enable the production of a high number of embryos from the most valuable donor cows in a shorter period of time than the traditional superovulation and *in vivo* embryo production in *Bos taurus* (Nibart *et al*., 1995) and *Bos indicus* (Pontes *et al*., 2009) cattle. Nibart *et al*. (1995) suggested that it is possible to obtain approximately 18 pregnancies in a period of three months using OPU/IVP, whereas only 5 pregnancies were obtained through the conventional embryo collection and transfer in the same period of time. Merton *et al*. (2003) have also shown that it is possible to obtain oocytes frequently (i.e every 3 to 4 days) in cows and heifers. The commercial application of IVP systems is greatly dependent on the number and quality of oocytes obtained from a highly valuable donor in an OPU session. Therefore, in order to increase production rates, different protocols for oocyte retrieval have been described in the literature. OPU has been performed on different days of estrous cycle (i.e. days 3-4, 9-10 or 15-16 after estrus) with better results when the OPU was performed in days 3-4 after estrus (Pieterse *et al*., 1991). Others also evaluated different intervals between OPU sessions such as 48 *vs*. 96 h (Simon *et al*., 1993), 4 to 5 days (Galli *et al*., 2001), weekly (Goodhand *et al*., 1999), or twice weekly (Gibbons *et al*., 1994). Furthermore, gonadotropins have also been used to increase the number of oocytes obtained in an OPU session in beef cattle (Goodhand *et al*., 1999; Pivato *et al*., 1999).

In superovulation and *in vivo* embryo production programs in beef cattle, follicle wave emergence has been synchronized by ultrasound-guided follicle aspiration of all follicles $\geq$5 mm in diameter (Bergfelt *et al*., 1994) or the two largest follicles present in the ovaries (Baracaldo *et al*., 2000). Treatments with gonadotropins are initiated at the time of follicular wave emergence, which occurs on average 1.5 days later in beef cows/heifers (Bergfelt *et al*., 1994, 1997). Another approach to synchronize the emergence of a follicular wave is by treating donor cows with estradiol and progesterone (P4; Bó *et al*., 1995). Gonadotropin

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treatments initiated 4 days after 5 mg of estradiol-17β or 2.5 mg of estradiol benzoate (EB) and 50 or 100 mg of progesterone (P4) have resulted in comparable or greater number of embryos than that obtained when treatments were initiated 8 to 12 days after estrus (Bó *et al*., 1995, 2002, 2006). The aim of using a superstimulation treatment prior to OPU is to increase the number of follicles suitable for puncture per aspiration session. The application of exogenous gonadotropins and control ovarian follicular dynamics may provide opportunities to maximize the number of oocytes obtained from superior beef and dairy cows (Chaubal *et al*., 2006, Sendag *et al*., 2008).Therefore, a series of four experiments were designed to test the hypotheses that: 1) follicle wave synchronization and 2) superstimulation treatments improve the number and quality of cumulus oocyte complexes (COCs) obtained by ultrasound-guided OPU in beef cattle. Experiment 1 was designed to evaluate the effect of synchronizing the emergence of follicular wave using EB and P4 on the number and quality of COCs aspirated by OPU. Experiment 2 was designed to evaluate and test the effects of: 1) induction of luteolysis three days prior to aspiration (to avoid the presence of the CL at the time of OPU) and 2) treatment with equine chorionic gonadotropin (eCG; to stimulate follicle development) on the number and quality of oocytes aspirated by OPU. Experiment 3 was designed to compare the effect of: 1) dominant follicle removal (DFR) by ultrasound guided follicle aspiration or EB+P4, to control follicular wave emergence, and 2) treatment with eCG or FSH, to superstimulate follicle growth, on the number and quality of COCs by OPU. Finally, experiment 4 was designed to evaluate the effect of inserting a progesterone releasing device (CIDR) during the supertimulation treatment on the number and quality of COCs aspirated by OPU.

Materials and Methods

Experiment 1

Brangus (3/8 Brahman and 5/8 Angus; n = 13) and purebred Angus (n = 32) non-lactating, multiparous cows, with a body condition score (BCS) between 2.5 and 3.5 (1 to 5 scale) were randomly allocated in two treatment groups and treated two times in a cross-over design (i.e. all cows received both treatments and all treatments were represented in each OPU session). On day 0, donors in Group EB+P4 received 2.5 mg EB (Estradiol Benzoate, Zoovet, Argentina) and 50 mg P4 (Progesterona, Syntex SA, Argentina) intramuscularly (i.m); whereas donors in the Control Group did not receive any treatment. On day 6, OPU was performed by ultrasound-guided follicular aspiration and COCs were classified based on cytoplasm appearance and cumulus cell numbers as described by Chaubal *et al*. (2006) and COCs grade 1, 2 and 3 were considered viable, while grade 4 (expanded cumulus cells and picnotic cytoplasm) were considered non-viable.

Experiment 2

Brangus (n = 20) non-lactating, multiparous cows, with a BCS between 2.5 and 4 were randomly allocated into three treatment groups in a cross-over design. On day 0 all donors received 2.5 mg EB and 50 mg P4 i.m. On day 4, donors from Group PGF received 150 µg D (+) cloprostenol (PGF, Ciclar, Zoovet, Argentina); whereas donors Group PGF+eCG received PGF2α plus 800 IU eCG (Novormon 5000, Syntex SA, Argentina) i.m. Cows in the Control Group did not receive any treatment. On day 7, COCs were collected and oocytes were counted and classified as in experiment 1.

Experiment 3

Brangus (n = 15) and Angus (n = 15) non-lactating, multiparous cows, with a BCS between 3 and 4 were treated four times by four treatments in a 2 by 2 arrangement. Again, the cross-over design was set up to have all cows receiving all treatments and all groups equally represented in each OPU session. Donors in Group EB/P4+eCG and Group EB/P4+FSH received 2.5 mg EB and 50 mg P4 i.m. on day 0; whereas donors in Groups DFR+eCG and DFR+FSH were subjected to ultrasound guided-follicle aspiration of all follicles >8 mm in diameter (a technique known as dominant follicle removal or DFR) on day 3. All cows received PGF on day 4, and those in Groups EB/P4+eCG and DFR+eCG also received 800 IU eCGi.m.; whereas donors in Groups EB/P4+FSH and DFR+FSH received 160 mg NIH-FSH-P1 Folltropin-V (Bioniche Animal Health, Canada) in four twice daily equal doses for 2 days (i.e. 40 mg bid on days 4 and 5). On day 7, OPU was performed and COCs were classified as in experiment 1.

Experiment 4

Brangus (n = 19) and Angus (n = 15) non-lactating multiparous cows, with a BCS between 2.5 and 3.5 were superstimulated by two treatments (DFR+PGF+FSH and DFR+PGF+FSH+CIDR) in a cross-over design. On day 0 all donors were subjected to DFR and received PGF and those in Group DFR+PGF+FSH+CIDR also received a CIDR (CIDR®, 1.9 g of P4, Pfizer Animal Health, Argentina). On day 1 and 2 all cows received 160 mg Folltropin-V in twice daily equal doses and OPU was performed on day 4.

Ultrasound-guided ovum pick-up (OPU)

Cumulus oocyte complex were recovered by

transvaginal follicular aspiration, as described previously by Chaubal *et al*. (2006). Briefly, donor cows were restrained in a chute and given 5-7 ml of epidural anesthesia (Lidocaine 2%). A Chison D 600Vet ultrasound scanner (Chison Medical Imaging Co., China) equipped with a 5-MHz microconvex transducer and a handle manufactured for transvaginal ultrasound scanning (WTA, Cravinhos, Brazil) was used. An 18 gauge needle (Terumo, Brazil) was passed through a needle guide along the handle of the transducer, and carried into the vagina and fixed dorsally to the fornix. After fixing the ovary against the transducer by manipulation per rectum, the needle was advanced to puncture the vaginal wall and enter the ovarian follicle. The needle was attached via silicon tubing to the oocyte collection tube (50 ml conic tube, Falcon, USA), and an aspiration pump (WTA, Brazil) was used to create a vacuum of 60-70 mm Hg, generating a fluid flow of 10-15 ml/min. The COCs were collected in PBS (PICTOR-GEN, Biogen Argentina S.A., Cordoba, Argentina) supplemented with heparin (10 IU/ml), and immediately following aspiration the PBS was poured through a 50 µm filter (Millipore, Brazil) that was subsequently washed with PBS. The contents were poured into a petri dish (Falcon, USA) to facilitate locating COCs under a stereomicroscope.

Statistical analysis

The number of follicles aspirated, number of COCs recovered and number of viable oocytes were analyzed by ANOVA for mixed models, using treatment and breed as a fixed variable and cow (i.d.) as a random variable. When the effect of treatment was found to be significant in experiment 2, differences between treatment groups were determined by the method of least significant difference (LSD). The software used was Infostat® (Infostat, 2010).

Results

Experiment 1

Results of this experiment are shown in Table 1. The mean number of follicles aspirated, the total number of COCs recovered and the number of viable COCs were higher in cows treated with EB+P4 six days prior to OPU than those in the control group ($P < 0.05$). There was also a breed effect (Table 2) because Brangus cows have a higher number of follicles aspirated, COCs recovered and viable COCs ($P < 0.05$) than Angus cows. Finally, there was no breed by treatment interaction in any of the end points analyzed ($P > 0.1$).

Table 1. Mean (± SEM) number of follicles aspirated, total COCs recovered and viable COCs in beef donors (Brangus and Angus) treated or not with EB+P4 four days prior to OPU. Effect of treatment.

Group	n	Follicles aspirated	COCs Recovered	Viable COCs
EB+P4	45	12.9 ± 1.1^{a}	7.9 ± 1.3^{a}	5.2 ± 0.9^{a}
Control	45	7.3 ± 0.7^{b}	3.2 ± 0.5^{b}	2.1 ± 0.4^{b}

[a,b]Means in the same column differ significantly ($P < 0.05$).

Table 2. Mean (± SEM) number of follicles aspirated, COCs recovered and viable COCs in Brangus and Angus donors. Effect of breed.

Breed	N	Follicles Aspirated	COCs Recovered	COCs Oocytes
Angus	64	9.6 ± 0.6^{b}	4.8 ± 0.6^{b}	2.9 ± 0.5^{b}
Brangus	26	14.5 ± 1.5^{a}	9.4 ± 1.4^{a}	6.2 ± 1.1^{a}

[a,b]Means in the same column differ significantly ($P < 0.05$).

Experiment 2

Results are shown in Table 3. The mean number of follicles aspirated, total number of COCs recovered and viable COCs were higher ($P < 0.05$) in cows treated with PGF on day 4 than in those treated with PGF and eCG and those in the control group. There were no differences between cows in the PGF + eCG group and those in the control group. Finally, there was no breed or a breed by treatment interaction in any of the end points analyzed ($P > 0.1$).

Experiment 3

Results are shown in Table 4. No significant effect ($P > 0.1$) of follicle wave synchronization treatment (DFR *vs*. EB + P4) was detected in the total number of follicles aspirated number of COCs recovered, and viable COCs. However, all the evaluated parameters were higher in cows treated with FSH than in those treated with eCG ($P < 0.05$). Finally, there was no breed effect or a breed by treatment interaction in any of the end points evaluated ($P > 0.1$).

Table 3. Mean (± SEM) number of follicles aspirated, COCs recovered and viable COCs in beef donors (Brangus and Angus) treated with PGF, PGF and eCG or not treated with PGF or eCG (control) four days prior to OPU.

Group	n	Follicles aspirated	COCs recovered	Viable COCs
PGF	20	18.3 ± 1.4[a]	11.4 ± 1.2[a]	7.9 ± 1.1[a]
PGF + eCG	20	11.8 ± 1.4[b]	7.5 ± 1.4[b]	4.5 ± 1.0[b]
Control	20	12.1 ± 0.8[b]	7.1 ± 0.8[b]	4.7 ± 0.7 [b]

[a,b]Means in the same column differ significantly (P < 0.05).

Table 4. Mean (± SEM) number of follicles aspirated, COCs recovered and viable COCs in beef donors (Brangus and Angus) superstimulated with FSH or eCG prior to OPU.

Group	n	Follicles aspirated	COCs recovered	Viable COCs
FSH	32	17.9 ± 1.2[a]	10.0 ± 0.8[a]	6.8 ± 0.6[a]
eCG	32	13.6 ±1.2[b]	7.3 ± 0.8[b]	3.7 ± 0.6[b]

[a,b]Means in the same column differ significantly (P < 0.05).

Experiment 4

Results are shown in Table 5. No significant effect (P > 0.1) of inserting a CIDR device was detected in the total number of follicles aspirated, number of COCs recovered, and number of viable COCs. Furthermore, there was no breed effect or a breed by treatment interaction in any of the end points analyzed (P > 0.1).

Table 5. Mean (± SEM) number of follicles aspirated, COCs recovered and viable COCs in beef donors (Brangus and Angus) superstimulated with FSH and treated with a progesterone releasing device (CIDR) prior to OPU.

Group	n	Follicles aspirated	COCs recovered	Viable COCs
DFR+PGF+FSH+CIDR	36	15.4 ± 1.2	8.6 ± 0.9	6.3 ± 0.7
DFR+PGF+FSH	36	15.9 ± 1.2	8.3 ± 0.9	5.8 ± 0.7

Means did not differ (P > 0.1).

Discussion

The hypothesis that synchronization of follicular waves increases the number and quality of COCs obtained by OPU was supported. The number and quality of COCs recovered by follicular aspiration depend largely on the stage of follicular wave in which the animals are at the time of OPU (Seneda *et al*., 2001). The greater number of follicles in a follicular wave can be found shortly after wave emergence and prior to the selection of the dominant follicle (Ginther *et al*., 1989, 1996). According to Seneda *et al*. (2001), oocyte quality is not affected by the size of the follicle, but mainly by the follicular phase of the donor. Furthermore, oocyte recovery rates are higher when the OPU is performed in follicles ≤6 mm in diameter than when follicles are larger (Seneda *et al*., 2001). The associated use of estradiol and P4 induces a suppression of FSH and follicular atresia (Bó *et al*., 1995) and the beginning of a new follicular wave has been shown to be on average 4 days after treatment with EB+P4 (Caccia and Bó, 1998). Therefore, when the OPU was performed two or three days after wave emergence (i.e. days 6 or 7 after EB+P4) most follicles aspirated were between 4 to 7 mm in diameter at the time of OPU, which is probably the ideal time and size of the follicles to have the best recovery rate and COCs quality after OPU (Seneda *et al*., 2001).

Although Kruip *et al*. (1991) and Bungartz *et al*. (1995) have reported that viable oocytes can be obtained in any stage of the cow's estrous cycle; our results suggest that more and better oocytes are obtained right after wave emergence. In agreement with our findings, Bols *et al*. (1997) and Hagemann (1999) have suggested that blastocyst production is higher when COCs are retrieved shortly after wave emergence, when follicles are not under the influence of a dominant follicle.

There was a breed effect observed in experiment 1, when we compared Angus (*Bos taurus*) and Brangus (1/3 *Bos indicus* 2/3 *Bostaurus*) cows.The difference was a reflection of the higher antral follicle population reported in *Bos indicus* compared to *Bos taurus* cattle (Dayan *et al*., 2000; Bó *et al*., 2003; Silva-Santos *et al*., 2014). Interestingly, these differences

were only apparent in experiment 1 when cows were not superstimulated. Conversely when cows were superstimulated the number of follicles aspirated and COCs retrieved was not different between Angus and Brangus cows. These results are in agreement with those reported elsewhere (reviewed in Baruselli *et al*., 2006). Although *Bos indicus* cows required a smaller dosage of FSH than *Bos taurus* cows, superovulatory response to the conventional treatment are usually comparable to those obtained in *Bos taurus* donors (Baruselli *et al*., 2006).

Another procedure evaluated in this study to facilitate the OPU technique is removing or reducing the diameter of a CL present by giving PGF four days prior to OPU. The absence of CL at the time of OPU has several favorable technical aspects. The visualization and the puncture of follicles is easier to perform when the CL is not present, moreover, there is a reduction of vascular perfusion with a lower uptake of blood. Consequently, a higher number of COCs were recovered. These results are consistent with a previous study (Bacelar *et al*., 2010). Conversely, the induction of luteolysis before OPU would reduce plasma P4 concentrations and potentially reduce oocyte quality. Although, this adverse effect was not seen in the morphology of the COCs evaluated in the present study, further studies must be performed to evaluate if the potential blastocyst production is affected when P4 concentrations are low at the time of OPU. It has been reported that embryo quality was seriously compromised in the absence of exogenous progesterone during FSH treatments in *Bos indicus* cows superstimulated during the first follicular wave (Nasser *et al*., 2011). Similarly, in lactating dairy cows superstimulated during the first follicle wave, embryo quality was improved when supplemental P4 was added to the treatment protocol (Rivera *et al*., 2011).

The hypothesis that gonadotropin treatment increases the number and quality of COCs obtained by OPU was only supported when cows were treated with FSH, but it was not supported when cows were treated with eCG. The results from experiment 2 and 3 suggest that eCG treatment is not effective in recruiting more follicles prior to OPU; at least in the dosages used in the present studies. Furthermore, in experiment 2 the addition of eCG confounded the beneficial effect of PGF on the number of COCs recovered. Conversely, the use of FSH significantly increased the number of COCs recovered.

The use of treatments that simulate follicle growth is a technique currently used by several practitioners that work with *Bos taurus* beef or dairy cattle (Bungartz *et al*., 1995; Goodhand *et al*., 1999; Van de Leemput *et al*., 1999; Blondin *et al*., 2002, 2012). It has been reported that the use of FSH facilitates the visualization of the follicles and also improves oocyte competence and blastocyst production (Chaubal *et al*., 2006; Blondin *et al*., 2012). Furthermore, Aller *et al*. (2012) have recently reported more oocytes recovered from pregnant beef cows after treatment with twice the dosage of eCG used in the present studies (1600 IU).

Both eCG and FSH have been used to superovulate donor cows for *in vivo* embryo collections for many years. Although some studies reported higher superovulatory responses in cows treated with FSH than with eCG (Monniaux *et al*., 1983; Rouillier *et al*., 1996; Goodhand *et al*., 1999; De Roover *et al*., 2005), others found no differences between pituitary extracts containing FSH and eCG (Mapletoft *et al*., 1990; Goulding *et al*., 1996). Nevertheless, the problems associated with the prolonged stimulation of the ovaries following a single injection of eCG reduced its use by practitioners around the world (Bo and Mapletoft, 2014). The half-life of eCG has been shown to be 40 h in the cow and eCG persists for up to 10 days in the bovine circulation (Murphy and Martinuk, 1991). Therefore, the long half-life of eCG causes continued ovarian stimulation, unovulated follicles, abnormal endocrine profiles and reduced embryo quality (Schams *et al*., 1977; Saumande *et al*., 1978; Mikel-Jenson *et al*., 1982; Moor *et al*., 1984). Furthermore, endocrine studies have revealed that eCG-treated animals more frequently had abnormal profiles of LH and progesterone (Mikel-Jenson *et al*., 1982; Greve *et al*., 1983), which was associated with reductions in both ovulation and fertilization rates (Callesen *et al*., 1986) when compared to FSH-treated cows. Although continued stimulation due to eCG's prolonged half-life may not be that detrimental in cows that are aspirated a few days after treatment, the continued stimulation with eCG may not permit that follicles be deprived of gonadotropin stimulation for some time prior to aspiration. It has been shown that oocyte competence and blastocyst rates are improved if cows are deprived of FSH for about 36 to 48 h prior to OPU in a procedure called "coasting" (Blondin *et al*., 2002). While coasting may be easily done by just stopping FSH administration for 36 to 48 h prior to OPU, it is not possible to do it in eCG stimulated cows, possibly affecting oocyte quality.

Another difference between eCG and FSH is the amount of LH in eCG. Radioreceptor assays and *in vitro* bioassays have revealed variability in both the FSH and LH activity of eCG, not only among pregnant mares, but also within the same mare at different times during gestation (Murphy and Martinuk, 1991). The effects of the FSH/LH ratio of eCG on superovulatory response has been examined and there was a positive correlation between the ratio of FSH/LH activity and superovulatory response. Lower ratios of FSH/LH activity resulted in a reduced ovulatory response in immature rats and LH added to eCG reduced superovulatory response in cattle (Murphy *et al*., 1984; Murphy and Martinuk, 1991). Furthermore, high levels of LH during superstimulation have been associated with premature activation of the oocyte (Moor *et al*.,

1984). Conversely, purified pituitary extracts with low LH contamination, like Folltropin-V that was used in the present study, have been reported to improve superovulatory response in cattle (reviewed in Bó and Mapletoft, 2014). Collectively, data support the notion that the detrimental effect eCG on oocyte quality may be due to an excess of LH during follicle stimulation and lack of a coasting period prior to OPU.

There was also no difference in the quality of oocytes among the groups that used different methods of synchronization. Although, Bacelar *et al*. (2010) observed a higher number of follicles available at the time of OPU and a higher number of COCs recovered by OPU session when they used BE + P4 to synchronize the wave emergence, other reports did not find differences in superovulatory response and embryo production in cows superstimulated 1.5 days after aspiration of all follicles >5 mm in diameter (Bergfelt *et al*., 1997) or the aspiration of the two largest follicles (Baracaldo *et al*., 2000) and cows superstimulated four days after treatment with estradiol-17β and progesterone.

Although high P4 concentrations seem to benefit oocyte quality and competence, the effect of adding a CIDR device in experiment 4 did not improve COC quality. Pfeifer *et al*. (2009) found that very high and very low levels on P4 affected oocyte quality in *Bos indicus* donors; whereas, Chaubal *et al*. (2007) found no significant effects when a progestin device was inserted prior to OPU. Low peripheral P4 concentrations have been reported to result in increased LH pulsatility, which may induce disturbances in nuclear maturation, reducing embryo quality and fertility (Callesen *et al*., 1986; Dieleman *et al*., 1993; Ahmad *et al*., 1995; Greve *et al*., 1995; Wehrman *et al*., 1997). Denicol *et al*. (2012) described higher pregnancy rates per AI in cows induced to ovulate the first wave dominant follicle following supplementation with exogenous progesterone during the development of that follicle compared with unsupplemented cows. Bisinotto *et al*. (2010) suggested that induction of ovulation of the first wave dominant follicle may compromise oocyte and embryo quality, due to the low concentrations of progesterone during development of the ovulatory follicle. However, in a recent study in which embryos were recovered from single ovulating cows that had follicles grow during low or high P4 there was no difference in embryo quality on day 7 (Cerri *et al*., 2011b). A follow-up study (Cerri*et al*., 2011a) indicated that although cows with low P4 had increased basal LH concentrations, altered follicular dynamics, and follicular fluid composition that could alter oocyte quality, a particularly distinct difference in cows with low P4 was the premature development of pathways leading to uterine prostaglandin F2α secretion. Thus, altered uterine function could also have an important role in reducing fertility in cows that have low P4 concentrations prior to AI.

In conclusion, the use of treatments that synchronize follicle wave emergence, prostaglandin F2α to avoid the presence CL and superstimulation with FSH were useful to improve the number of COCs recovered by OPU in beef cattle. Conversely, the insertion of a CIDR device during the superstimulation treatment prior to OPU did not improve the number of COCs recovered nor their quality.

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