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Ovulatory response of Beef and Dairy cows subjected to two follicular emergence synchronization protocols before superovulation

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ABSTRACT

The aim of this study was to evaluate the superovulation response of Beef and Dairy donor cows to two different follicular emergence synchronization protocols. Twenty-two beef and dairy cows were divided into two groups viz. Conventional group (n=8) having four Holsteins and four Charolais cows between days 10 and 11 of their estrous cycle and IVD+EB group (n=14) with six Holsteins and eight Charolais cows treated with an intravaginal device (IVD) containing 1.9 g of P_4 + 2 mg of estradiol benzoate (EB) between days 10 and 11 of their estrous cycle. The superovulation protocol consisted of intramuscular application of FSHp twice a day for four days, in decreasing doses (850 IU for Dairy cows and 500 IU for Beef cows). The number of follicles (13±1.1 vs. 7.5±0.9) and embryos collected (11.7±2.1 vs 6.1±1.0) were significantly affected by the treatment in the Beef cows but, the protocols did not significantly affect these variables in Dairy cows (12.2±0.9 vs 10.4±0.7, respectively). Regarding the production of non-viable embryos, a significant difference was only found in the group of Beef cows for both treatments (8.2±2.3 vs. 1.3±0.3, respectively). Results showed that IVD+EB is not necessary for the superstimulation of the emergence of a new follicular wave before superovulation when it starts in the mid-luteal phase of the estrous cycle in Holstein cows and beef cows, since they had similar results without significant differences between both treatments.

Keywords: Biotechnology, Cattle, Embryo transfer, Embryos, Superovulation

Superovulation protocols (SOV) favour the development of *in vivo* embryo production for breeding programs and improve cattle's reproductive efficiency (Alkan *et al.* 2020). These programs have significantly increased the production of transferable embryos in dairy, beef, and dual-purpose cows (Barucelli 2006, Callejas *et al.* 2008). The SOV includes the management of endocrine processes through hormonal treatments, mainly based on gonadotropins to control the estrous cycle until the ovulation of the dominant follicles (Soria 2017). However, variability in SOV response and embryo viability had been reported. It has been documented that around 20 to 30% of cows subjected to superovulation treatments do not respond (Lonergan *et al*. 2011, Mogollón *et al*. 2019). One of the factors that undoubtedly have a great effect on the SOV response of donor females is the timing of the emergence of a new follicular wave, that is, such a response depends mainly on the number of follicles sensitive to gonadotropins present in the donor at SOV. Traditionally, when the SOV protocol (conventional protocol) was started, it was between days 8 and 12 of the estrous cycle corresponding to the middle luteal phase of the donor female, approximately coinciding with the appearance of the second follicular wave. However, a limitation of this protocol was the strict monitoring and observation of the start of the estrous cycle (estrus) of the donor females, since this event was of the utmost importance for the start of the SOV protocol. In this same sense, in the 1990s, the use of progestogens and estradiol to induce the synchronous appearance of a new follicular wave was reported (Bó *et al.* 1995), without considering the stage of the estrous cycle and without considering the need to observe estrus or wait for 8 to 12 days to start the SOV protocol (Soria *et al*. 2017). However, one of the greatest limitations to the use of this method is the use of estradiol, since in many countries this hormone is prohibited, due to the residues of these steroid hormones in the food chain (Lane *et al.* 2008, Mapletoft *et al.* 2018). Therefore, this study was designed to compare the response of dairy cows and beef donor cows to embryos with or without progesterone plus estradiol benzoate (EB). Although recent studies report good superovulation results using progesterone plus EB, we hypothesize that the conventional protocol without progesterone and EB could

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match or improve the rate of embryonic production in dairy and beef cows.

MATERIALS AND METHODS

The management of the experimental units used in this study was in strict accordance with the guidelines for the ethical use, care, and welfare of animals in research at the international level (FASS, 2010), national levels (NAM, 2002), and institutional with reference number UAAAN-UL/005/22-BO-PR-LN.

The study on Holstein cattle was carried out on a commercial dairy farm in the Comarca Lagunera (25° 44' 36'' N and 103° 10' 15'' W) which is characterized by an extremely hot climate, with maximum temperatures in summer (43°C), and minimum in winter (2 to 9°C). The average annual rainfall is 240 mm and the relative humidity ranges from 29 to 83%; the altitude is 1,111 meters above sea level (CONAGUA 2015). While the study on Beef cattle was carried out on a commercial cattle ranch of the Angus breed (27°46'N, 105°43'W), the area is characterized by a very dry semi-warm climate, temparature ranges from 18 to 20°C, with a rainfall of 200 to 400 mm and an altitude of between 1,100 and 1,400 meters above sea level (INEGI 2020).

Experimental animals: Twenty-two cows were used, 10 Dairy cows and 12 Beef cows divided first according to their zootechnical function and then randomly assigned to one of the two follicular wave synchronization protocols. The first group was Holstein cattle (n=10) in which six (6) Holstein cows were synchronized with a protocol where a device (IVD) with progesterone (CIDR®, Zoetis, Mexico) and estradiol benzoate (EB) was used, and 4 Holstein cows synchronized with a protocol without IVD or EB (conventional). While in Angus cattle (n=12), 8 cows were synchronized with the IVD+EB protocol and 4 cows synchronized with the conventional protocol.

Holstein cows had a body condition score (BCS) of 2.75 to 3.5 on a scale of 1 to 5 (Lowman *et al*. 1976), and Angus cows had a BCS of 4 to 5 on a scale of 1 to 9 (Hall *et al*. 2000), with an age of 40 to 60 months, with more than 90 days postpartum and clinically healthy, without having had any pathological alteration in the genital organs during their reproductive life. All the cows were managed under the same environmental and nutritional conditions according to their exploitation system.

Treatments

IVD + EB: In this protocol, on day seven of the estrous cycle, cows received an intravaginal device (IVD) impregnated with 1.9 g of progesterone (CIDR®, Zoetis, Mexico), followed by the application of 2 mg of estradiol benzoate (EB; Sincrodiol®, Eurofino, Mexico) intramuscularly (IM), the SOV protocol began on day eleven of the estrous cycle prior to this, on day ten an ultrasound was performed to detect the presence of the corpus luteum (CL). Superovulation treatment was performed by applying decreasing doses of porcine follicle-stimulating hormone (FSHp; Pluset®, Calier, Spain) and on day fourteen the IVD was removed as shown in Fig.1.

Conventional protocol (without IVD or EB): Day zero (D0) was established as the day when cows showed signs of heat. On day ten of the estrous cycle, an ultrasound was performed to detect the presence of the corpus luteum (CL). On day eleven, the SOV treatment was started with applications of porcine follicle-stimulating hormone (FSHp; Pluset®, Calier, Spain).

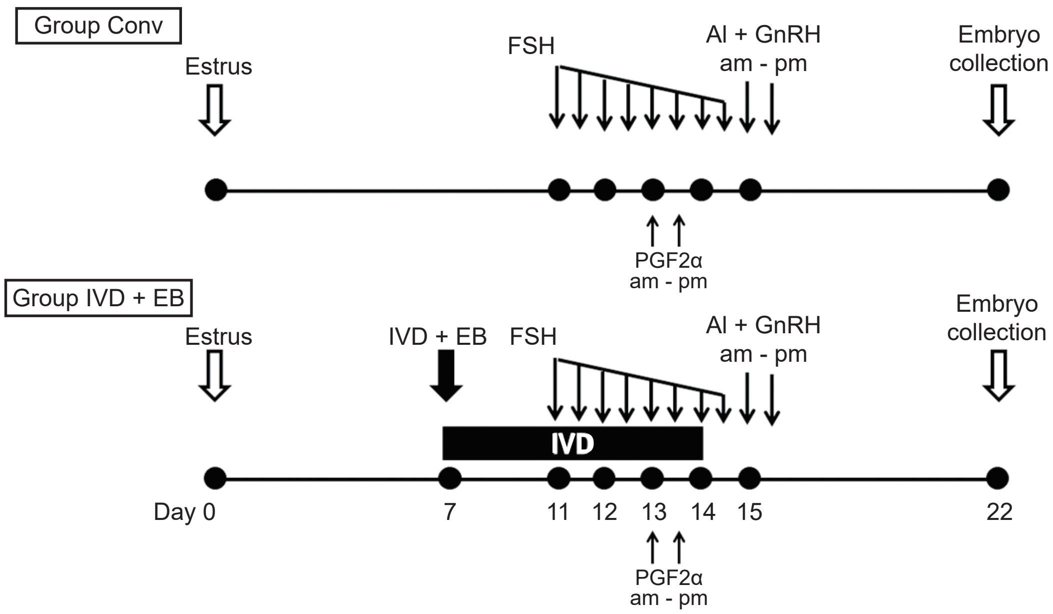

Fig. 1. Schematic description of the synchronization and SOV protocols. Conventional group and group Estradiol Benzoate (EB) plus progesterone. The doses of FSHp used in dairy cattle were 175 IU, 175 IU, 125 IU, 125 IU, 75 IU, 75 IU, 50 IU, and 50 IU morning and evening. While in beef cattle it was 100 IU, 100 IU, 75 IU, 75 IU, 50 IU, 50 IU, 25 IU, and 25 IU of FSHp morning and afternoon. Artificial Insemination (AI), Follicle Stimulating Hormone (FSH), Progesterone (P4), intra-vaginal device (IVD), embryo transfer (ET).

The total doses of FSHp for superovulation in the case of dairy cows in both synchronization protocols (IVD+Prgesterone and Conventional) was 850 IU and, for beef cows, it was 500 IU of FSHp IM twice a day (6:00 AM and 6:00 PM) in decreasing doses for four days as shown in Fig. 1. Seven days after AI, the embryos were collected with the non-surgical method (transcervical) described by Roberson (2015).

Embryo collection and evaluation: All the materials used for the collection of the embryos were sterile. The donor cow was placed in a chute, the base of the tail and the vulva were shaved and washed, and 5 mL of epidural anesthesia (2% lidocaine) was applied to immobilize the animal's tail and prevent rectal contractions. Rectal palpation was then performed to determine the number of *corpora lutea*, verifying this count by transrectal ultrasound. Subsequently, the hand was introduced through the rectum to manipulate the cervix and the uterine horns, then a mandrel covered with an 18-gauge Foley catheter was introduced vaginally, the cervix was crossed, and the anterior end of the catheter was directed towards the ipsilateral horn of the ovary that presented a greater number of *corpora lutea*, locating the anterior end 5 cm cranially from the bifurcation of the uterine horns. The catheter balloon was inflated according to the size of the uterine horn. Once the Foley catheter was inside, the mandrel was removed and connected to a "Y" circuit to introduce and extract the wash medium and drag with Hartman solution at 37.5°C (approximately 1000 mL per uterine horn). This same procedure was performed on the other uterine horn.

After recovering the washing medium containing the embryos, they were isolated from the total volume by filtration (Em-Com Filter). With a phase contrast stereoscopic microscope (MARK), the embryos were evaluated to classify them according to their quality based on their morphology (Bo *et al.* 2018). A maintenance holding medium was used and they were subsequently classified according to their stage of development. The characteristics that were observed were compaction of the cells, regularity in the shape of the embryo, variation in the size of the cells, colour and texture of the cytoplasm, presence of vesicles, extruded cells, diameter and regularity of the zona pellucida. The classification used was that proposed by Bó *et al*. (2018). The variables that were evaluated in this study were the superovulation response based on the number of follicles, the superovulation response was also considered based on the number of corpora lutea, quantity, quality, and viable embryos defined as those embryos compact and spherical with blastomeres of similar size with a homogenous colour, texture and homogeneous citoplasm (Bó *et al.* 2018).

Statistical analysis: The number of total follicles, total corpora lutea, and total embryos were analyzed using the PROC GLM option of SAS (Statistical Analysis Systems Inc., Cary, North Caroline, USA). The number of viable (transferable) embryos and degenerated embryos was also compared with the SAS GLM procedure, before a normality analysis of these data with the SAS UNIVARIATE option; data that were not normally distributed (Shapiro-Wilks test) were transformed with Log10X. Differences between means were considered statistically significant if $P<0.05$.

RESULTS AND DISCUSSION

The effect of the synchronization protocols prior to superovulation on the production of follicles, corpora lutea, and collected embryos is shown in Table 1. The two protocols were effective in stimulating the emergence of a new follicular wave. Significant statistical difference in the variables of the number of follicles and number of embryos in favour of the conventional treatment in the group of Beef cows ($P<0.05$) was found. On the other hand, in the group of Dairy cows, only a statistical difference was found in the variable of the number of corpora lutea in favour of the conventional treatment ($P<0.05$).

These results agree with the observations of Son *et al.* (2007) who showed that cows treated with an intravaginal progesterone device plus EB administration prior to superovulation resulted in response and embryo production comparable to that seen with conventional superovulation. It has been suggested that treatment with progesterone plus estradiol prior to superovulation synchronizes the appearance of follicular waves in cows and reduces the variability associated with superovulation treatments initiated at random stages of the follicular wave development (Mapletoft *et al.* 2002, Barrett *et al.* 2007). In this sense, some studies mention that treatment with 2.5 mg EB at the time of CIDR insertion results in the synchronized emergence of a new follicular wave 3-4 days later (Caccia and Bó 1998). It is important to point out that although it is mentioned that cows treated with progesterone through an intravaginal device, plus the administration of estrogen esters before superovulation treatment, at any stage of the estrous cycle, effectively synchronizes the emergence of the follicular wave, the development follicle and subsequent ovulation (Mapletoft *et al.* 1991, Andrade 2003), in the present study an increase in the number

Table 1. Superovulation response (Mean±SEM) of Beef and Milk cows subjected to two follicular emergence synchronization protocols (CONV and IVD+EB)

Variable	Beef cows		P value	Dairy cows		P value
	CONV	IVD+EB		CONV	IVD+EB	
Follicles (n)	13±2.7[a]	7.5±0.9[b]	0.02	7.7±1.1	8±0.9	0.86
Corpus luteum (n)	12.2±0.9	10.4±0.7	0.11	8.7±0.6[a]	6.3±0.6[b]	0.01
Embryos (n)	11.7±2.1[a]	6.1±1.0[b]	0.01	4.7±0.5	4.0±1.4	0.67

Table 2. Viable embryo (Mean±SEM) of Beef and Dairy cows subjected to two follicular emergence synchronization protocols (CONV and IVD+EB)

Variable	Beef cows		P value	Dairy cows		P value
	CONV	IVD+BE		CONV	IVD+BE	
Viable embryos	3.5±1.1	4.7±0.8	0.38	2.5±1.1	1±0.8	0.06

CONV, Conventional; IVD, Intravaginal device; EB, Estradiol benzoate.

of follicles was observed after conventional treatment compared to treatment with IVD+EB in the group of the Angus breed (P=0.02) not so in the Holstein breed group (P=0.86), however, in this breed the number of corpora lutea were higher when IVD+EB was not administered (P=0.01), although there was no difference in Beef cattle (P=0.7), these results contrast with what was reported by Bo *et al*. (1995), Broadbent *et al.* (1995) and Bo *et al*. (1996) in Bos taurus cows, where it has been proposed that the use of a IVD plus EB improves the superovulation response.

Table 2 shows the number of viable embryos of Beef and Dairy cows subjected to two follicular emergence synchronization protocols. In the group of Beef cows, the number of embryos was higher for the conventional protocol (P<0.05), but in Dairy cows, there was no statistical difference in the number of viable embryos for conventional protocol (P>0.05). The results of this study in the two breeds contrast with the observations of Nasser *et al.* (2011) who observed that embryo quality was compromised by the absence of exogenous progesterone during FSH treatments. In the same sense, Wiley *et al.* (2019) also showed that the removal of endogenous progesterone during superovulation can decrease the total number of embryos.

Regarding the results of the production of viable embryos, it was not affected by the synchronization protocols in the group of Beef cows (P>0.05), but in Dairy cows, there was a tendency (P=0.06) to favour the conventional protocol (Table 2). These results are consistent with the data from Andrade *et al*. (2003), who found that the number of total ova, transferable embryos, degenerated embryos, and unfertilized ova were the same when follicular wave emergence was synchronized with 2 mg EB in CIDR-treated cows as for a conventional protocol. Our results are also consistent with the reports of Mitchell *et al*. (1998), in which total ovules and transferable embryos did not differ between treatments, using 2.5 mg of EB, and without EB to synchronize the follicular wave in cows treated with the use of CIDR. Another study reported that transferable embryos, degenerated embryos, and unfertilized ova did not differ when follicular wave emergence was synchronized with 2.5 mg EB in CIDR-treated cows than when a conventional protocol was used (Meyer *et al.* 2000).

The fact that in this study no statistical difference was found in favour of the use of IVD+EB in both synchronization treatments, coincides with Wiley *et al.* (2019) who reported an increase in the percentage of grade 1 embryos in a conventional protocol in beef-producing cows, which suggests that the conventional protocol does not affect embryo quality and that starting superovulation on day 11 of the estrous cycle with the presence of corpus luteum and its production of progesterone is sufficient for a favourable response to superovulation and embryo production. This study is one of the few that exist where it is shown that the use of exogenous progesterone or estradiol esters is not necessary, even though there is literature where it is mentioned that the combination of progesterone plus estradiol in superovulation protocols not only impacts at the level of the superovulation response but also tend to improve embryonic quality (Bó and Mappletoft 2014). However, in the present study, the viability of the embryos produced from cows to which estradiol and exogenous progesterone were applied was not different compared to the cows to which it was not applied.

Taken together, these results suggest that pre-superovulation synchronization protocols using estrogen ester-induced follicular wave emergence synchronization in progesterone/estradiol-treated cows are not necessary as they give the same result as the conventional protocol (mid-luteal phase), the latter has the advantage of using fewer hormones, in addition to not requiring the use of steroids, which affect the food chain (Lane *et al*. 2008.), which allows dispensing with the use of these hormones that can present a risk in residues and also make superovulation treatments more expensive.

Administration of intravaginal devices impregnated with progesterone plus estradiol benzoate is not necessary for superstimulation of the emergence of a new follicular wave prior to SOV when initiated in the mid-luteal phase of the estrous cycle in Holstein and beef cattle. Similar results were found without significant differences between both treatments. These results are very helpful as an alternative to SOV protocols in countries where the use of steroidal hormones is prohibited.

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