**PeerJ- 59336**

**Title: GnRH or Estradiol benzoate combination with CIDR improves in-vivo embryo production as compared to conventional superovulation in bovine (Bos indicus and Bos taurus) under subtropics**

**Article Type: Research article**

**Reviewer comments:** This study assessed the effect of using GnRH or estradiol benzoate together with CIDR to synchronize the follicular wave prior to the start of the SO protocol in Holstein and crossbred cows in a subtropical environment. The effect was evaluated mainly on the number of transferrable embryos. This topic has been extensively studied and therefore the contribution of this manuscript to the field of knowledge is not clear. The English language should be improved because the current writing makes many sections of the manuscript difficult to understand. I suggest you have your manuscript revised by a professional language editing service. Please find some particular comments below.

**Abstract**

**Lines 35-37:** The present study aimed to compare three different superovulation protocols (CIDR-GnRH, CIDR-EB and conventional/control group) **= Correct as indicated below for lines 109-110. Also check the title accordingly to make it more representative of what the study was about.**

**Introduction**

**I suggest replacing the terms "exploit" or "exploitation" by other that does not imply abusive use of animals. Please replace in all the document.**

**Line 75-77:** Crossbreeding is an effective tool for quick genetic improvement of low milk-producing cows, but commercial dairy farms cannot sustain economically with F1 cows from nondescript animals. **= This idea is somewhat contradictory. Please clarify.**

**Lines 77-79:** Raising a purebred stock of dairy cows to produce F1 cows has its own constraints. **Such as?** However, exploiting high milk-producing already available XB and exotic cows is a practical model. = **What do you mean by "exploiting high milk-producing XB and exotic cows is a practical model"? A practical model for what? What do you mean by exotic cows?**

**Lines 79-80:** Crossbreeding may be used for formation of synthetic breeds, rotational crossing and up-grading **= Delete.**

**Lines 86-90:** The non-availability of technical staff and fixed timeline-based superovulation protocols in subtropical conditions is the root cause of ET's low adaptability in developing countries. No specific superovulation protocol with promising results has been reported for exotic (Bos Taurus) and XB (Bos taurus x Bos indicus) cows under subtropical conditions as per our knowledge. **= First, why should SO protocols be different for temperate than for tropical conditions? Please explain. Second, this information is not precisely accurate. In recent years some articles have been published on the use of different hormone combinations for induction of SO in donor cows in the tropics. For example:**
**1) Embryo production after superovulation of bovine donors with a reduced number of FSH applications and an increased eCG dose. Theriogenology 2020.** https://doi.org/10.1016/j.theriogenology.2019.09.018 **This article cites several references that can be useful for your manuscript.**
**2) Superovulatory response of bovine donors using five or eight injections of swine FSHp. Acta Sci Vet 2006;34(Suppl. 1):s289.**

**And some other articles that are available.**
**In addition, in different tropical countries where *Bos taurus* cattle is not considered exotic, there are some SO protocols that are used routinely. Some of this information can be found in the articles just mentioned. I suggest that you revise them.**

**Lines 102-103:** Meanwhile, treatment with gonadotrophin on the day of follicle wave emergence results in better super-stimulatory response and vice versa **= Not clear; why viceversa?**

**Lines 109-110:** the present study aimed to evaluate CIDR-GnRH and CIDR-EB-based superovulation's efficiency compared to the conventional super-stimulatory protocol **= Use of CIDR + GnRH or EB is not intended to induce SO, but to synchronize the follicular wave prior to SO, which is based on FSH injections. You said it in line 107: progesterone, estradiol and GnRH have been used to induce the surge of follicular waves. Therefore, I suggest the following objective: "…to evaluate the use of GnRH or EB along with CIDR for synchronizing the follicular wave before induction of SO with the conventional FSH protocol" or something like that.**

## Materials and methods

**In what country and area was the study conducted? Geographic location? Type of climate that prevails in the study area?**

**Line 121:** The study was conducted during moderate or colder months (October to March) **= What was the mean temperature?**

**Line 125:** at the time of initial heat… **= Does it mean that you selected the cows based on the display of estrus?**

**Line 129:** In the CIDR-GnRH group, presence of CL was confirmed on Day 8 of the estrus cycle through ultrasonography and 2 ml GnRH was administered… **= Do you mean that all the donors were synchronized to estrus before the start of the study? Did they all start the protocol on Day 8 of the estrus cycle? Any particular size of CL to include the cow in the study? Please clarify.**

**Line 137:** After confirmation of heat signs… **= How did you detect estrus?**

**Lines 141-143: Did these cows also have an active CL? I sounds obvious but you have to make it clear. Same comment for line 144, for the control group.**

**The number of donors from the control treatment is twice as large as the number of donors from each of the other groups. This may be a problem for the statistical analysis. I suggest reducing in half the number of donors from the control group and run again the statistical analysis.**

**Indicate the number of cows from each genotype in each group.**

**Lines 151-152:** ...to check the existence of ovulatory follicle and CL on "Day 0" and "Day 8" of the estrous… **= Same question again, does it mean that you synchronized estrus before the start of the study?**

**Line 153:** …rule out the presence of dominant follicle **= Did you find any cow that had a dominant follicle? What happened to these cows?**

**Line 154:** …ovarian scanning of donor animals was correspondingly performed on the day of super-estrus… **= What do you mean by correspondingly? In correspondence to what?**

**Line 184:** Non-descriptive heifer **= I suggest replacing the term "non-descriptive" for crossbred or undefined genotype. Check this in all the manuscript.**

**Line 184:** first cows **= Do you mean primiparous cows?**

**Line 186:** a good quality CL **= What do you mean by good quality? Any particular size?**

**Lines 187-188:** Thirty embryos (15 from HF and 15 from XB) from each group **= All embryos were from different donors?**

**Line 190:** pregnancy was checked on the 45th day after estrus **= day 45 after estrus or after ET? How did you check pregnancy, by transrectal palpation or US?**

**Lines 191-197: I suggest including a section for "Study variables" in which you explain clearly the variables that were evaluated, apart from the statistical tests used. Then, in the section of Statistical analysis, indicate the statistical tests used for each of the variables. It would be clearer this way, because, for example, you haven't mentioned what you evaluated as "embryo fertility" and why you analyzed it through Chi-square (fertile? non-fertile?).**

**Results**

**Line 205:** The total structures recovered **= What structures did you include in the total? Degenerated embryos, unfertilized ova, or only quality 1 to 3 embryos?**

**Lines 205-209: The statistical significance must be included (P<0.05, P>0.05).**

**Lines 216-219: Same comment as above.**

**The title of Fig. 3 is not representative of the figure itself, only of part of it. Please check.**

**Line 228:** Overall fertility percentage of embryo transfer **= Do you mean pregnancy rate? What were the results for each group?**

**The captions for the figures and table are not clear; please revise.**

**Discussion**

**Lines 234-235:** Enhanced TEs production is most acknowleged criteria of any experimented bovine superovaltion protocol **= Not clear what you mean; please clarify.**

**Lines 237-238:** inadequate super-stimulatory response of domestic breeds **= by domestic do you mean native breeds?**

**Line 247:** exotic blood **= Do you mean European cattle breeds?**

**Lines 262-264:** …CIDR's application supplemented with GnRH or EB before initiation of super-stimulatory treatment yields a higher number of TEs than conventional super-ovulatory protocols. **= Clarify that conventional SO protocols do not include GnRH or EB to synchronize the follicular wave. Otherwise, it is confusing, because it appears that sometimes you consider CIDR + GnRH or EB as part of the SO protocol.**

**Line 278: The number or transferrable embryos in this study was within the mean number reported worldwide. Please discuss on this.**

**Lines 300-302:** The experimented timeline may be correlated to reduced uncertainty about superovulation results and eliminating the confusion about a suitable time to initiate FSH administration. **= It's not clear what you mean. Please revise.**

**Lines 310-312:** The present study exhibited first heat after 17.33±3.50 days and 16.07±3.36 in CIDR-GnRH and CIDR-EB groups, respectively. Days taken by these groups for RTE were significantly lower than the control group **= How would you explain that? What is the relationship between the hormones used to synchronize the follicular wave and the days to return to estrus?**

**Lines 314-316:** Overall fertility percentage of embryo transfer trial remained 56.67+5.25. On statistical analysis, the fertility of TEs was neither affected significantly by the superovulation protocol used nor by breed differences among donor cows. **= If by embryo fertility you mean pregnancy rate after ET, the mean rate that you obtained was high, compared to most of the studies, where a 50% pregnancy rate is considered as good. How would you explain that?**

**Did you consider the cost of using GnRH or EB prior to administering the SO protocol? What could you say about that, taking into account your results? Please explain.**