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Potential for Producing Viable Embryos in Post Pubertal Heifers. Comparison during the Dry and Wet Seasons in the Tropics Using a Protocol Based on FSH or eCG

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Abstract

The use of pubertal heifers to produce embryos before their natural breeding period which is normally at 3 years is a possibility that needs to be researched thus the objective of this assay was to superovulate pubertal *Bos indicus* heifers to obtain embryos in the dry and wet season, two phases were fodder availability is quite distinct.

Twenty Brahman heifers 18 to 24 months and weighing 300 to 330 kg were synchronized with a progesterone releasing device (CIDR, Pfizer, Mexico) for 9 days and concomitantly applying a superovulatory regimen, using a total of 280 mg of FSH (Folltropin-V Bioniche Mexico) in eight applications with decreasing dosages 12 h apart, starting with two dosages of 50 mg followed by another two of 40, two of 30, and ending with two of 20 mg.

One day before the device was withdrawn; an injection of 25 mg of prostaglandin F2α (Dinoprost Pfizer, México) was administered. For treatment with eCG an injection of 2000 I.U. of Equine Chorionic Gonadotropin eCG (Folligon, Intervet México) was administrated at day 6 after CIDR insertion.

There were differences in the total follicular response between the dry season average n = 84 and the wet, average n = 51 ($p < 0.05$) but not between treatments. The number of good quality embryos recovered in either season was not more than 4. Response in relation to number of corpus luteum (CL) present and number of embryos was affected by season ($p < 0.05$) regardless of the treatment utilized.

The production of embryos in post pubertal heifers is not feasible based in our results. Heat stress, nutritional inconsistencies, season when treatment applied and age might be some of the main driving factors for this poor response.

Keywords: embryo transfer, animal production, zebu cattle, tropics

1. Introduction

The application of embryo transfer (ET) has grown considerably in the cattle industry the last twenty years. In particular, livestock raised under tropical conditions (Baruselli et al., 2010). This biotechnology, if economically feasible, would be an excellent tool for improving milk yield as well as production traits. As it has been reported in several communications, the F1 crossbred animals *(Bos taurus x Bos indicus*) provides genetic advantages in terms of milk production and disease resistance (for reviews see McDowell, 1985; Madalena, 1993). Bó and Mapletoft (2013) in a recent review postulated that although the number of transferable embryos per donor cow superstimulated has not increased, the protocols that are used today have improved the numbers of transferable embryos recovered per unit of time and have facilitated the application of on-farm embryo transfer programs. Nonetheless, in the situation of small farm enterprises, recent economic studies have not been demonstrating a reliable and economically feasible tool (Alarcon et al., 2010; Bolivar & Maldonado, 2008).

A review on different superovulation regimes and various hormones used (Baruselli et al., 2010) a variety of treatments for superovulation have shown exceptional results, for example Souza et al. (2009) have reported that animals receiving 400 IU eCG have greater plasma progesterone concentrations during the subsequent luteal phase. In F1 crossbred recipients, eCG treatment resulted in an increase of CL diameter compared with controls. Treatments based on FSH appear to have a good outcome, for instance, Peixoto et al. (2006) using a large sample of various types of *Bos indicus*, found that the average number of viable embryos ranged from 4.1 to 7.3. In another large Brazilian study, Silva et al. (2009) concluded that management in the farm and the age of the donor, were the most prominent sources of variation.

The question that needs to be addressed is how important is the selection of donor animals. Many of the studies consulted relay on using females not suited for superovulation due to their dubious fertility or aging (Desaulniers et al., 1995; Duby et al., 1996; Kafi & McGowan, 1997). One feasible approach is to superovulate nulliparous heifers from reputable stud farms prior to the time when needed to be bred to meet the standards for age at first calving. In effect, several studies have afforded information that this parameter will be extended up to 35 months of age. This in spite that puberty is reached around 15 months (Abeygunawardena & Dematawewa, 2004).

The objective of the present study is to research on the possibility of utilizing post pubertal *Bos indicus* heifers and to obtain embryos from them prior to their breeding season.

2. Material and Methods

The methods were approved by the Ethical committee for experimentation in animals of the Faculty of Veterinary Medicine, University of México in accordance to The Code of Ethics of the World Medical Association.

Females were located at "El Clarín" farm belonging to the Faculty of Veterinary Medicine, National Autonomous University of México, located in Tlapacoyan, Veracruz. This unit is situated in the central part of Veracruz State at 19°58′N and 97°13′W at an altitude of 151 m above sea level. The climate in this area is hot and humid with a mean annual temperature of 23.4 °C and a mean of 1,840 mm of rain distributed in about 10 months of the year.

A total of twenty Brahman heifers 18 and 24 months and weighing 300 to 330 kg were selected based on their health status and a healthy reproductive tract evaluated by rectal examination and ultrasound to measure follicle size and the presence of a corpus luteum (CL). Animals were super ovulated in the dry season March through May (average temperature 25 °C with a minimum of 13 and a maximum of 35; average rainfall 351 mm and relative humidity of 50%) and again in the wet season July until October (average temperature 25 °C with a minimum of 17 and a maximum of 36; average rainfall 628mm and relative humidity of 59%). A representative sample for age and weight was included in both seasons.

Cows were randomly assigned to either receive a superovulatory treatment based on FSH or eCG. This was followed up by a synchronization protocol consisting of a progesterone releasing device (CIDR, Pfizer, México) for 9 days and at the time of insertion an injection of 2.5 mg of estradiol benzoate. Cows receiving the FSH treatment were subjected to a super ovulatory regimen starting at day 6 of CIDR insertion consisting in a total of 280 mg of FSH (Folltropin-V Bioniche México). The total dose was divided in 8 applications with decreasing dosages 12 hours apart starting with two dosages of 50 mg followed by other two of 40, two of 30, and end with two of 20 mg.

Finally, one day before the device withdrawal at day 8 after starting the treatment, an injection of 25 mg of prostaglandin F2α (Dinoprost, Pfizer, México) was administered. In relation to treatment with eCG an injection of 2000 I.U. of Equine Chorionic Gonadotropin eCG (Folligon, Intervet México) was administrated at day 6 after insertion of the progesterone releasing device.

For both treatments, an ultrasound examination of the reproductive tract was performed to assess the number of follicles with a diameter of 8 mm or larger regardless the number of CL's. Estrus detection was performed continuously for 72 h after CIDR withdrawal according to the method proposed by Orihuela et al. (1983). Heifers not displaying estrus behavior were inseminated at fixed time (72 hours) following a gynecological examination to determine the presence of a turgid uterus and plentiful cervical mucus. Embryo collection was performed 7 days after the first AI using a Foley catheter.

2.1 Statistical Analysis

A student "T" test was used to compare the response to estrus in the wet and dry season. A two way ANOVA with two factors, treatment and time of the year was used for the response following a superovulatory regimen.

3. Results

There were no significant differences in the number of animals displaying overt signs of estrus in the two seasons of the year the values are not significant at $p > 0.05$. In the dry season 14/20 showed estrus for 18/20 in the wet season.

Table 1 contains the individual response of the animals in relation to the number of follicles with at least 8 mm in diameter; the number of corpus luteum formed contains the response of the two treatments in the two seasons tested. The number of follicles bigger than 8 mm was significantly different between seasons ($p < 0.05$), with a greater extent in the dry season. The number of CL's formed was also significantly different favoring the wet season. It is noticeable the amount of CL's developed from presumably follicles smaller than 8 mm. This disparity was more apparent in the wet season utilizing FSH.

Table 1. Number of follicles bigger than 8 mm and CL formation. Averages correspond to 20 heifers per season and treatment

Season	**Follicles ≥ 8mm**	**Average**	**Corpus luteum**	**Average**
Dry	84^a	4.2 ± 2.4	105^a	5.2 ± 1.3
Wet	51^b	2.5 ± 3.7	233^b	11.6 ± 2.8
Treatment	**Follicles ≥ 8mm**	**Average**	**Corpus luteum**	**Average**
FSH	67^a	3.3 ± 3.0	208^a	10.4 ± 10.1
eCG	68^a	3.4 ± 3.3	130^b	6.5 ± 7.4

Note. Different letters indicate a significant difference ($p < 0.05$).

Table 2 contains the total embryo production on the two seasons (dry and wet) and the treatments (FSH and eCG). There were significant differences between seasons and also in treatments ($p< 0.05$).The total of frozen embryos was significantly higher in the wet as compared to the dry season ($p< 0.05$).

Table 2. Total numbers of embryos collected and total of frozen embryos during both seasons (dry, wet) and treatments (FSH, eCG). The averages correspond to 20 heifers per season and treatment

	Total number of embryos (n= 20)		Average frozen embryos (n=20)	
	Total	Average + SD	Total	Average + SD
Dry	11	0.6 ± 1.3^a	3	0.2 ± 0.5^a
Wet	44	2.2 ± 2.8^b	34	1.7 ± 2.4^b
FSH	34	34 ± 2.6^a	16	0.8 ± 1.7^a
eCG	21	21 ± 2.01^b	21	1.0 ± 2.1^a

Note. Different letters in columns indicate a significant difference ($p < 0.05$).

Only two animals responded to the superovulatory treatment in the dry season regardless of the treatment utilized. In contrast in the wet season 5 and 7 animals did not respond. The total of embryos recovered were only three for the eCG treatment in the dry season and 8 for the FSH group. In contrast in the wet season, corresponding values were 18 for the eCG and 26 for the FSH treatments. It is worth noting that the recovery of embryos was not an apparent issue in spite that the animals were all heifers. There were no differences on embryos recovered between treatments.

Table 3 shows the total of embryos which was significantly superior ($p < 0.05$) during the wet season regardless treatment. This finding is in accord to the number of CL's formed as there more present in the wet season as compared to the dry and treatment had no effect on this result.

Table 3. Good quality embryos and corpus luteum on each season (dry, wet), between treatments (FSH, eCG), averages correspond to 10 heifers

Dry	**Total number of embryos**	**Average**	**Total number of CL'S**	**Average**
eCG	3[a]	0.3 ± 0.67	40[a]	4 ± 5.4
FSH	8[a]	0.8 ± 1.75	65[a]	6.5 ± 8.1
Wet	**Total number of embryos**	**Average**	**Total number of CL'S**	**Average**
eCG	18[b]	1.8 ± 2.62	90[b]	9 ± 8.5
FSH	26[b]	2.6 ± 3.06	143[b]	14.3 ± 10.8

Note. Different letters in columns indicate a significant difference ($p < 0.05$).

4. Discussion

The response to the treatment and embryo production was better during the wet season. This could be explained as a consequence of heat stress during the dry season. The production of embryos is often reduced in periods of hot weather, this is due to the reduced superovulatory response and lower embryo quality (Hansen et al., 2001). Our results are similar to Satrapa et al. (2011) who reported that heat stress decreased the rates of blastocyst yield in *Bos indicus* breeds. Nonetheless, heat stress cannot be the only factor influencing the response, as a comparable poor response was obtained in the less hardy wet season.

Nutrition could also have played a very important role in the superovulatory response. In a retrospective study in the USA, Stroud and Hasler (2006) found that donor females grazing in palatable green pastures have a much better response than those fed hay, silage or raised on poor pastures. In the case of animals in the tropics, the dry season is marked by food scarcity which tends to affect the growth of the animal (Maquivar & Galina, 2010). In contrast, during the wet season, the availability of fodder to the heifer still in the process of growing tends to minimize the metabolic status of the animal (Maquivar et al., 2010; Rhodes et al., 1995). In spite that most of the females responded to treatment forming CL's one cannot rule out the possibility (Fajersson et al., 1991) that although heifers have reached puberty defined as the presence of a CL, there might be a possible interaction between age and adult weight involved in the maturation of follicles or the formation of CL's that do not support a viable embryo.

The numbers of follicles ≥ 8 were similar between the two treatments but superovulatory responses (CL's) were considerable more using FSH in the wet season. In most studies, Rouillier et al. (1996), Goodhand et al. (1999), and Roover et al. (2005) comparing the two procedures, the FSH treatment has resulted in a better response and a higher number of usable embryos. Lower superovulatory responses with eCG may be associated with its relatively long circulating half-life, resulting in excessive follicular development and its failure to ovulate (Siddiqui et al., 2002). Bo et al. (2006) found that delaying the removal of a progestagen releasing device, combined with the administration of GnRH or porcine LH (pLH) 12 or 24 h latter, results in predictable synchronous ovulations, permitting fixed-time AI without reducing the numbers or quality of embryos.

The number of quality frozen embryos decreased during the dry season and as well, heat stress could be one of the major reasons. In a recent study Silva et al. (2013) observed that heat stress increased the percentage of apoptotic blastomeres in embryos cultured at 41 °C relative to embryos cultured at 38 °C, this might be a factor that affects the embryo grading during the morphological evaluation in which qualities 1 and 2 are viable to be frozen (Hasler, 2001). In a recent retrospective study, Nasser et al. (2004) reported that season affected conception rate following the transfer of fresh *in vitro*-produced embryos in *B. taurus* × *B. indicus* beef recipients. Lower pregnancy rates were observed during the autumn and winter compared with the spring and summer (41.1% (448/1090) *v.* 48.1% (1760/3658), respectively). These results are probably related to the dry weather and/or the lower availability/quality of forage during the autumn/winter period. In addition, the average rate of pregnancy loss (between 30 and 60 days) was 11.7% (258/2208) across all seasons. In accord, Slenning and Wheeler (1989) using a simulation model based on 115 published papers in bovine embryo transfer concluded that PMSG treatment averaged more pregnancies per flush than FSH treatment (4.4 vs 3.9) but showed greater variation in response (64 vs 51%). Decision analysis suggests that a PMSG-induced flush would net more than an FSH-induced flush, and that either superovulation strategy would yield approximately 10 times the net *income* of a non-superovulated flush.

Torres-Junior et al. (2008) found that exposure of Gyr cows to heat stress had no immediate effect on reproductive function, but exerted a delayed deleterious effect on ovarian follicular growth, hormone

concentrations, and oocyte competence. Heat-stress (HS) increased the diameter of the first and second largest follicles from days 28 to 49. Indeed, HS increased the number of > 9 mm follicles (characterized as follicular codominance) during this phase. Zeron et al. (2001), results may explain the differences in the ability of oocytes to develop to the blastocyst stage at different seasons. Thus, temperature may lead to changes in membrane properties, which, in turn, can influence oocyte functional fertility. Similarly, Bastidas and Randel (1987) in an old retrospective study involving 1841 records in 813 Brahman cows found that the number of blastocysts recovered per donor was lower (2.0) during the winter season than during the fall season (3.1). These data establish the concept of seasonal effects on embryo donor reproductive performance in Brahman cows similar to the results in the present study in heifers. The variability in the results of the studies reviewed tend to suggest an interaction between, nutrition, weight and age that is not clearly expressed based on the conditions expressed in the material and methods.

Based in our results, it is possible to conclude that the production of embryo in post pubertal heifers is not feasible under the conditions of this experiment. Heat stress, age and maturity and nutritional inconsistencies might be four of the main driving factors for this poor response and research on this topic is important in other to commence the economical input of heifers in the enterprise as soon as possible.

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Conflict of Interest

All authors declare that there are no actual or potential conflicts of interest between the authors and other people or organizations that could inappropriately bias their work.

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