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Responses of Holstein and Boran X Holstein Crossbred Cows to Super-ovulatory Hormones used for *In vivo* Embryo Production

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የቦስ ኢንዲከስ እና ቦስ ታውረስ የተባሉት የከብቶች ዝርያ ወይም ዲቃሎቻቸው በአንድ ጊዜ በዛ ያለ ዕንቁላሎችን እንዲያኮርቱ ለማድረግ ለሚሰጣቸው የሆርሞን ህክምና የተለያየ ምላሽ መስጠታቸው መሰረታዊ የሥነ-ተዋልዶ ባህሪይ ልዩነት እንዳላቸው ጥናቶች ያመለክታሉ፡፡ በመሆኑም በፅንስ የማምረት ሂደት ውስጥ ተጨማሪ ዕንቁላሎችን እንዲያኮርቱ ለማድረግ ተመሳሳይ የአሰራር ሥልት መጠቀም የማይቻል መሆኑን ያሳያል፡፡ ስለዚህ የዚህ ጥናት ዋና ዓላማ ለቦስ ታወረስ የተመረጠ የአሰራር ሥልትን የሆሊስቲን ዲቃላ ለሆኑት የቦረና ላሞች ላይ በቀጥታ ከመጠቀም ይልቅ ምላሻቸውን በመመርኮዝ የአሰራር ሥልት መረጣ ለማድረግ የተደረገ ጥናት ነው፡፡ ይህንን ጥናት ለማድረግ ከ3 - 8 ዓመት ዕድሜ ያላቸውን 36 የቦረናና ሆሊሲቲን ዲቃላ ላሞችና ንፁህ ሆሊስቲን ላሞች ለሙከራው ተጠቅመናል፡፡ በሙከራው ጅምር ላይ ለ7 ቀናት የሚቆይ በብልታቸው ውስጥ ፕሮጀስትሮን ሆርሞን የተነከረ ሲ.ዳ.ር. የተቀመጥ ሲሆን ላሞቹን በሦስት ቡድን ተከፍለው 500፣ 650 እና 800 አይ.ዩ. ፕሉሴት (የኤፍ.ኤስ.ኤች. እና ኤል ኤች ሆርሞን ድብልቅ) ከ4ኛ ቀን እስከ 7ኛ ቀን በ12 ሰዓታት ልዩነት ጧትና ማታ ተሰጥቷቸዋል፡፡ በ7ኛ ቀን ከብልታቸው ውስጥ የተቀመጠው ሲ.ዳ.ር. ሲወጣላቸው ኢስትሩሜት ሆርሞን በመውጋት ለ72 ሰዓታት የኮርማ ፍላጎት እንደሚያሳዩ ለማረጋገጥ ክትትል ተደርጓል፡፡ በመቀጠልም ተጨማሪ ዕንቁላል ማኮረታቸውን ለማረጋገጥ አድገው ነግር ግን ያልተለቀቁ ዕንቁላሎችና የተለቀቁ ዕንቁላሎች ብዛት በአልትራሳዎንድ መሳሪያ ተቆጥሯል፡፡ ያልተፀነሱ ዕንቁላሎችና የተፈጠሩ ፅንሶች ተሰብስበው ተቆጥረው ተመዝግቧል፡፡ ተጨማሪ ዕንቁላል እንዲያኮርቱ ሆርሞን ከተወጉ ፅንስ ሰጪ ላሞች ውስጥ ከንፁህ ሆሊስቲን (8.67± 3.06) ይልቅ የቦረና እና የሆሊስቲን ዲቃሎች (15.45 ± 0.5) በቁጥር ከፍ ያለ ዕንቁላል መልቀቃቸውን የሚያሳዩ ምልክቶች እንዳላቸው ታይቷል፡፡ የተሰበሰቡት ፅንሶች ወይም ያልተፀነሱ ዕንቁላሎችን ቁጥር ሲታይም የቦርና ዲቃሎች ከንፁህ ሆሊስቲን የበለጠ በዛ ያለ ቁጥር የሰጡ መሆኑ ታይቷል፡፡ በጣም የጎላ ልዩነት ባይኖርም ከሦስቱ የፕሉሴት ዶዝ መጠን ውስጥ የ650 አይ.ዩ. የወሰዱት እንስሳት የተሻለ የፅንስ ቁጥር እና የተለቀቁ ዕንቁላሎች ብዛት አስገኝቷል፡፡ ከአምስቱ የአካል ሁኔታ ምዘና መስፈርት (ከ2.5 እስከ 5 መካከል ያሉት ተወስደው) በ3.5 እና 3.8 ነጥብ መካከል ያሉት ላሞች ከፍተኛ ቁጥር ያላቸው ዕንቁላሎችን (15.74 ± 0.59፤ 14.33 ± 2.90 በቅደም ተከተል) የሰጡ ሲሆን በ3.8 ነጥብ ላይ ያሉት በቁጥር የተሻለ ዕንቁላል/ፅንስ ሰጥተዋል፡፡ ከዚህ በመነሳት የቦረና እና የሆሊስቲን ዲቃላ ላሞች ተጨማሪ ዕንቁላል እንዲያኮርቱ ለተሰጣቸው የሆርሞን ህክምና ለአነስተኛ ዶዝ የተሻለ ምላሽ የሚሰጡ መሆኑንና ባለ 650 አይ.ዩ. ዶዝ ሆርሞን በሁሉም መስፈርት በቦረና እና ሆሊስቲን ዲቃላ ላሞች ላይ የተሻለ ውጤታማ መሆኑ ተረጋግጧል፡፡

Abstract

Variability in the response to super-ovulatory treatments in Bos indicus, Bos taurus, and their crosses was reported to be a fundamental biological difference in reproductive function between them and has been a major barrier to the adoption of similar superovulation protocols. Therefore, this study was designed to optimize the super-ovulatory protocol for the Boran x Holstein crossbred cows. A total of 36 Boran x Holstein crossbred and pure Holstein cows 3-8 years of age were utilized for this study. A 7-day CIDR treatment was initiated on Day 0, and cows were treated on Days 4-7 with 500, 650, or 800 IU of Pluset® (combined FSH and LH *product) administered at 12-hour intervals in a decreasing dose regimen. Estrumet (Prostaglandin F2α analogue) was given on day 7 before CIDR withdrawal. Each super-ovulated cow was monitored for 72 hours to assess behavioral estrus in response to super-ovulatory treatment. Ovarian response was also assessed by counting the number of corpora lutea and number of un-ovulated follicles via transrectal ultrasound scanning, the number of total collections, and the number of viable embryos. From the super-ovulated donor cows, Boran X Holstein crosses had a higher (P<0.009) number of corpora lutea (15.45 ± 0.5) than pure Holstein (8.67± 3.06). The number of collected embryos/oocytes and the number of viable embryos collected was significantly higher for crossbred cows (p<0.01, p=0.06) than the pure Holstein cows. Although it was not statistically significant, from the three Pluset® (combined FSH and LH product) dose levels the 650 IU dose have produced a higher response in terms of corpora lutea number and number of collected viable embryos. From the five-scale body condition scoring (Recorded 2.5 to 5) cows with 3.5 and 3.8 scales have produced a higher number of corpora lutea (15.74 ± 0.59; 14.33 ± 2.90, respectively), while those with 3.8 scores had the highest total number of collections (6.33 ± 2.60). From the findings, it can be concluded that Boran X Holstein crossbred cows have a better ovarian response to lower doses of exogenous gonadotropins than pure Holstein cows. The 650IU Pluset® dose level is the optimal level for superovulation of crossbreeds.*

Keywords: Crossbreed, Pluset® dose, in vivo embryo production, pure Holstein, Superovulation

Introduction

The development of biotechnology tools in assisted animal reproduction (ART) become the most emblematic technological advance in livestock development, global genetic exchange, and conservation. The 1st and 2nd generations of ART (AI, gamete freezing, and MOET) had a major impact on livestock improvement in the developed world through speeding up genetic progress and expanding the number of animals that can be bred from the superior parent (FAO, 2007; Foote, 2002). Accordingly, the reward of these technologies enabled the utilization of the immense reproductive potential of female calf exploited by natural breeding.

There are reports which indicated that there is a greater sensitivity of *Bos indicus* to gonadotropins than in *Bos taur*us cattle, which show fundamental biological differences in reproductive function between them (Sartori *et al.,* 2010). As a result, variability in the response to super-ovulatory treatments has been a major barrier to the adoption of similar superovulation protocols. For instances, the dose of exogenous FSH required for superovulation of *Bos indicus* cattle was reported to be less than (25-30%) in *Bos taurus* cattle (Mikkola et al., 2019; Tamrat *et al.,* 2018; Hussein et al., 2017; Gaddis et al., 2017; Mapletoft and Bo, 2015). And hence, the variability problem with the response to super-ovulation treatments for *in vivo* embryos production remains a challenge (Tinda *et al.,* 2020; David *et al.,* 2019; Tamrat *et al.,* 2018; Mapletoft and Bo, 2015). Similar problems are believed to exist among Ethiopian cattle breeds, and hence, adopting and verifying different protocols that alleviate the problem and optimize the process for the local breeds would have paramount significance. Hence, insight into physiological differences between *Bos taurus* and *Bos indicus* breeds that have not yet been extensively studied would be useful for the development of specific superovulation protocols. Therefore, this study was undertaken to assess the superovulation response on different Pluset® (combined FSH and LH product) dose levels on Boran X Holstein crossbreed and pure Holstein cows kept under commercial farms in and around Bishoftu town, Ethiopia.

Material and Methods

Study animal

The study was conducted at Debre Zeit Agricultural Research Center (DZARC) in collaboration with the voluntary private commercial dairy farms in the vicinity of Bishoftu town. Twenty-eight Boran X Holstein crossbred cows from on station herds in Bishoftu Agricultural Research Center and eight pure Holstein cows from Av-Nordeli Dairy Farm were super-ovulated and used as embryo donors were fed with grass hay, green alfalfa fodder, and commercially prepared concentrate supplement at a rate of 3% body weight, had *adlib* water and kept in the door throughout the experimental period. All cows were selected on the history of best performance in terms of production, cycling, temperament, ease of flushing and good body condition score, and freedom from any gynecological problems.

Super-ovulatory treatment

The 36 cows (in the 1st and 2nd parity) from BiARC and AV-Nordeli farms were grouped into three dosage rates of Pluset® (combined FSH and LH – Chlorocresol 0.021g, LABORATORIOS CALIER, S.A) for the response evaluation in Boran X Holstein cross: Treatment I = 500 IU, Treatment II = 650 IU and Treatment III = 800 IU Pluset®. Intravaginal Controlled Internal Drug Released (CIDR, AEZI BREED CIDR® impregnated with 1.38g progesterone, DEC International, NZ, Ltd.) was inserted in all selected donors on day zero. A decreasing dose of Pluset® was injected "bid" at intervals of 12 hours from day four to day 7 of CIDR insertion and 2 ml Estrumate® (Cloprostenol sodium 263µg Intervet, South Africa (Pty) Ltd.)" was injected IM on day six after CIDR insertion. CIDR was removed from all donors on day 7 at the time of injecting the last shot of Pluset®.

Insemination and data collection

Cows/heifers showed behavioral signs of estrus (standing heat) were inseminated twice (in the morning and afternoon) by the researcher to capture multiple follicles ovulation in response to superovulation. The ovarian response (number of ovulated follicles in terms of CL and un-ovulated follicles) was examined and recorded by ultrasonography (intra-rectal 7.5- MHz linear probe; Digital Veterinary Diagnostic Imaging CTS- 3300V, VOLT: 220v, FREQ: 50HZ, SIUI) on day seven at the time of flushing. Embryo flushing with a non-surgical gravitational procedure by Y-tubing and foley catheter using commercial flushing media (BoviFlush recovery medium for bovine embryos, Minitube GmbH, Germany) was done on the 7th day after the second insemination. Collected embryos were evaluated under a stereomicroscope (Motis SMZ 140/143®) at magnification 10X - 40X) for developmental stage (from stage 1 = 1-cell to stage 9 = expanded hatched blastocyst) and quality (from quality 1 = excellent to quality 4 = degenerate) (Bó and Mapletoft, 2013, and Jahnke *et al.* 2015).

Data analysis

Descriptive statistics and univariate analysis of the SPSS were used to analyze the genotype response to Pluset® (combined FSH and LH) treatments. Frequency distribution, chi-square test, and one-way ANOVA were used to analyze the data for the rate of embryo recovery and results of laboratory evaluation. The level of significance was held at P<0.05.

Result and Discussion

Statistically significant difference was observed between pure Holstein and Boran X Holstein crosses on the number of ultrasonically counted CL (p<0.000) and total collection (embryos and unfertilized oocytes; p<0.009). Boran X Holstein crosses had a higher number of CL (15.5 ± 0.49 Vs 8.67 ± 3.06; Table 1), total collection of embryos, and/or unfertilized ovum (4.3 ± 0.59 Vs 0.5 ± 0.34). The crossbreeds had also a higher number of transferable (quality grade 1-3) embryos (2.6 ± 0.52 Vs 0.33 ± 0.33). Previous reports indicated that the dose and genotype effects on response to superovulation, resultant number of ovulations, and number of transferrable quality embryos recovered which is in line with current finding (Tinda *et al.*,2020; David *et al.*, 2019; Mapletoft and Bo,, 2015). A higher number of un-ovulated follicles (>11 mm diameter) were counted in both breeds of cows (Boran X Holstein crosses= 15.49 ± 1.59; Holstein = 13.83 ± 4.51). However, the difference between the two breeds was not statistically significant.

The difference in the number of CL, un-ovulated follicles, total collections, and transferable embryos flushed were not statistically significant between the three Pluset® dose levels. However, the 500 IU and 650 IU doses produced a greater number of unfertilized oocytes (16.6 ± 2.12 and 16.29 ± 2.28, respectively) than the 800 IU dose. The number of transferable embryos was higher for the 650IU Pluset® dose (2.69 ± 0.76). This finding goes in line with the previous report from the DZARC farm that stated Boran cows had a greater ovarian response to lower doses of superovulation than Boran X Holstein crossbred cows (Tamrat *et al.*, 2016) which indicates the contribution of Boran genetics on the Boran X Holstein crosses to respond to lower doses of Pluset® when compared with Holstein. The current finding confirmed that similarly Boran X Holstein crossbreed cows responded better to lower Pluset® doses (650 IU) when compared to the pure Holstein cows treated with similar doses of Pluset®.

Table 1. Mean number of CL, Un-ovulated follicles (UOF), total collection (Oocytes/embryos) and viable embryos produced from Holstein and Boran*Holstein breed (Mean ± SE)

Measured variables	Breed	Mean (SE)	F	P-value
CL	Holstein	8.67 ± 3.062	16.107	.000
	Crossbreds with Boran	15.50 ± 0.49		
UOF	Holstein	0.17 ± 0.167	.165	.687
	Crossbreds with Boran	1.60 ± 0.42		
Unovulated follicles	Holstein	13.83 ± 4.51	7.773	.009
	Boran X Holstein crosses	15.49 ± 1.60		
Total collection	Holstein	0.50 ± 0.34	7.773	.009
	Crossbreds with Boran	4.30 ± 0.60		
Viable embryo	Holstein	0.33 ± 0.33	3.601	.066
	Crossbreds with Boran	2.60 ± 0.52		

The body condition score recorded for all cows included in the study were ranged between three and four on the five scale. From the four-body condition scored in the range of 3 to 4 on the five scale cows with BCS 3 had produced a higher number of total collections, un-ovulated follicles (17.29 ± 3.3) and transferable embryo (4.14 ± 1.57) whereas BCS of 3.5 and 3.8 had a higher number of CL (15.74 ± 0.39 and 14.33 ± 2.9, respectively; Fig 1).

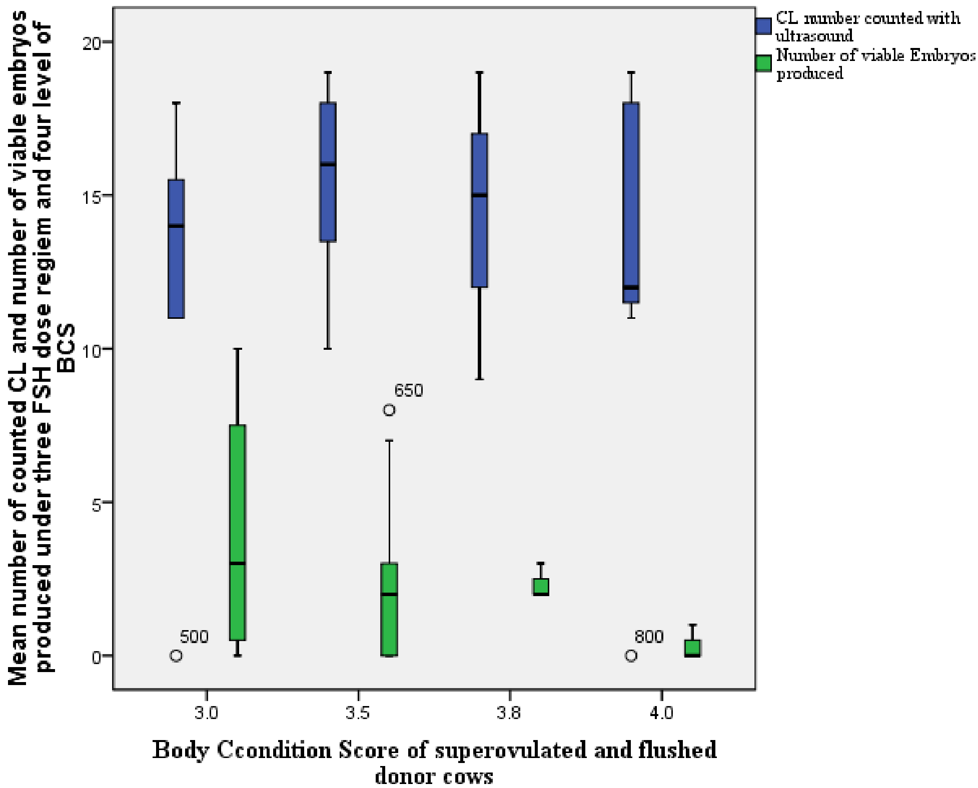

Fig 1. Response of cows with different BCS in terms of mean total number of CL and viable embryos produced under three FSH dose levels

The previous findings on purebred Boran and the current finding on crossbred Boran*Holstein cows are in strong agreement with the ample biological evidence that stated zebu cattle (*Bos indicus*) and their crosses (Boran X Holstein) required less exogenous FSH than pure *Bos taurus* breeds to achieve optimal super ovulatory response in terms of the number of ovulated as well as flushed embryos (Mikkola et al., 2019; Gaddis et al., 2017; Tamrat et al., 2016; Sartori et al., 2010).

Conclusion

The increase in knowledge of the physiological differences between *Bos taurus* and *Bos indicus* breeds, and within the tropical zebu breeds is useful to develop specific protocols or strategies for reproductive management to maximize production in different breeds of cattle raised in a tropical environment. It was repeatedly reported that *Bos indicus* breeds have a reduced capacity for LH secretion and a greater sensitivity to exogenous gonadotropins than *Bos taurus* cattle. Therefore, the 650 IU FSH dose level is the optimal level for superovulation in Boran X Holstein crosses. And hence, further research is needed for refinement of the doses of super-ovulatory agents.

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