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Variations in Responses to the Superovulatory Hormone Doses using Crossbred HF Dairy Cows in Ethiopia

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ABSTRACT

Response to the superovulatory hormone treatments varied for the type of breed and doses of superovulatory hormone used. Ovarian response of 42 crossbred HF (50%, 75%) and pure dairy cows were evaluated based on ultrasonographic count of CL and embryo recovery for quality assessment. Ovarian response rate was significantly higher (P<0.05) in the crossbreds than in the pure bred cows. The maximum response achieved (CL count, mean ± SE) was 14.6±1.1 and the least with 11.2±1.1, 75% HF at 650IU and pure HF at 800IU, respectively. Significantly higher (p<0.05) number of UFO were collected at a dose of 500 IU while transferable embryos were higher at 650IU in both the 50% HF and 75% HF cows. Pure bred cows responded fairly with more CL and produced higher number of transferable embryos at a dose of 800 IU FSH. In conclusion, the optimal dose for maximum response (CL count) exhibited in cross breed HF cows is 650 IU.

***Keywords*:** CL count, dairy cows, embryo quality, FSH dose, ovarian response

1. INTRODUCTION

Different kinds of superovulation protocols have been reported in multiple ovulatory embryo transfer techniques (MOET) [1-3]. But the results of these superovulatory protocols

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were highly characterized by variations in their outcome [1]. Beside to that, results of superovulatory treatments were inconsistent in different dairy breed under different production systems [3, 4]. These differences are believed to be due to the difference in biological response of the animals and the nature of the hormones used beside to other intrinsic and extrinsic factors [2, 4].

The differences of outcomes in using superovulatory treatments reported as a challenge to hinder the enhancement of outcome of in vivo embryo production [5]. Different dairy breeds respond differently for different FSH dose treatments stated as factors affecting effectiveness of the protocols [3, 6, 7]. Even to the extent, the dose of FSH to be used for a donor cow also varies according to the breed of the donors, age and/ or parity as well as body condition [7]. For instance, heifers generally receive a lower dose of FSH for superovulation than do mature cows. Due to the variations of response of donors, testing and optimizing the best responsive doses of FSH hormone in the targeted breed is critical during any superovulatory procedures intended to maximize the number of preovulatory follicles. This will help to upgrade the outcome of the superovulatory procedures [4, 8].

Furthermore, Bos indicus breeds have lower threshold of response to the superovulatory treatments compared with Bos taurus breed though it is not well characterized [8]. This implies that cross breed of this family have also different responses that believed to be different from taurus. The misunderstanding of dose application has a drawback effect in embryo production by affecting the biological mechanism of fertilization [7, 8]. When administering booster dose of superovulatory hormone it will be reason for poor response by inhibits release of FSH from anterior pituitary, and increasing LH concentration which luteinize follicles and reason for premature ovulation of the dominant follicle [8, 9].

Few attempt of embryo production have been conducted in Ethiopia as well in east African countries [10-12]. Recent study carried out by [12] in dose optimization for Boran breed has shown that the best ovarian response was seen comparatively at lower dose of FSH (250 IU). While proper FSH dose-response information for cross breed dairy cows in MOET procedure is still not established and well documented under tropical environment. The objective of this study was to determine the optimal dose of FSH used for superovulatoy procedure in cross bred HF donor cows.

2. MATERIAL AND METHODS

2. 1. Study animals

A total of 42 dairy cows were categorized in to three groups based on the blood level as Holstein Frisian (Pure HF) (n=14), 75% cross HF*Boran (n=13) and 50% HF*Boran cross dairy cows (n=15) and also each group further categorized into three different treatment groups as 500 IU, 650 IU and 800 IU.

All selected dairy cows were from parity one to three and have been checked for their gynecological soundness using ultrasound through rectum. Controlled Internal Drug Release (CIDR) inserted (Progesterone 1.38 gm, Hamilton, New Zealand) for 7 days. On day 4, Follicular Stimulating Hormone (FSH) (Pluset®, Spain) injected intra muscular at decreasing rate for four consecutive days [13]. On day 6, all cows injected 2 ml PGF2α (Estrumate®, France) for estrus induction. One liter of lactated ringer solution was used to flush both uterine horns having 0.1% fetal calf serum [14].

All dairy cows selected were non-lactating and provided with grass, concentrate feed, ad-libitum water during the experiment. BCS scale used was 1 to 5 and all of the herd in the research station was classified and rated as 1-Poor, 2-Fair, 3-Good, 4-Very Good, and 5-Fat based on [15] while BCS of rate 1 and 5 were excluded from this experiment.

2. 2. Experimental design

A factorial of factor effect combinations used to construct the study designs [16]. The factor effect test was done based on the blood level of donors and three FSH dose levels (Table 1). Based on this, three-way comparisons of blood level of donors and the three levels of superovulatory doses were assigned to treat study animals and evaluated the superovulatory response based on the following parameters presented in Table 2.

Table 1. The three-way combination of treatment groups

Treatment groups	500 IU	650 IU	800 IU
50% HF	No. of cows assigned	No. of cows assigned	No. of cows assigned
75% HF	No. of cows assigned	No. of cows assigned	No. of cows assigned
Pure HF	No. of cows assigned	No. of cows assigned	No. of cows assigned

Table 2. Parameters considered for superovulatory response

<table>
<tr><td colspan="4">Levels of Parameters</td></tr>
<tr><td>Breed*</td><td colspan="3">Dose</td></tr>
<tr><td>50% HF</td><td>500 IU</td><td>650 IU</td><td>800 IU</td></tr>
<tr><td>75% HF</td><td rowspan="2">POF, CL, Embryo recovered</td><td rowspan="2">POF, CL, Embryo recovered</td><td rowspan="2">POF, CL, Embryo recovered</td></tr>
<tr><td>>87% HF</td></tr>
</table>

POF (Preovulatory follicle) **= CL+ PUF (**Post unovulated follicles)

This experiment was done by the following superovulatory procedure (**Figure 1**) conducted according to the presented diagram.

Criteria's used for categorizing range of responses were: CL<5 as Poor response; CL between 5 to 10 as Fair response; CL between 11 to 14 as Good response; and CL ≥15 as Excellent response.

$$\text{Rates of responses} = \frac{\text{Number of cows responded}}{\text{Total number of cows treated}} \times 100\%$$

Source: Modified from [11] and [12]

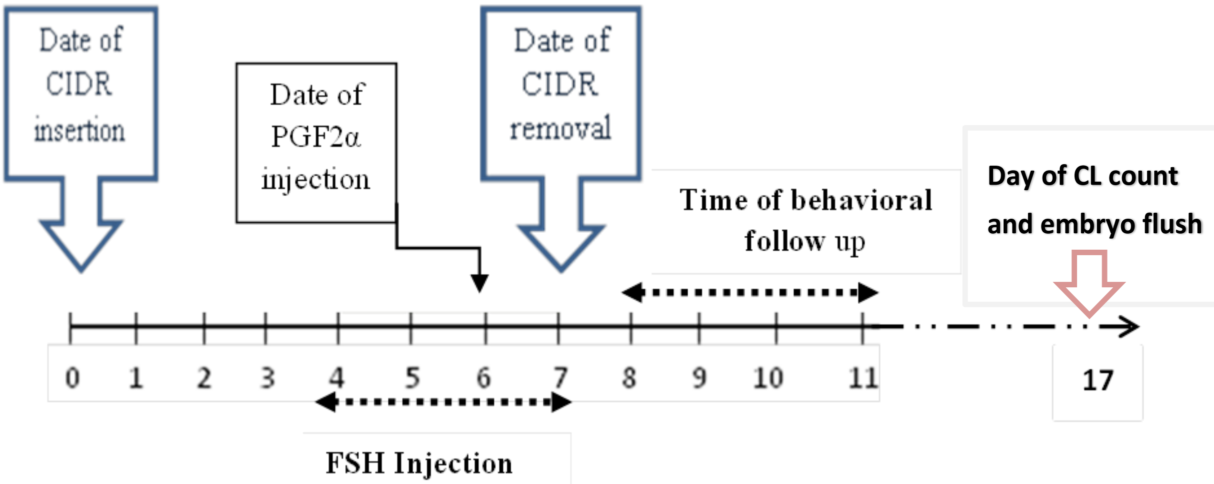

Figure 1. Diagrammatic presentation of hormone application and schedule of embryo recovery

Quality of embryos were graded based on IETS guideline. Superovulatory response was evaluated based on an ultrasonic count of the corpus luteum (CL) and POF [POF= CL + PUF (post unovulatory follicles)]. The embryos were graded for their quality (from Grade 1 = excellent to Grade 4 = degenerate/poor) according to the standards given by the International Embryo Transfer Society as described and illustrated by [17]. Transferable embryos were categorized as grades (G) 1, 2, and 3 and UFO were all unfertilized embryos.

2. 3. Statistical analysis

All dependent variables of the superovulatory response were tested for their normality and Zero-skewness log transform technique used to transform skewed variables, and tested for their significance effect and interaction terms using STATA® version 14.0. GLM was run to test differences between variables (blood level of dam and dose differences). The effect of the dam and dose was computed by Three-way factorial ANOVA with Duncan's pairwise mean comparison test and significance determination of the model was done at 95%.

3. RESULTS

Data of superovulatory response (POF and CL) meticulously scanned for counting, and total recoveries (UFO/ embryos, UFOs, degenerated and transferable embryos) were conducted to all superovulated cows (n=42). The descriptive statistics of the superovulatory response based on blood levels of donor HF dairy cows (n=42) were tabulated as described in **Table 3** below.

Table 3. The overall superovulatory response of donor cows based on the blood levels (Mean ±SE)

Dam blood level	Parameters	Mean±[SE]	Max	Range
50% HF Cross	POF	13.2±0.7	18	9
	CL	11.2±0.9	16	12
75% HF Cross	POF	13.4±0.9	18	10
	CL	11.7±1.1	17	12
Pure HF	POF	12.1±0.6	16	9
	CL	10.5±0.8	15	11

Ovarian sizes of the donors were larger for higher doses with fewer CL than the lower doses in cross breeds. Regardless of the dose FSH applied, the overall response rates of the donors were analyzed to see status of the response variations under the different blood level for the donors. Response rates were calculated for individual blood level based on submitted dairy cows for the experiment **Table 4**.

Table 4. Variations of the superovulatory response rate of donor cows based on the blood levels

Response parameters	50% HF	75% HF	Pure HF
N	14	13	15
Poor response (%)	7.14 (n=1)	15.4 (n=2)	13.3 (n=2)
Fair response (%)	21.43 (n=3)	23.0 (n=3)	46.7 (n=7)
Good response (%)	50.0 (n=7)	38.5 (n=5)	33.3 (n=5)
Excellent response (%)	21.43 (n=3)	23.1 (n=3)	6.7 (n=1)
n = number of cows			

The three-way Anova comparisons of superovulatory response of the categories donor cows (for POF and CL) have shown significant variations (P<0.05) for the FSH dose administered, as presented in **Table 5**.

Table 5. Effect of blood level of donors and dose variation in response to CL and POF (Mean ± SE).

Donor	Dose (IU)	POF	CL	PUF
50% HF		13.0 ± 1.0	11.7 ± 1.3	1.2 ± 0.5
75% HF	500	12.5 ± 1.0	11.0 ± 1.3	1.5 ± 0.5
Pure HF		10.2 ± 0.8	8.5 ± 1.0	1.7 ± 0.4
50% HF		15.2 ± 0.9**	13.6 ± 1.1*	1.6 ± 0.4
75% HF	650	16.0 ± 0.9**	14.6 ± 1.1*	1.4 ± 0.4
Pure HF		14.5 ± 1.0 *	13.2 ± 1.3*	1.2 ± 0.5
50% HF		11.4 ± 0.9	8.4 ± 1.1	1.8 ± 0.4
75% HF	800	10.2 ± 1.0	7.7 ± 1.3	1.5 ± 0.5
Pure HF		12.6 ± 0.9	11.2 ± 1.1	1.4 ± 0.4

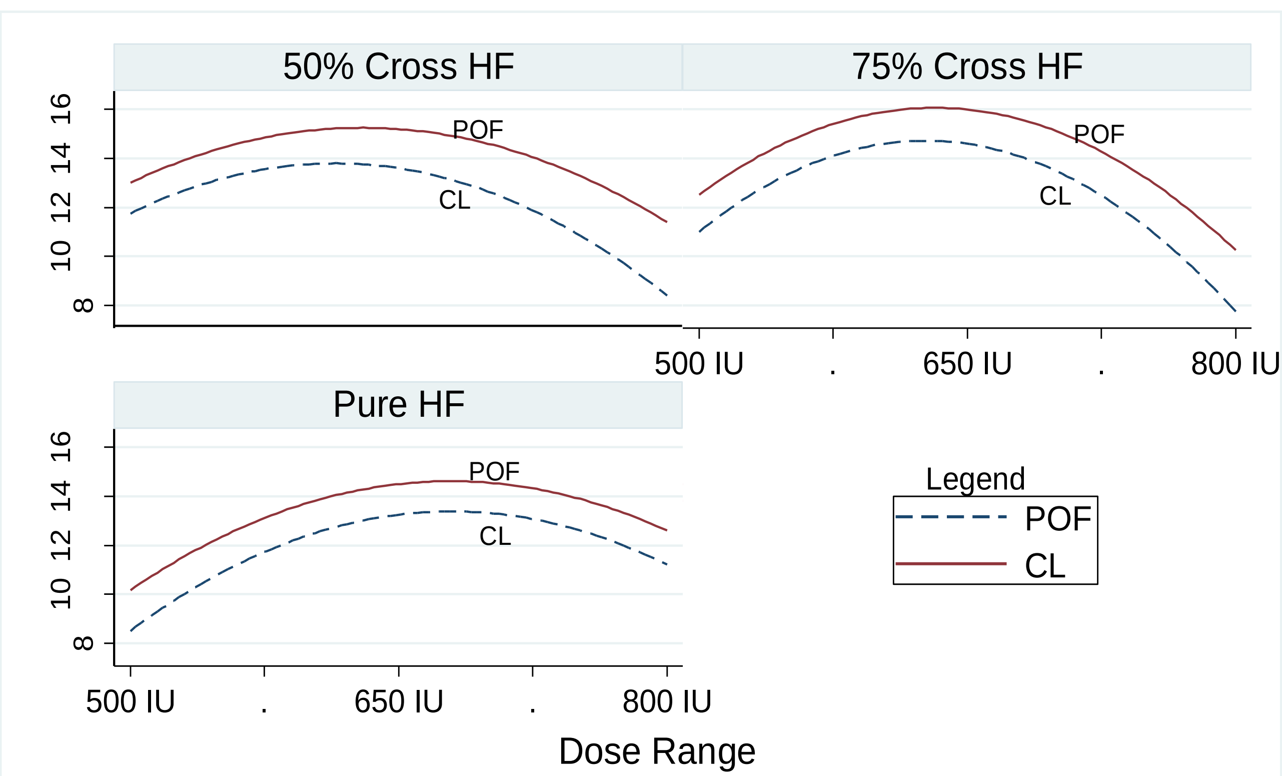

Figure 2. The combined plotting of the different superovulatory hormone dose response for mean of CL counts.

Superovulatory response was plotted with combined linear prediction and trajectory of the fitted plot to show that the response of both cross breed increasing for the dose 500 IU and picked for the dose 650 IU. Unfortunately, the lowest record in response was at 800 IU. Differently pure HF has shown the lowest response for 500 IU and increasing as dose increased, as shown in **Figure 3**.

The performance of embryo production for both breed and dose difference was evaluated and total embryo recovery result was confounded with both cross breed effect and dose. Hence, degenerated embryos and transferable embryos were used as a comparison for dose effect, as shown in.

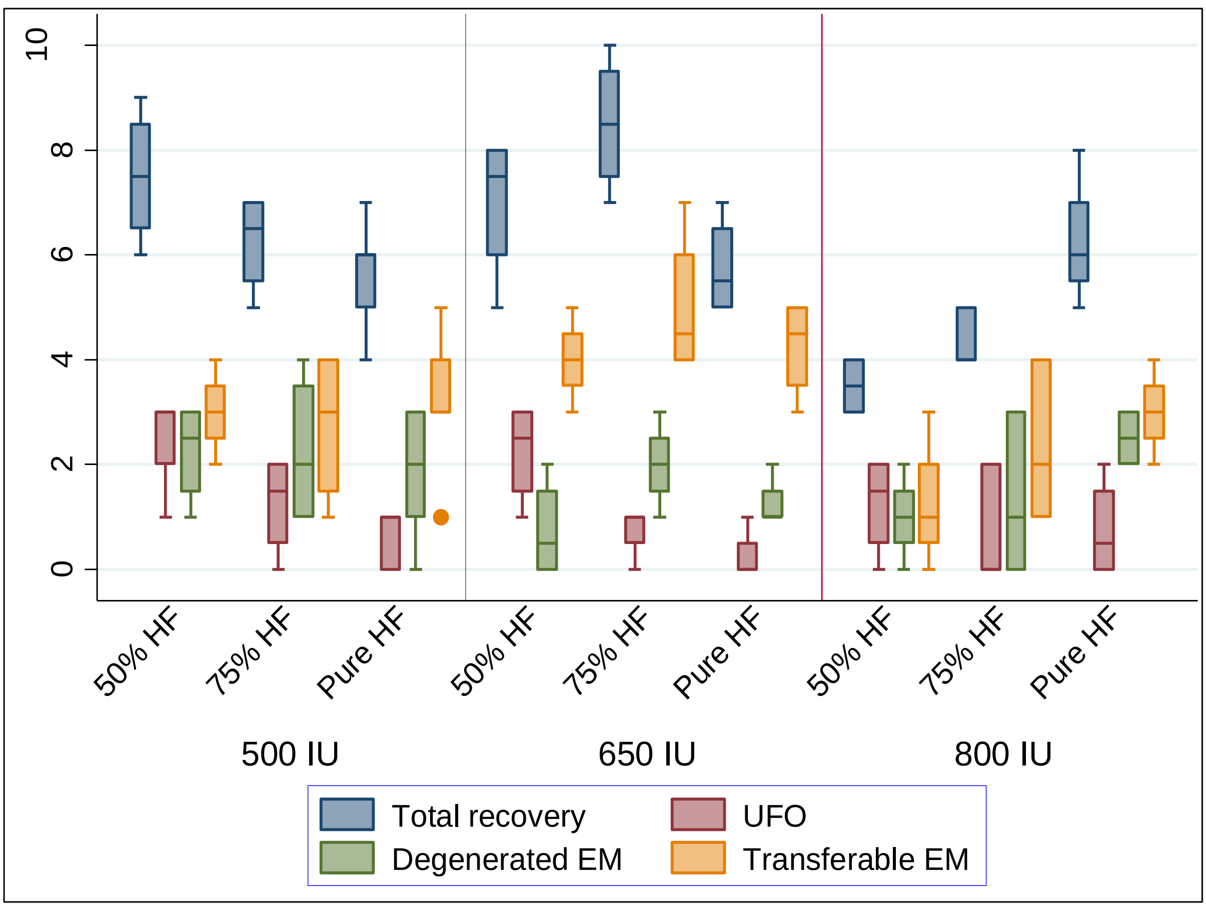

Figure 3. Status of the embryo recoveries for both categories of superovulatory hormone doses and donor's blood level

4. DISCUSSION

In this study, there was significant variation of responses ($p<0.05$) for POF and CL among donor treatment groups. Cross HF donors have shown higher response (number of CL count) for lower dose of FSH (650 IU) than Pure HF. This aligns with the previous study done by [12] on the other breed (Boran) and more CL count in the current study than the other report by [10].

In this research cross breeds have shown hyper stimulation (swelling) of ovaries for 800 IU with significant ($P<0.05$) decrease in CL count. This might be due to the lower threshold of the breeds for administered dose [8]. The breed dose response in pure HF shows that there is needed more dose of FSH to attain the maximum response. This indicated that there is a tendency of Pure HF required the recommended dose of FSH (1000 IU) to the maximum response [8, 18].

Range POF in this study was in the range of other studies [19-20] which reported the range of variations 8 to 20 preovulatory follicles per an ovary. Number of CL count at the time of embryo collection was similar and consistent with [21] which was 14 for 650 IU and 18 in OPU case reported by [22]. Though breed did not show significant effect ($P>0.05$) for CL count in this study, Boran genetics might have the contribution for the greater count of CL in cross breed HF [12].

More proportion of cows have shown good and excellent response rates in 50% and 75% cross breed HF while the greater proportion of pure HF breed shown fair response rate but with few excellent response rates. This can be due to the variations in blood level of the breed that hampers ovarian response [14, 17].

Cross breed HF responded lower for 800 IU FSH than Pure HF and detected with large ovarian size on day of flushing, and concurrently with low recoveries which align with different reports [4, 8, 12], which explained a reason as the higher the dose with the lower the response and this is due to the effect of superstimulation of cows might bear with the larger the ovarian size. In such cases the fimbriae is incapable to envelop the ovarian surface and fail to receive and pass ovum from ovulatory follicle to the oviduct [23].

The overall embryo yield in this study was not influenced ($P>0.05$) by either of the donor or the dose of the FSH treatments, and this is similar with other reports by [7, 24]. The range of embryo yield was in the range of other studies [7, 21] while transferable embryos were significantly higher ($p<0.05$) in 75% HF cross breeds with 650 IU treated cows. This finding was consistent with [22]. Yield of UFO was significantly higher in 50% HF compared with 75% HF and pure HF. On the other hand, number of UFO in the current study was significantly higher compared with the report data from [21]. The higher UFO yield in 50% HF might be due to the Boran genetics [12]. Yield of transferable embryos consistent with the superovulatory response was significantly higher ($p<0.05$) in cross bred HF than Pure HF but lower than [8] report.

5. CONCLUSIONS

Breed of the study animals clearly shows that differences in response to the different hormone doses as blood level of dairy cows differs. The reduced dose of FSH (650 IU) recommended for the cross HF has shown an improvement in ovarian response than higher doses (800 IU). Subsequently, higher superovulatory response recorded at dose of 650 IU in both 50% and 75% cross breed HF was also associated with higher number of transferrable embryos. The higher the blanket recommendation for cross bred HF comes up with the lower ovarian response and subsequently with lower recovery of embryos in cross-bred HF. Higher UFOs were counted in 50% cross HF than the other group of donor HF cows. Effects of the different level of hormone dose treatments to the subsequent reproductive parameters in embryo production require further study in other Ethiopian dairy breeds.

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