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Assessment of Two Superovulation Programs in Dairy Cows in the Ecuadorian Central Highlands

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ABSTRACT--- ***In different dairy farms in the Ecuadorian Central Highlands, we assessed the effect of superovulation in dairy cows of different breeds based on intravaginal device implants P4 (DIB) + EB additional to the application or not of GnRH during the 1st Artificial Insemination (AI), using 24 dairy cows, each being an experimental unit and distributed under a completely randomized design (CRD), the experimental results were subjected to Chi-squared (X^2) and Students' t tests to compare the effect of treatments. Determining that the superovulation programs used showed statistically similar responses, however, cows receiving GnRH showed a greater number of animals superovulating (91.7 % vs. 83.3 %), the highest total number of structures collected 10.7$\pm$7.0 vs 8.4 $\pm$4.1 per cow and viable embryos 9.4$\pm$6.1 vs 7.1$\pm$3.5 cow, corresponding to 87.30 % and 84.50 % respectively. Breed had a direct influence on the assessed parameters. It's recommended to induce superovulation with intravaginal implants P4 (DIB) + EB plus the application of GnRH during the first artificial insemination to improve the quantity and quality of embryos to collect.***

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1. INTRODUCTION

The techniques for handling the reproductive process that have received the most attention and development in recent years are: multiple ovulation and embryo transfer, embryo freezing, twins production, in vitro embryo production, embryo multiplication, bisection or nuclear transfer, embryo and fetal sexing, gene transfer (González, 2012). Cattle has traditionally been genetically improved from the paternal side via artificial insemination (AI). Using the Embryo Transfer (ET) technique, livestock improvement can be accelerated from the maternal side. Among these improvements are: reducing the generation interval, accelerating the selection of animals with productive traits, increasing animal's production and productivity and obtaining a large amount of progeny from valuable donors. Embryo transfer is the technique by which embryos (Fertilized ova) are collected from the donating female's cervix before nidation, and transferred to the recipients' cervix to complete gestation. Superovulation and ovulation synchronization are based on a series of physiologic events. Among these would be certain endocrine processes occurring from the estrous cycle until the ovulation of follicles, developed in response to the supply of a specific hormonal treatment. Inducing this hormonal response produces an improvement in the number of salvaged embryos and their quality (Bolívar y Maldonado, 2008). An embryo transfer program' success is measured by the number of calves born alive per female donor in a period of time (Pérez et al., 2006). Results are affected by factors inherent to the donor, embryo, technique application and recipients, who receive a strange embryo in their uterus allowing the gestational development (Duica et al., 2007; Peres et al., 2006). Among embryonic factors, quality clearly influences the transfer's results, independent of embryos being fresh, cryopreserved, micromanipulated and/or produced in vitro (Cutini et al. 2000a). Gallegos et al. (2003) formed two groups of 18 cows, each group of the Beefmaster breed. In group I, the rut previous to superovulation was synchronized with PGF2α, in a dual injection program, and 24mg of porcine pituitary gonadotropin was used for superovulation, obtaining 6.7 $\pm$1.6 quality 1 embryos and 1.8$\pm$0.7 degenerate embryos. In group II a progestogen was applied intravaginally plus a 2.5mg benzoate estradiol IM injection one day after applying the progestogen. Afterwards, superovulation was induced with 22mg FSH (purified extract of porcine pituitary) obtaining: 3.8$\pm$ 1.1 quality 1 embryos and 3.5 $\pm$ 0.9 degenerate embryos. The goal of this study was to assess the response of dairy cows to superovulation, using two hormonal protocols and determining superovulation percentage and quality of embryos collected for each protocol.

2. MATERIALS AND METHODS

Twenty-four dairy cows of breeds: Brown Swiss, Holstein Friesian and Jersey, were used. They belong to different dairy farms in the Ecuadorian Central Highlands (Chambo and Chuchi cantons in Chimborazo province, and Mejía canton in Pichincha province). A cow selection criteria was used based on production and reproductive records, and a gynecological examination to determine the ovaries' physiological state. Next were chosen the dairy cows meeting the following requirements: 1) Being disease free and having given birth at least once, 2) No presence of anatomical anomalies nor diseases in the genital tract, 3) Presence of structures indicating ovarian functionality (corpus luteum, follicles) and 4) Milk production in ranging from 20 to 25 liters according to milk production records in the studied farms under a grazing management system. Cows selected previously to the application of hormonal treatments were immunized according to each locality's health calendar; internal de-worming was also carried out, while external de-worming was carried out using spray baths. Hormonal treatments were randomly applied to cows (Table 1).

Seminal material from bulls of different commercial establishments was used for the artificial insemination, thus guaranteeing its quality. Embryo collection process was carried out using the non-surgical technique, which consists of cleansing the uterus with a saline solution added to albumin serum and antibiotics. Intravaginal devices of P4 (DIB) + EB were used, plus the application or not of GnRH during the first week of artificial insemination, so we had two experimental treatments, each one with twelve repetitions. The experimental units were distributed under a completely randomized design (CRD). Measures of central tendency (averages) and dispersion (standard deviation) were obtained to express data acquired from breed, age and body conditions in cows; also from the amount and quality of embryos obtained per animal. Chi-squared test (X^2) was used to determine if treatments cause or not significant differences in the superovulatory responses and the amount of viable embryos collected. Students' t-test, considering unequal variances, was used to compare the effects of treatments in the number of structures and embryos collected, and in the amount of viable embryos.

Table 1. PROTOCOLS USED FOR SUPEROVULATION.

Day		Treatment 1	Treatment 2
0		Inserting device with P4 + 1 ml (1mg) EB	Inserting device with P4 + 1 (1mg) ml EB
Day 4	AM	2.5 ml (50mg) de FSH (Foltropin)	2.5 ml (50mg) de FSH (Foltropin)
	PM	2.5 ml (50mg) de FSH (Foltropin)	2.5 ml (50mg)de FSH (Foltropin)
Day 5	AM	2.5 ml (50mg) de FSH (Foltropin)	2.5 ml (50mg) de FSH (Foltropin)
	PM	2.5 ml (50mg) de FSH (Foltropin)	2.5 ml (50mg) de FSH (Foltropin)
Day 6	AM	2.5 ml (50mg) FSH + 5 ml (25mg) PGF2α (Lutalyse)	2.5 ml (50mg) FSH + 5 ml (25mg) PGF2α (Lutalyse)
	PM	2.5 ml (50mg) FSH + 5 ml (25mg) PGF2α (Lutalyse)	2.5 ml (50mg) FSH + 5 ml (25mg) PGF2α (Lutalyse)
Day 7	AM	Device removal 2.5 ml (50mg) de FSH (Foltropin)	Device removal 2.5 ml (50mg) de FSH (Foltropin)
	PM	2.5 ml (50mg) de FSH (Foltropin)	2.5 ml (50mg) de FSH (Foltropin)
Day 8	AM	I.A.	I.A. + 2.5 ml GnRH (0.011mg) (Gonaxal)
	PM	I.A.	I.A.
Day 9	AM	I.A.	I.A.
Day 15	AM	Collecting Embryos	Collecting Embryos

3. RESUTLS AND DISCUSSION

Becaluba (2007) specifies that the donor's age influence on the superovulatory responses has been studied in dairy cattle, obtaining a greater number of transferable embryos from donors aged 3-6 than from heifers and cows older than 10 years. Holstein Friesian cows weighted 560+41 kg, Brown Swiss cows 496 +41 kg and Jersey cows 377+21 kg. Brady (1996) indicates that these are appropriate weights to carry out the testing according to their genotype. To achieve successful conception the cow must be within the optimal weight range, if it's below it the reproductive capacity diminishes, and if above the cow tends to be infertile (Fraure, 2004). Brown Swiss cows presented a body condition of 3.6+0.3, Holstein Friesian 3.3+0.2 and Jersey 3.3+0.2 in agreement with Peña, et al., (2001), where they state that the donor must present a body condition between 3 and 4 out of a total of 5. It can be observed that the donors' inherent traits are within a normal range so they should not influence the results of superovulation.

Table 2. CHARACTERISTICS OF DAIRY COWS SUBJECTED TO TWO SUPEROVULATION PROGRAMS TO OBTAIN AND PRESERVE EMBRYOS.

		Age, years			Weight, kg			CC (5 puntos)		
Breed	Nº obs.	Mean		D.E	Mean		D.E	Mean		D.E
Brown swiss	13	7,5	±	3,3	495,5	±	41,4	3,6	±	0,3
Holstein Friesian	6	6,5	±	1,6	560,7	±	41,4	3,3	±	0,2
Jersey	5	9,0	±	4,0	378,6	±	21,4	3,5	±	0,2
Average		7,68			478,27			3,38		
Standard dev.		1,26			92,26			0,16		

Nº obs.: Number of observations. D.E.: Standard deviation. CC: Body condition.

SUPEROVULATORY RESPONSES

Superovulatory responses do not differ statistically ($P > 0.05$) among treatments according to the Chi-squared test ($X^2cal < X^2\ tab$), however, numerically, was found that the greatest proportion (91.67 %) of cows superovulated when applied the GnRH during the first insemination, contrary to those who did not receive it, as the obtained results were 91.7 and 83.3 % respectively (Table 3). These results agree with that stated by De la Fuente, (2004), who indicates that greater risks in embryo transfer are caused by superovulation, since 20-25% of cows don't respond to the stimulatory treatments and those that display variability in superovulatory responses

Table 3. EFFECTS OF TWO SUPEROVULATION PROGRAMS IN DAIRY COWS OF DIFFERENT BREEDS

	Superovulated		Non-ovulated		Total			
	Nº	%	Nº	%	Nº	%	X²cal	Prob.
Treatment 1	10	83,33	2	16,67	12	100	0,38	> 0.05
Treatment 2	11	91,67	1	8,33	12	100		
Brown swiss	13	100,00	0	0,00	13	100	38.51	< 0.01
Holstein Friesian	4	66,67	2	33,33	6	100		
Jersey	4	80,00	1	20,00	5	100		

T1: Intravaginal devices of P4 (DIB) + EB without GnRH during 1st A.I.
T2: Intravaginal devices of P4 (DIB) + EB + GnRH during 1st A.I.
$X^2cal < X^2\ tab$: There are no statistical differences.

Superovulatory responses by breed differed significantly ($X^2cal > X^2\ tab$, $P < 0,01$), because of the 13 Brown Swiss cows assessed, 100% presented superovulation; this was not the case in the Jersey breed, where only 80% presented it, and 67% in Holstein Friesien. These results agree with those obtained by Palma, (2001), who indicates that in a study about superovulation treatments carried out in 13 different breeds was found that the donor's breed is influences aspects such as: number of superovulations, number of oocytes and embryos collected, number of transferable embryos, amount and percentage of pregnancies.

STRUCTURE AND EMBRYOS COLLECTED

Total Amount of Structures Collected

The number of structures recovered per collection in this study was statistically similar ($P > 0.05$), although, there are numerical differences because out of the 12 cows that weren't applied GnRH, 10 presented superovulation and 8.4+4.1 structures on average. While of the 11 cows that superovulated with the application of GnRH, 10.7+7.0 structures were collected, noting that this difference could be consequence of the application of GnRH because at the moment of artificial insemination it guarantees the synchronization between insemination and ovulation, prevents delayed ovulation and improves development of the corpus luteum (Ayala and Castillo, 2010).

TABLE 4. STRUCTURES AND EMBRYOS COLLECTED FROM COWS OF DIFFERENT BREEDS, AS RESULT OF TWO SUPEROVULATION PROGRAMS.

	T1				T2					
	Obs.	Mean		D.E.	Obs.	Mean		D.E.	Tcal	Prob.
Total	10	8,40	±	4,14	11	10,73	±	7,00	-0,937	0,181
Ovum	0				3	2,33	±	2,31		
Early morula	1	4,00			4	7,75	±	2,22	-3,382	P < 0.01
Compact morula	5	6,40	±	0,55	8	7,50	±	2,22	-0,413	0,346
Expanded morula	3	3,67	±	2,31	2	3,00	±	2,83	0,277	0,404
Blastocyst	6	4,00	±	1,67	1	5,00			-1,464	
Early blastocyst	0				1	1,00				
Hatching blastocyst	1	2,00			0					
Degenerate embryo	4	2,75	±	2,87	5	1,60	+	0,55	0,789	0,244

T1: Intravaginal devices of P4 (DIB) + EB without GnRH during 1st A.I.
T2: Intravaginal devices of P4 (DIB) + EB + GnRH during 1st A.I
D.E.: Standard deviation.
Prob. > 0.05 there are no statistical differences.
**: There are statistical differences.

Ova
Presence of ova during embryo collecting is considered as non-viable or non-fertilized structures. In three cows that received the GnRH were found 2.3+2.3 ova/cow, while in cows not receiving the GnRH during collection no ova were found.

Early Morula
Only one of the cows not being applied GnRH to showed, on average, four early morulae, however, from cows that did receive the GnRH were observed four animals with 7.8+2 early morulae on average per animal; these differences can be attributed to the GnRH, which prolongs the corpus luteum's half-life via follicle luteinization, hence embryo survival could be improved by increasing the corpus luteum's half-life.

Compact Moruala
With the application of GnRH to 8 cows, 7.5+2 compact morula/animal were collected. Unlike cows that didn't receive the GnRH from which five presented 6.4+0.5 compact morula/animal, thus establishing that GnRH application produces a better ovulatory response

Early Blastocyst
This embryo stage was infrequent during collection as it was found only once, and the cow received the GnRH during the 1st A.I.

Blastocyst
Cows that didn't receive the GnRH presented a higher frequency of embryos in the blastocyst stage, because from the twelve subject cows, six of them presented, on average, 4.0+1.7 blastocysts, however from the twelve cows that did receive the GnRH only one animal presented 5 embryos in this stage. This shows that when not applied the GnRH cows' ovulation happened earlier because their fertilized ova achieved the blastocyst stage in greater proportion.

Hatched Blastocyst
This embryo stage was collected from a cow that didn't receive the GnRH, it presented two hatched blastocysts in total. These are considered unfit for use.

Degenerate Embryo
In the GnRH-free treatment, four animals presented 2.8+2.9 degenerate embryos; while in the treatment with GnRH five animals presented an average of 1.6+0.6 degenerate embryos. The differences can be attributed to the GnRH, because it extends the corpus luteum's half-life via luteinization of follicles that should normally cause luteolysis and/or induce an accessory CL (Cervera, et al., 2011).

VIABLE AND NON-VIABLE EMBRYOS

As seen in table 5, the largest amount of viable embryos were collected from cows that received the GnRH than from those that didn't. 9.4+6.1 vs a 7.1+3.5 viable embryos/cow, respectively. These results agree with that exposed by Maldonado and Paula (2008), who, after inspecting about hormones use in superovulation schemes and donor synchronization, concluded that the rate of transferable embryos does not appear to change significantly, obtaining in average 6.2+1.4 viable embryos/cow. Another study assessed the superovulatory response and embryo quality in dairy cattle of high genetic merit, and used a CIDR impregnated with 1.9 g of Progesterone jointly with 5 mg of Burnet 17β-estradiol seven days before starting the treatment; 9.1 ± 0.6, viable embryos/cow were collected (Castrillón, 2011).

Table 5. VIABLE AND NON-VIABLE EMBRYOS COLLECTED FROM COWS OF DIFFERENT BREEDS, AS RESULT FROM TWO SUPEROVULATION PROGRAMS.

	Viable			Non-viable		
	Mean		D. Est.	Media		D. Est.
T1	7,10	±	3,54	3,25	±	3,86
T2	9,36	±	6,09	2,50	±	2,35
Tcal	-1,050			0,350		
Prob.	0,154			0,373		
Brown swiss	10,00	±	5,46	3,25	±	3,06
Holstein Friesian	6,00	±	2,45	1,00		
Jersey	5,00	±	3,27	1,00		

T1: Intravaginal devices of P4 (DIB) + EB without GnRH during 1st A.I
T2: Intravaginal devices of P4 (DIB) + EB + GnRH during 1st A.I
D.Est.: Standard Deviation. Prob. > 0.05 there are no statistical differences.

Strong differences per genetic group were found; as from Brown Swiss cows were collected 10+5.5 viable embryos/cow, 6+2.5 viable embryos/cow from Holstein Friesian and only 5+3.3 from Jersey breed. These results prove that stated by Rodríguez et al., (2007). They showed that breed affects directly the ovulatory follicle's size and the number of granulosa cells, this can have a favorable or adverse effect on the ova's fertility rate.

PERCENTAGE OF VIABLE AND NON-VIABLE EMBRYOS

The percentages of viable embryos collected from the superovulation programs weren't significant according to the Chi-squared test ($X^2cal < X^2$ tab) as the achieved values were 84.5 % y 87.3 % of viable embryos with respect to the total amount of collected structures, belonging to the group of cows that didn't receive the GnRH during the 1st A.I. (Table 6) and to those that did, respectively. Thus, it is established that applying the GnRH doesn't statistically improve the productivity of embryo collection.

Table 6. PERCENTAGE OF VIABLE AND NON-VIABLE EMBRYOS COLLECTED FROM COWS OF DIFFERENT BREEDS, AS RESULT FROM TWO SUPEROVULATION PROGRAMS.

	Viable	Non-viable	Total	X^2cal	X^2 tab
Treatments				0,32	3,84
T1	84,50	15,50	100		
T2	87,30	12,70	100		
Raza				13,02	5,99
Brown swiss	83,33	16,67	100		
Holstein Friesian	96,00	4,00	100		
Jersey	95,24	4,76	100		

T1: Intravaginal devices of P4 (DIB) + EB without GnRH during 1st A.I
T2: Intravaginal devices of P4 (DIB) + EB + GnRH during 1st A.I
X^2cal < X^2 tab: There are no statistical differences; X^2cal < X^2 tab: There are statistical differences.

Cows' breed influenced the percentage of viable embryos collected as the Chi-squared test established significant differences between genetic groups ($X^2cal > X^2tab$ $P < 0.01$). Holstein Friesian cows presented the largest percentage of viable embryos (96 %), followed by Jersey cows with 95.2 %, and finally Brown Swiss with 83.3 %. This variability in responses according to the genetic group is always present, and it's uncontrollable in the treatments to induce superovulation and viable embryos collection. Apreza, (2009) states that the multiple superovulatory response occurs depending on the follicles' development the moment the program begins, since it's very difficult to predict the beginning of the follicular waves. Also, the difference in quality among embryos can be due to high multiple ovulatory response, because from a large quantity of collected embryos, the production of bad quality embryos increases, as may be the case in the Brown Swiss breed.

4. CONCLUSIONS

The superovulation programs used presented statistically similar responses, however, cows receiving GnRH displayed a larger number of cows superovulating, greater amount of total structures collected, and viable embryos.
The animals' breed had a direct influence in the assessed parameters; Holstein Friesian cows presented the largest percentage of viable embryos, followed by Jersey cows, and in lower proportion by Brown Swiss cows.

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