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An Integrated Framework for Genetic Improvement and Artificial Breeding of Beef Cattle: a Case Study in Livestock Industry of Colombia

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ABSTRACT

Milk and meat production has slowed down and endangers food safety. To address this problem, this paper presents a framework to ensure the effectiveness of genetic improvement and cattle breeding programs in farms supported by the implementation of a Decision Support System (DSS). Here, the DSS is used to monitor and control critical process variables derived from some components of these programs, specifically in-vitro fertilization, embryo collection and artificial breeding. An application in three Colombian farms is shown to validate this approach. Through this intervention, the average calf weight gain increased by 46.58%.

Key words: Artificial breeding, decision support system (DSS), embryo collection, in-vitro fertilization, livestock industry.

1. INTRODUCTION

Double-purpose cattle productivity (beef and dairy) have slowed down and consequently endangers food safety, rural development and competitiveness of livestock industry. In accordance with the most recent reports, only 35% of livestock is oriented to double-purpose production whilst 6.4% is involved in specialized dairy [1] which evidences a weak intention of being productive. On the other hand, in 2015, the milk production (6623 millions of litres) declined by 1.4% compared to 2014 (6717 millions of litres) which is and alarm for both national government and livestock sector institutions. Another aspect of concern is the fact that the number of slaughtered cows has significantly increased by 44% [1] which negatively impacts upon cattle restocking. Additionally, it is important to mention that 81% of farms have less than 50 beef cattle heads [1]. All the above-mentioned aspects are evidence of food safety problems and must be therefore analysed to create effective interventions.

Both meat and milk mostly contribute protein to the colombians' diet. In addition, the beef consumption (kg/year) tends to increase (more than 20 kg/person) which demands greater response capacity by the current cattle herd. This is confirmed by the fact that 18% of food expenditure is represented by beef and dairy. The increased demand averagely create 7.9 and 5.5 jobs (per 100 inhabitants) due to specialized dairy and meat production activities respectively which are highly significant for any rural region.

Another aspect of interest is that, over the past decade, the pregnancy rate was 54.1%. In this respect, a meaningful endeavour is essential for implementing genetic improvement and factory farming programs. This is widely recommended by livestock sector institutions upon considering that transfer techniques should be adopted since, in spite of the high costs involved, provides better results compared to other methods. These techniques are also suggested to improve production efficiency and reduce poverty whilst small farmers can have access to superior genetics for increasing production levels and subsequently contribute to socio-economic development of the rural regions [2]. However, the aforementioned techniques must be carefully implemented to ensure meaningful outcomes during calf birth and rearing. In this regard, critical process variables should be identified and controlled through Decision Support Systems (DSS) in order to inform veterinarians and practitioners about the evolution of genetic improvement and cattle breeding programs. As a result, deviations and out-of-control patterns can be detected and on-time interventions can be then deployed to ensure high productivity. Therefore, this paper aims to develop a combined framework for increasing production efficiency of cattle herds based on in-vitro fertilization, embryo collection and factory farming supported by DSS.

The remainder of this paper is organized as follows: In Section 2, a literature review on techniques for genetic improvement of cattle is presented while methods are explained in Section 3. In Section 4, a case study in the livestock industry is described. Then, in Section 5, results and analysis are shown. Finally, Section 6 presents conclusions.

2. PRIMARY STUDIES ON GENETIC IMPROVEMENT AND ARTIFICIAL BREEDING: A BRIEF LITERATURE REWIEW

A reduced productive efficiency results in a low milk and calf production rates per year, which, in a dual purpose system, means significant losses of meat and milk annually. In this regard, the Girolando race, which is the cross-breeding between Gyr race (G) and Holstein race (HOL), has become in one of the genetic proposals of the global livestock sector to deal with the production requirements. The bovine animals belonging to Girolando race are characterized by attaining a better adaptation to environmental conditions of tropical and sub-tropical areas [3]. Also, they have been identified to have a dual purpose, becoming the ideal cross-breeding to tolerate the genetic renovation process of bovine by adopting the standard 5/8 HOL + 3/8 G (Girolando race Synthetic pure). The average milk production of this race is 5116 l, the lactation period is 279 days and the average age at the first calf birth is 1080 days [4].

In the renovation process of bovine genetic, the assisted reproduction technologies are an efficient alternative when increasing the milk and meat production of bovine herds. These biotechnologies shorten the generational intervals and the spread of genetic material in the bovine population [5]. Here, In Vitro Fertilization (IVF) and Embryo Transfer (ET) are proposed. The ET application helps to prevent the transmission of diseases in bovine cattle. ET is a more integrated approach for the genetic distribution compared to the artificial insemination (AI). Although ET could be considered as a methodology for the genetic improvement (80% of embryos with viability before transfer), the technology has not yet reached the optimum point due to the variability of the ovarian response to the superovulatory gonadotropin treatment [6]-[8]. Likewise, it has a high yield under wet conditions and requires the synchronization of the donors and recipients so that the embryos can be recovered and synchronically transferred to ensure appropriate elongation of pregnancy by recipients [9]. The embryo transfer should not be done immediately. In this regard, bovine embryos should be frozen through slow cooling with ethylene – glycol while ensuring their safe storage and a better management of the genetic resources [10]. It can be noted that the embryo collection technique applied to synchronized recipients (superovulation) is the most used variation and evidences better results in relation to other protocols [11]. An average pregnancy rate of 50 % is globally accepted for embryo transfer programs [12]-[14]. On the other hand, FIV has a good yield in the embryonic production, although there is still suboptimal oocyte maturation [15], [8].

Under the assumption of providing better-assisted reproduction techniques, a study presented in [5] suggests that the use of transvaginal follicular puncture and guided ultrasound could make the oocyte recovery more effective with a collection up to 1000 per cow-year. Herein, the embryo transfer and in vitro fecundation will be combined as an innovation strategy of this study to achieve higher pregnancy rates, increased percentage of viable embryos before transfer and increased number of first-level and second-level embryos in the process of genetic improvement. By applying this hybrid method, it is expected to reduce the physiological affectations of the ovaries and the normal estrous signs. Another contribution in this process is presented by [16]. This article describes that the use of sexed sperm (90 % - female calf) in the in-vitro embryo production could be an innovative and promising application to balance the production capacity of the bovine herd with the current demand. Nevertheless, the biological causes of damaged embryos in the in-vitro technique should be investigated. This aspect will be addressed in this case study so that we can contribute to the development of this knowledge area. It should be also mentioned that, in accordance with the obtained data, the percentage of first-level or second-level embryos of the process is 50%, the percentage of viable embryos before the transfer is 50% and the percentage of recipients with lute body is equal to 75 % [5].

The efficient growth of the Girolando calves is an essential factor for the profitability of both milk and meat sectors since it impacts upon its future production capacity, quality standards and, consequently on the food safety programs. This growth depends on the implementation of feeding systems decreasing the low intake problem. With this in mind, the effects of different feeding schemes in young calves have been studied by several authors and have shown that the liquid intake when using milk replacer increases the weight gain [17] and a greater fat and protein production [18]. At present, weight increase (17.8%) is expected in the first month [18], [5]. Nevertheless, despite the milk replacer advantages, it is necessary to counteract its negative effects on the calf rumen development and its pancreas enzymes [19]-[21] which is evidenced by low levels of ADG (Average daily gain) in the next months. Therefore, it is proposed to implement gradual weaning schemes where the milk provided to the calves can be progressively increased until 23 days of life, and slowly reduced until the day 49 [22]. To control this process, it is necessary to implement a Decision Support System (DSS) to assess the livestock-raising system in terms of animal performance [23]. However, software for supporting genetic improvement process of Girolandas is largely limited. This is even more important when addressing the need of controlling the variability of these genetic techniques [24], [5], [25], [26]. It should be also mentioned that the selection optimal of recipients is an aspect of interest since it has become a preponderant factor to obtain positive results in terms of pregnancy and calf-birth [5]. This variable has to be also controlled through DSS in order to ensure high-effective selection of donors and recipients based on performance data.

In light of the above-mentioned findings, the conducted literature review practice showed that studies directly concentrating on integrated frameworks for increasing the effectiveness of genetic improvement and artificial breeding programs. Therefore, we implemented a hybrid approach in this study in order to provide a useful decision-making tool that can be used in realistic scenarios of the livestock industry.

3. PROPOSED METHODOLOGY

The proposed methodology (refer to Figure. 1) aims to increase the effectiveness of genetic improvement and artificial breeding programs. It is comprised of 6 main phases as detailed below:

Phase 1 – Software design, development and implementation: In this phase, a technological platform (DSS) is designed for the compilation, representation and analysis of the resulting information on critical process variables regarding the selection of donors and recipients through the combined application of In-vitro fertilization, embryo collection and care-feeding scheme.

Phase 2 – Diagnosis of physiological state: It consists of an initial diagnosis of the physiological state of donors and recipients through sonograms for the pre-selection of those participating in the application of the combined method. Afterwards, the sanitary protocols against abortion are applied in both pre-selected donors and recipients. Finally, the most suitable donors and recipients are selected to be part of the genetic improvement and artificial breeding programs.

Phase 3 – Design and implementation of a care and feeding scheme: It encompasses the design of the nutritional and environmental control plans that are necessary to ensure satisfactory results regarding the percentage of recipients with corpus luteum and viable embryos before transfer.

Phase 4 – Combined implementation of In-vitro fertilization and embryo collection techniques: In this step, the hormonal protocols are implemented in the selected donors and recipients. Herein, the cow estrous status is continuously measured and monitored. Afterwards, the uterine wash and follicular aspiration are applied. Finally, the oocytes are fertilized with sexed sperm and then transferred to the recipients.

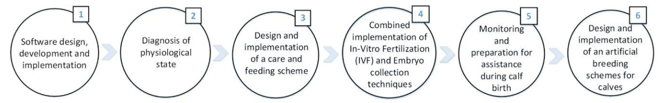

Figure. 1: The proposed framework for increasing the effectiveness of genetic improvement and artificial breeding programs in the livestock industry.

Phase 5 – Monitoring and preparation for assistance during calf birth: After the transferring process, a diagnosis is done on the pregnancy status of recipients through ultrasound scanning test (45 days after the process start). The pregnancy status and fetal sex are then monitored after 70, 90 and 120 days of process start. Finally, assistance activities during calf birth will be implemented while monitoring the critical process variables through control charts.

Phase 6 – Design and implementation of an artificial breeding scheme for calves: In this phase, a scheme for the suitable artificial breeding of calves is designed based on resulting conclusions from the literary review where the implementation of milk replacers together with milk supply and weaning methods are highly recommended.

4. RESULTS AND DISCUSSION

4.1 Software Design, Development and Implementation

CEMG was designed for monitoring and controlling the critical statistical parameters involved in both genetic improvement and artificial breeding programs. Previously, key performance indexes were created to measure the effectiveness of the main activities throughout the implementation (refer to Table 1). The software assisted veterinarians and livestock managers in selecting the most suitable donors and recipients so that high process performance can be ensured. In addition, it provided a good support for decision-making processes in both IVF and embryo collection techniques which is relevant when considering that the aforementioned techniques must be carefully implemented to guarantee meaningful outcomes during calf birth and rearing. On the other hand, it was useful to graphically control the average calf weight gain which is the most relevant result of this process.

Table 1: Specifications of recipients and donors.

Activity	Key performance indexes
Diagnosis of physiological state	• Cervical diameter • Uterine horn diameter • Ovarian diameter • Presence of functional corpus luteum/dominant follicle • Readability of cattle identification number
Selection of donors and recipients	• Presence of functional corpus luteum/dominant follicle
Estrous detection	• Percentage of estroused cows
In-vitro fertilization and embryo collection	• Percentage of first-level or second-level embryos • Percentage of viable embryos before transferring
Embryo transfer	• Presence of functional corpus luteum/dominant follicle
Pregnancy monitoring (45, 70, 90 and 120 days after process start)	• Pregnancy rate • Percentage of female calf in in-vitro fertilization • Percentage of female calf in embryo collection
Parturition	• Percentage of cows with dystocic parturition in in-vitro fertilization • Percentage of cows with dystocic parturition in embryo collection • Percentage of live births in in-vitro fertilization • Percentage of live births in embryo collection
Artificial breeding	• Average calf weight gain

The software here described is comprised of four modules (Administration, Configuration, Genetics and Reports) through which the users can effectively deploy data analysis and evidence-based decisions. Also, a code is assigned to each recipient and donor in order to ensure process traceability. This is important when correlating final results with specific parameters of any process step. Furthermore, it enables us to mathematically model response variables in terms of predictors which are significant when implementing corrections or preventive actions. The software also provides a full report of each donor, recipient and calf so that further analysis can be performed for the continuous improvement of the programs.

4.2 Diagnosis of Physiological state

In this phase, veterinarians proceeded to measure the following parameters: cervical diameter, uterine horn diameter, ovarian diameter, the presence of functional corpus luteum/dominant follicle and readability of cattle identification number. The potential recipient or donor is concluded to be SUITABLE for the improvement genetic and artificial breeding programs whether specifications (refer to Table 2) are fully satisfied.

The results evidenced that the average cervical diameter is greater than lower-specification-limit (15mm) which is highly beneficial for an embryo transfer program since during artificial inseminations, it is necessary to penetrate the cervix with ease (refer to Figure. 2). In this regard, favourable uterine conditions and the subsequent early embryonic development can be generated evidencing a potential good reproductive performance during genetic improvement programs (PPM = 0; PpK = 5.84).

Table 2: Specifications of recipients and donors.

Key performance indexes	Specification
Cervical diameter	LSL = 15 mm
Uterine horn diameter	LSL = 15 mm
Ovarian diameter	LSL = 15 mm
Presence of functional corpus luteum/dominant follicle	The corpus luteum is functional if its diameter is equal or greater than 8.5 mm. Similarly, the follicle is concluded to be dominant if the aforementioned condition is satisfied
Readability of cattle identification number	If the cattle identification number is not blurred, it is concluded to be readable.

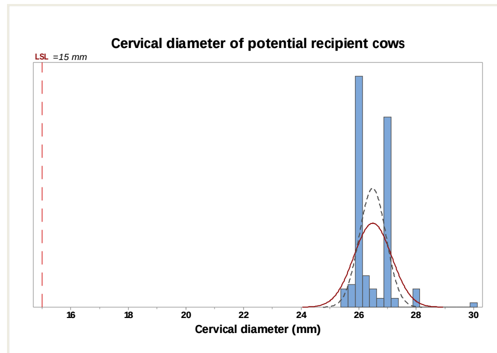

Figure. 2: Cervical diameter of recipients.

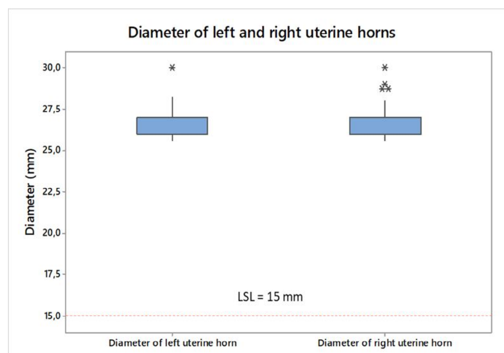

Figure. 3: Uterine horn diameter.

This is also confirmed by the outcomes related to uterine horn diameter (refer to Figure. 3) which was also confirmed to be higher than LSL (15 mm) in both right (PPM = 0; PpK = 4.74) and left (PPM = 0; PpK = 5.17) uterine horns. Evaluations of these parameters are significant predictors of fertility in beef [27].

On the other hand, considering ovarian diameter, it can be concluded that the donors and recipients satisfy with anatomical requirements and they are therefore qualified for embryo transfer program (refer to Figure. 4). In this case, the minimum diameter value was 15.6 mm in both ovaries which confirms the above-mentioned statement. Some atypical values were also observed in both cases; however, they cannot be classified as ovarian cysts (diameter > 25 mm). Another parameter of concern is the presence of functional corpus luteum which indicates an excellent ovary activity with 88% (refer to Figure. 5 and Figure. 6). The corpus luteum is a transient reproductive gland producing progesterone, a substance that is needed for setting and maintaining pregnancy [28]. In addition, prolonged luteal function is positive associated with high milk production which is one the primary aims of this intervention.

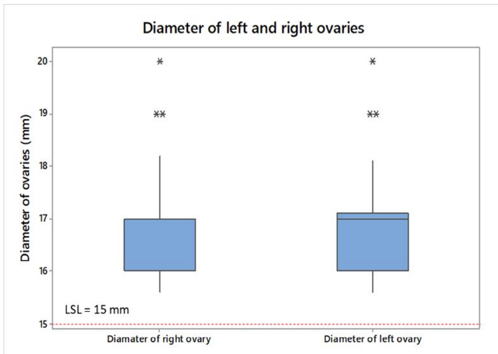

Figure. 4: The ovarian diameter of donors and recipients.

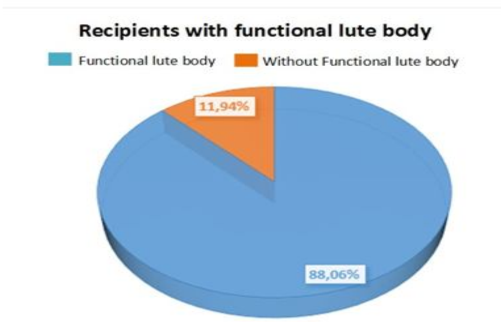

Figure. 5: Percentage of functional corpus luteum.

Figure. 6: Ultrasound scanning for detecting corpus luteum.

4.3 Design and implementation of care and feeding scheme

As next step, a care and feeding scheme was designed for both donors and recipients. Here, nutritional and environmental plans were created to ensure an adequate absorption of essential amino acids, good digestibility and duodenal flow of nutrients. Particularly, distiller grains from the fermentation of sorghum and corn were utilized as components of the cattle diet. Distillers represent a significant protein and energy source. Furthermore, wet products have been demonstrated to have a better performance compared to dry concentrates. On the other hand, Palm kernel cake was incorporated as an innovative component of the cattle feeding behavior since it provides, with more than 15% of inclusion, high efficiency of rumination and better orientation to improvement genetic and artificial breeding programs [29].

4.4 Combined Implementation of In-vitro Fertilization and Embryo Collection Techniques

After incorporating feeding and care scheme into the process, IVF and embryo collection techniques were applied (refer to Figure. 7 and Figure. 8).

Particularly, the percentage of estrous recipients was 91.21% (refer to Figure. 9), which evidences a higher performance value than the expected (65%). In this regard, a bigger group of recipients in suitable conditions can be effectively used for the embryo transfer program. Cows with estrus evidenced better ovarian responses [30]: higher ovulation rate, larger corpus luteum and high P4 levels). Also, the percentage of first-level and second-level embryos that were obtained through embryo collection technique was found to be 91 %. This measure shows the high genetic compatibility between the bull sperm and the donors (refer to Figure. 10). The probability of successful pregnancy and the potential milk and meat production can be increased due to grade 1 and 2 embryos have minor and moderate irregularities in the overall shape of the embryonic mass respectively. Additionally, they can survive the thawing and freezing procedures which are part of the genetic improvement programs.

Figure. 7: Embryo transfer process

Figure. 8: Synchronization protocol for recipients

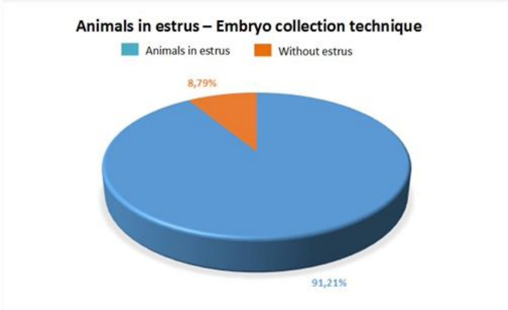

Figure. 9: Estrous cattle

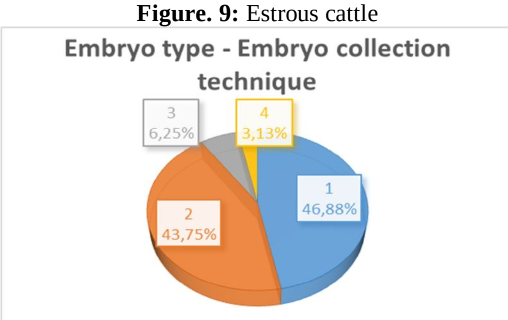

Figure. 10: First-level and second-level embryos

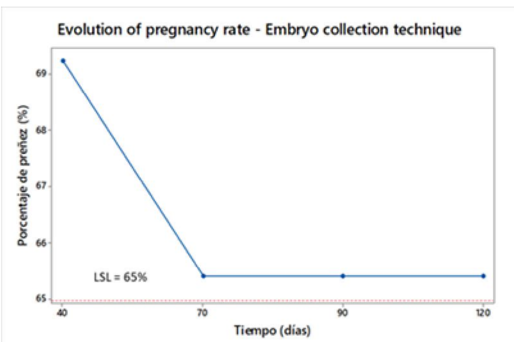

Figure. 11: Evolution of pregnancy rate

Particular attention should be also paid to pregnancy rate (Figure. 11) which was calculated to be 65.38% which is higher compared to the average behaviour identified in the literature (50%). It can be observed a 3.85% of pregnancy loss (n = 1 cow) between t = 0 and t = 70 days. The following potential causes can explain the aforementioned fact: infection, nutritional deficiencies, toxins and genetic problems. Despite this, the pregnancy rate was found to be equal during the next 50 days which demonstrates a better control on the program outcomes.

4.5 Monitoring and Preparation for Assistance during Calf Birth

Several training programs were implemented to effectively as-sist recipients during parturition and improve the normality of pregnancies. In this respect, it is important to avoid bacterial contamination of the uterine lumen and provide assistance to newborn calves during initial suckling. Furthermore, focused training is necessary to monitor the intensity of labor in recipients, diminish the risk of dystocia and minimize calf mortality. The results in terms of calf birth type and birth rate are shown in Figure. 12, Figure. 13, Figure. 14 and Figure. 15. It can be fully appreciated that the percentage of dystocic calf birth is lower in in-vitro fertilization which is consistent with the findings in the literature [31]. In this regard, no correlation was concluded between "dystocia" and "calf weight gain" (p-value = 0.904; T = -0.13)

On the other hand, IVF (86%) is also superior to embryo collec-tion (75.9%) regarding birth rate. In addition, it is cost-effective since it can diminish boarding and semen costs due to a single straw of semen can be used to fertilize several embryos. Also, IVF can utilize sexed semen provided by almost any bull; nonetheless, protocols for IVF embryo export still remains.

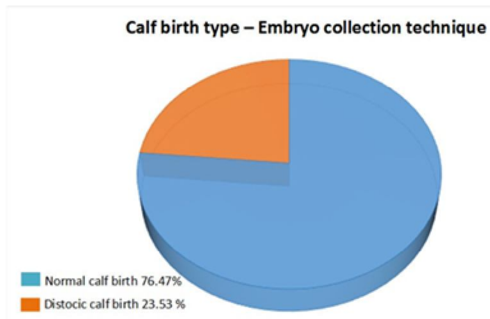

Figure. 12: Calf birth type in Embryo collection

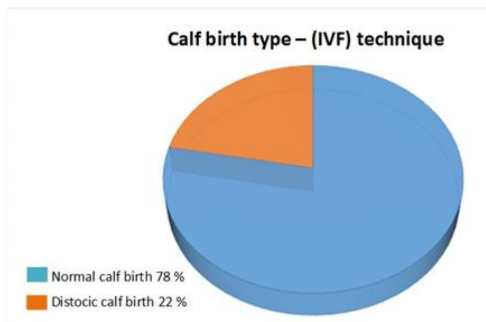

Figure. 13: Calf birth type in IVF

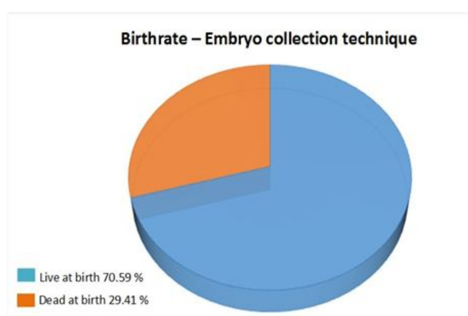

Figure. 14: The birth rate in Embryo collection

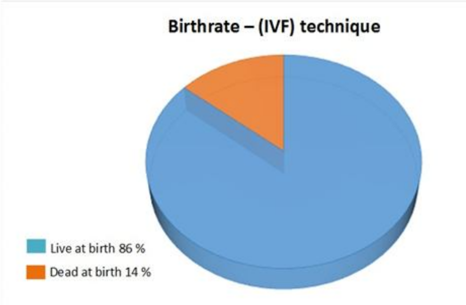

Figure. 15: The birth rate in IVF

4.6 Design and Implementation of an Artificial Breeding Scheme for Calves

Finally, an artificial breeding scheme was designed for the girolando calves in order to ensure high average calf weight gain. The efficient growth of young calves is relevant to guarantee the high profitability of the livestock sector [32]. In this regard, calves were fed with increasing amount of milk replacers before the weaning period. Their weight gain was measured after 30, 45 and 70 days of birth. The resulting data was introduced in the CEMG software to monitor the evolution of this critical parameter (refer to Figure. 16). The final average calf weight for the program was 46.58% (t = 60 days). It was calculated that, in this program, calves gained 0.329 kg/day on average. In addition, small variations were noticed in each period which denotes a high performance of the artificial breeding scheme. On the other hand, no correlation was observed between "embryo grade" and "average calf weight" (p-value = 0.669; ρ = -0.088).

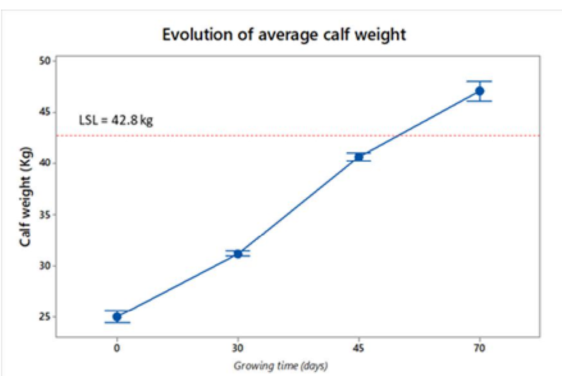

Figure. 16: Evolution of average calf weight gain

5. CONCLUSION

Genetic improvement and cattle breeding programs are complex to implement due to the wide variety of critical variables and conditions that may derive from the cattle biological nature. Therefore, it is highly suggested that veterinarians apply DSS and statistical techniques underpinning decision-making process throughout the programs. This is to ensure significant outcomes contributing to a higher calf weight gain.

A critical aspect is the continuous measurement and monitoring of key process parameters. This is an accurate guide for selecting cows with high reproductive efficiency, controlling pregnancy period, provide effective assistance during calf birth and verify the progress of calf weight. Of course, this ends up with increasing the reliability and robustness of the resulting decisions. Additionally, it is relevant to count on the engagement of the livestock farms involved in the study with the goal of ensuring greater levels of milk and meat production in the future.

The proposed methodology enables veterinarians and livestock managers to effectively implement integrated production programs for addressing the growing food safety problem. Nevertheless, it can be complemented with other interventions to minimize health problems and improve the ease of milking. For future work, it is intended to include programs aiming to control seasonal calf/cow weights in addition to designing complete feeding programs where components with low environmental impact can be incorporated.

The intervention here described presented the use of the integrated approach in three livestock farms of Colombia. Based on the outcomes, it can be concluded that the average calf birth gain was 46.58% whilst the percentage of first-level and second-level embryos was found to be 90.68%. On the other hand, the pregnancy rate was calculated to be 65.38% and the percentage of recipients with functional corpus luteum was concluded to be 88.06%. The aforementioned facts evidence that the proposed methodology offers superior results compared to the reported findings provided by [12]-[14] and can be thus replicated in other practical scenarios.

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REFERENCES

1. L. Mahecha, L. A. Gallego, and F. J. Peláez. **Situación actual de la ganadería de carne en Colombia y alternativas para impulsar su competitividad y sostenibilidad**, *Revista Colombiana de Ciencias Pecuarias*, Vol. 15, no. 2, pp. 213-225, Agosto 2002.
2. A. Tonamo. **A review on cattle husbandry practices in Ethiopia**, *International Journal of Livestock Production*, Vol. 7, no. 2, pp. 5-11, February 2016. https://doi.org/10.5897/IJLP2015.0276
3. A. W. Canaza-Cayo et al. **Genetic parameters for milk yield and lactation persistency using random regression models in girolando cattle**, *Asian-Australasian Journal of Animal Sciences*, Vol. 28, no. 10, pp. 1407–1418, October 2015. https://doi.org/10.5713/ajas.14.0620

4. D. C. Carvalho, R. M. Palhares, M. G. Drummond, and T. B. Frigo. **DNA Barcoding identification of commercialized seafood in South Brazil: A governmental regulatory forensic program**, *Food Control*, Vol. 50, pp. 784-788, April 2015. https://doi.org/10.1016/j.foodcont.2014.10.025
5. H. Rodriguez-Martinez. **Assisted reproductive techniques for cattle breeding in developing countries: A critical appraisal of their value and limitations**, *Reproduction in Domestic Animals*, Vol. 47, no. 1, pp. 21-26, January 2012. https://doi.org/10.1111/j.1439-0531.2011.01961.x
6. R. J. Mapletoft, K. B. Steward, and G. P. Adams. **Recent advances in the superovulation in cattle**, *Reproduction Nutrition Development*, Vol. 42, no. 6, pp. 601-611, November-December 2002. https://doi.org/10.1051/rnd:2002046
7. K. J. Betteridge. **Farm animal embryo technologies: Achievements and perspectives**, *Theriogenology*, Vol. 65, no. 5, pp. 905-913, March 2006. https://doi.org/10.1016/j.theriogenology.2005.09.005
8. P. Lonergan. **State-of-the-art embryo technologies in cattle**, *Society of Reproduction and Fertility Supplement*, Vol. 64, pp. 315-325, January 2007.
9. H. Rodriguez-martinez and E. Sato. **Feasibility of a Nylon-Mesh Holder for Vitri cation of Bovine Germinal Vesicle Oocytes in Subsequent Production of Viable Blastocysts**, *Biology of reproduction*, Vol. 72, no. 6, pp. 1416-1420, June 2005. https://doi.org/10.1095/biolreprod.104.037051
10. J. Saragusty and A. Arav. **Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification**, *Reproduction*, Vol. 141, no. 1, pp. 1-19, January 2011. https://doi.org/10.1530/REP-10-0236
11. G. N. O. Vianna et al. **Comparison between different protocols to estrus synchronization and artificial insemination time fixed in Nellore cows in anestrus,** *Archives of Veterinary Science*, Vol. 13, no. 4, pp. 247-254, November 2008.
12. J. F. Hasler. **Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle,** *Theriogenology*, Vol. 56, no. 9, pp. 1401-1415, December 2001. https://doi.org/10.1016/S0093-691X(01)00643-4
13. M. Thibier. **The zootechnical applications of biotechnology in animal reproduction: current methods and perspectives**, *Reproduction Nutrition Development*, Vol. 45, no. 3, pp. 235-242, May-June 2005. https://doi.org/10.1051/rnd:2005016
14. L. Oyuela and C. Jimenez. **Factores Que Afectan La Tasa De Preñez En Programas De Transferencia De Embriones**, *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*, Vol. 57, no. 3, pp. 191-200, Septiembre-Diciembre 2010.
15. J. S. Merton et al. **Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry**, *Theriogenology*, Vol. 59, no. 2, pp. 651-674, January 2003. https://doi.org/10.1016/S0093-691X(02)01246-3
16. R. D. Wilson et al. **In Vitro Production of Holstein Embryos Using Sex-Sorted Sperm and Oocytes from Selected Cull Cows**, *Journal of Dairy Science*, Vol. 88, no. 2, pp. 776-782, February 2005. https://doi.org/10.3168/jds.S0022-0302(05)72741-7
17. E. Eckert, H. E. Brown, K. E. Leslie, T. J. DeVries, and M. A. Steele. **Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage**, *Journal of Dairy Science*, Vol. 98, no. 9, pp. 6315-6326, September 2015. https://doi.org/10.3168/jds.2014-9062
18. U. Moallem et al. **Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production**, *Journal of Dairy Science*, Vol. 93, no. 6, pp. 2639-2650, June 2010. https://doi.org/10.3168/jds.2009-3007
19. S. J. Morrison et al. **The effect of calf nutrition on the performance of dairy herd replacements**, *Animal*, Vol. 6, no. 6, pp. 909-919, June 2012. https://doi.org/10.1017/S1751731111002163

T. M. Hill, J. M. Aldrich, R. L. Schlotterbeck, and H. G. Bateman. **Effects of Feeding Calves Different Rates and Protein Concentrations of Twenty Percent Fat Milk Replacers on Growth During the Neonatal Period**, *The Professional Animal Scientist*, Vol. 22, no. 3, pp. 252-260, June 2006. https://doi.org/10.15232/S1080-7446(15)31101-3

20. M. Terré, M. Devant, and A. Bach. **Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning**, *Livestock Science*, Vol 110, no. 1-2, pp. 82-88, June 2007. https://doi.org/10.1016/j.livsci.2006.10.001
21. A. M. de Passillé, T. F. Borderas, and J. Rushen. **Weaning age of calves fed a high milk allowance by automated feeders: Effects on feed, water, and energy intake, behavioral signs of hunger, and weight gains**. *Journal of Dairy Science*, Vol. 94, no. 3, pp. 1401-1408, March 2011. https://doi.org/10.3168/jds.2010-3441
22. R. D. Galvis, D. Agudelo, and A. Saffon. **Condición corporal , perfil de lipoproteínas y actividad ovárica en vacas Holstein en lactancia temprana**, *Revista Colombiana de Ciencias Pecuarias*, Vol. 20, no. 1, pp. 16-29, March 2007.
23. E. C. Jones, M. M. Parast, and S. G. Adams. **A framework for effective Six Sigma implementation**, *Total Quality Management & Business Excellence*, Vol. 21, no. 4, pp. 415-424, April 2010. https://doi.org/10.1080/14783361003606720
24. M. O. Barrios and H. F. Jiménez. **Reduction of average lead time in outpatient service of obstetrics through six sigma methodology**. *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, volume 9456, pp. 293-302, December 2015.
25. M. A. Ortiz Barrios and H. Felizzola Jiménez. **Use of Six Sigma Methodology to Reduce Appointment Lead-Time in Obstetrics Outpatient Department**. *Journal of Medical Systems*, Vol. 40, no. 10, pp. 1- 15, October 2016. https://doi.org/10.1007/s10916-016-0577-3
26. C. Orgel, I. Ruddat, and M. Hoedemaker. **Prevalence and severity of lameness in early lactation in dairy cows and the effect on reproductive performance**. *Tierarztl Prax Ausg G Grosstiere Nutztiere*, Vol. 44 no. 4, pp. 207-117, August 2016.
27. D. Schams and B. Berisha. **Regulation of corpus luteum function in cattle - An overview**. *Reproduction in Domestic Animals*, Vol. 39, no. 4, pp. 241-251, August 2004. https://doi.org/10.1111/j.1439-0531.2004.00509.x

28. L. R. Pimentel et al. **Feeding behavior of lactating cows fed palm kernel cake in the diet**. *Acta Scientiarum. Animal Sciences*, Vol. 37, no. 1, pp. 83-89, January – March 2015.
29. M. F. Sá Filho, J. E. P. Santos, R. M. Ferreira, J. N. S. Sales, and P. S. Baruselli. **Importance of estrus on pregnancy per insemination in suckled Bos indicus cows submitted to estradiol/progesterone-based timed insemination protocols**. *Theriogenology*, Vol. 76, no. 3, pp. 455-463, August 2011. https://doi.org/10.1016/j.theriogenology.2011.02.022
30. F. Morotti et al. **Pregnancy rate and birth rate of calves from a large-scale IVF program using reverse-sorted semen in Bos indicus, Bos indicus-taurus, and Bos taurus cattle**. *Theriogenology*, Vol. 81, no. 5, pp. 696-701, March 2014. https://doi.org/10.1016/j.theriogenology.2013.12.002
31. J. D. Quigley, T. A. Wolfe, and T. H. Elsasser. **Effects of Additional Milk Replacer Feeding on Calf Health, Growth, and Selected Blood Metabolites in Calves**. *Journal of Dairy Science*, Vol 89, no. 1, pp. 207-216, January 2006. https://doi.org/10.3168/jds.S0022-0302(06)72085-9