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APPLICATION OF MICROSATELLITE-BASED PARENTAGE VERIFICATION USING VeriSire™ IN SELECTED CATTLE FARMS IN THE PHILIPPINES

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ABSTRACT

Parentage verification using microsatellite markers is yet to be fully appreciated in the local cattle industry. Breeder experiences have proven that parentage testing, in combination with well-run breeding programs, can ensure accurate pedigrees which are necessary for genetic improvement and to avoid unintentional inbreeding. However, keeping such records is a widespread challenge among cattle raisers specifically in keeping their bull records. Thus, this study aims to validate the pedigree of animals in farms with different breeding methods using VeriSire™ - a DNA-based test to verify the true sire of a calf. Blood and hair follicle samples were collected from four local cattle farms. Subsequent processes of DNA extraction, PCR amplification using 16 markers pooled into 4 panels, gel electrophoresis, capillary electrophoresis, and parentage assignment were performed. The study underscores the practicality of using gDNA extracted from hair follicle samples as an alternative to blood samples. Our results revealed that through parentage verification, the pedigree information of the target animals can be corrected using VeriSire™. The analysis identified the true sire of 20 animals in a farm utilizing more than 20 bulls for breeding. In three other farms that utilize AI and a combination of both, sire mismatches were identified, and the pedigree of 26 animals was corrected and validated. These results are strong indications that either one breeding method can be compromised when breeding programs are not properly implemented. This research emphasizes the importance and efficacy of rigorous pedigree verification methods in ensuring the integrity and genetic progress of breeding programs in the livestock industry.

Keywords: cattle, microsatellite markers, parentage verification, pedigree, VeriSire

INTRODUCTION

Livestock biotechnologies generally aim to improve animal productivity through techniques for enhancing genetic potentials for improved nutrition and nutritional utilization, improved animal health and welfare, and enhanced reproduction. Initiatives on the use of other reproductive biotechniques such as embryo transfer, in-vitro embryo production, and

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ovum pick up as part of national genetic improvement are mostly confined to water buffaloes and a very limited extent on bovine and small ruminants. To maximize the female genetics' contribution to the gene pool, the use of embryo production through multiple ovulations or in-vitro embryo production can be explored in local cattle.

The local cattle industry has not fully utilized these biotechnologies in their operation which could potentially increase their productivity. The recent projects of the PCC on the application of livestock biotechniques in the field of reproduction and molecular genetics on buffaloes, swine, and goats prompted the members of the Federation of Cattle Raiser's Association of the Philippines (FCRAP) and some individual dairy farms to signify their intention to collaborate with PCC in these fields.

Among the challenges in the animal industry is that due to low artificial insemination (AI) efficiency farms use multiple bulls for natural service to sustain production. The impact of a bull with superior genetics is therefore limited and this affects the productivity of the industry. Aside from that, improper implementation of breeding programs was observed since some use the combination of both AI and natural mating which affects the animal records. Inaccuracy in parentage records leads to diminished precision in genetic parameter estimation and genetic evaluation (Hu *et. al.,* 2021).

For decades, horse and cattle breeders from all over the world, especially in North America and Europe, have utilized pedigree verification in their registration programs. Breeder experiences have proven that parentage testing, in combination with well-run breeding programs, can ensure accurate pedigrees. In a study by Makkar and Viljoen (2005), the effectiveness of using microsatellite or simple sequence repeats (SSR) marker analysis to demonstrate DNA polymorphism of Arabian, Thoroughbred, and Anglo-Arab horses was shown, thereby providing an effective and useful tool for horse breeding and horse registries. To date, nine microsatellite markers are already recommended for use in cattle for pedigree verification. Single nucleotide polymorphism (SNP) markers are also available. However, due to the bi-allelic nature of SNPs, the test will require two to three times more markers to approximate the power of the markers. SSR markers are also available and are being tested for goats, sheep, swine, and horse among others.

Currently, the Department of Agriculture – Philippine Carabao Center (DA-PCC) has a working set of 16 SSR markers for parentage verification registered IPOPhil (VeriSire™) with Registration No. 2/2020/050497 as a utility model that works for both cattle and buffaloes. VeriSire™ is a DNA-based test to verify the true sire or father of your calf. Specific DNA microsatellite markers are passed on from parents to offspring and it is this set of markers that are matched between parent and offspring to verify and determine paternity. Mismatches between putative parent and offspring on paired microsatellite markers will exclude non-parent.

This parentage testing is a pioneering technology in the Philippines and once applied on a larger scale, would be a game-changer in the local cattle industry in strengthening the breeding programs for the next several years. The set of markers used was taken from the International Society for Animal Genetics (ISAG) and Food and Agriculture Organization (FAO) of the United Nations recommended markers for parentage testing and genetic diversity and from previous DOST-PCAARRD funded projects. As the data set of cattle genetic profiles is small and does not include the current sires of dairy and beef cattle, capturing the genetic profiles of current sires is necessary for the service to be offered widely to the industry.

VeriSire™ addresses the challenge of accurately determining the true sire in situations where multiple clean-up bulls are used. This biotechnology developed by DA-PCC utilizes advanced genetic testing protocols, offering valuable applications for both beef and dairy cattle operations, including purebred and seed stock farms. By contributing to the enhancement of reproductive efficiency and enabling the identification of genetically superior animals, VeriSire™ supports the growth and sustainability of the livestock industry. This study aims to validate existing pedigree records from participating local cattle farms employing various breeding programs, such as the use of multiple bulls through natural mating, artificial insemination (AI), and combinations of these methods. Additionally, it seeks to demonstrate the practicality of using hair follicle samples for reliable genetic analysis.

MATERIALS AND METHODS

Animals

Animals with pedigree, production and reproduction records owned by two dairy cattle farms in Batangas, and a beef cattle farm in Laguna and Masbate were used in this study. Sampling at least 15% of the breeding herd ensures sufficient representation of genetic diversity across herds (Cochran, 1977). This proportion is aligned with standard practices in population genetics and livestock studies.To have good representation from various herds in different locations, at least 15% of the breeding herd were sampled. Blood and hair follicle samples were collected and processed following protocols adopted by DA-PCC with regards to genetic material sample collection, processing, and handling. The collection of blood samples was accomplished following policies and rules on animal care and welfare under the Republic Act 8485.

DNA extraction

DNA samples were extracted using two protocols – for blood and hair follicle samples. The genomic DNA (gDNA) from blood samples was isolated using a Promega Wizard® DNA Purification Kit (Promega Corporation, USA) with minor modifications. On the other hand, gDNA from hair follicle samples were extracted using Qiagen DNeasy® Blood and Tissue Kit (Qiagen, Germany). The eluted DNA samples were stored at 4°C. The DNA concentration and purity were quantified using the Thermo Fisher Scientific NanoDrop Spectrophotometer (USA).

Polymerase chain reaction amplification

Amplification of genomic DNA was performed using an ESCO thermal cycler through the process of polymerase chain reaction (PCR). The PCR component includes sdH2O, 4 µL of 10x PCR buffer with 15mm MgCl2, 1.6 µL of 2.5mM dNTPs, 10ng Forward and Reverse Primers, 0.4 µL of Taq Polymerase, and 2 µL DNA. In this study, sixteen labeled primers grouped into panels according to their dyes, sizes, GC contents, and co-amplification compatibility were used. The following PCR profile was the optimum setting for co-amplification to all panels which subsequently follows initial denaturation at 95°C for 10 minutes, 30 cycles of denaturation at 95°C for 15 seconds, annealing temperature at 58°C for 30 seconds, extension at 72°C for another 30 seconds, and then with a final extension

of 72°C for 10 minutes. PCR products were loaded on 2% agarose gel to check and semi-quantify the amplicons. Fifty base pair ladder was used to determine the sizes of amplicons. Mupid-Ex gel electrophoresis was used to run the PCR products while Enduro GST Gel documentation was used to view gel products.

Fragment analysis

After quantifying PCR products using slab gel, it was then subjectively graded from 1-100 dilution before fragment analysis using ABI 3500xl Genetic Analyzer in the Molecular Genetics Laboratory of the DA-PCC. The exact allele size of the microsatellite loci was determined through the detection of fluorescently labeled primers. GeneScan 600 LIZ size standard was used for standard sizing. Ten microliter mixture of highly deionized (Hi-DiTM) formamide and GeneScan 600 LIZ size standard was used to resuspend sample before electrokinetic injection on Capillary Electrophoresis (CE) using Applied Biosystems 3500xl Genetic Analyzer by Thermo Fisher Scientific.

Gene mapping and parentage analysis

The generated .ab1 file that contains the loci sizing was analyzed using GeneMapper® software for individual allele size calling. The important generated file of the software was the allele calls of the samples and since the markers used in this study were dinucleotides, two allele calls were expected to be generated. Further, Cervus version 3.0.7 was used for the computation of genetic parameters and the simulation of parentage assignment. Parentage reassignments with 95% strict confidence were assigned as true parents. Assignment with 80% strict confidence suggests a close relationship but does not establish parentage.

Statistical analysis

Comparison of the mean between groups was calculated using SPSS version 16. One-way analysis of variance was used to assess the difference among means.

RESULTS AND DISCUSSION

This study evaluated the gDNA concentration and purity of blood and hair samples to assess whether hair samples can serve as an efficient alternative to blood samples for isolating gDNA for parentage verification. Additionally, it aimed to determine the long-term viability of the samples for storage, examining whether viable DNA could still be extracted over time.

Table 1. Comparison of mean concentration and purity of samples between groups of sampling method.

SAMPLE	CONCENTRATION	PURITY
	Mean ± SD	Mean ± SD
Blood	392.74 ± 580.41	1.81±0.055[b]
Hair	326.71 ± 175.19	1.97±0.076[a]

*Superscript between columns mean significant at 95% level of confidence

Table 1 indicates that gDNA extracted from blood and hair samples was comparable in terms of concentration. However, a significant deviation was observed in the concentration of blood samples, likely due to differences in collection periods. Blood is more prone to degradation over time because of enzymatic activity and environmental factors which can reduce the integrity of its genetic material, unlike the more stable structure of hair follicles (Tan & Yiap, 2009; Bhardwaj *et al.*, 2020). Moreover, a significant difference was observed between groups in terms of gDNA purity. This difference may be attributed to the presence of single-stranded DNA and slight degradation in blood samples, which impacts their purity compared to hair follicle samples. Studies suggest that single-stranded DNA or partially degraded material can reduce the purity ratio in spectrophotometric analyses, further contributing to the observed variation (Wilfinger *et al.*, 1997).

Although blood samples are the preferred source of DNA for genetic studies, their collection poses several challenges. Collecting blood requires specialized skills, proper tools, and assistance, particularly for restraining animals to obtain sufficient quantities. Additionally, blood samples must be stored under strict conditions, such as at 4°C or lower, or cryopreserved, to maintain their integrity. Transporting blood also demands careful handling, with storage temperatures maintained between 4°C and 20°C, which can add to logistical complexities and costs.

In comparison, hair follicle samples are much easier and more practical to collect. They require minimal tools and assistance and are less sensitive to handle. For example, in this study, hair follicle samples were transported inter-island via standard courier services, a cost-effective alternative to the more expensive transport methods needed for blood samples. Unlike blood, hair follicle samples can be stored at room temperature without compromising their quality. These factors make hair follicle samples a more practical and convenient choice for local cattle farms opting to avail the parentage testing services offered by the Molecular Genetics Laboratory of DA-PCC.

Sequential multiple bulls

The use of multiple bulls for natural mating is quite common in some local cattle farms in the Philippines. It is assumed that using multiple bulls is a good strategy for successful breeding. However, Hamilton (2007) concluded that an increase in the probability of a successful breeding season can be achieved through proper selection and management of cleanup bulls. In this study, beef cattle farm A uses multiple bulls in their breeding program since they manage a large cattle population. Artificial insemination (AI) was also applied seasonally. The request for parentage testing is to identify the dominant bulls in terms of reproduction for line breeding and preserve the superior genetics to be used for future breeding programs. Fifteen samples out of 28 bulls (MULTIPLE) were used for breeding, and ten offspring were sent for analysis. As presented in Table 2, the parentage test revealed that among the samples submitted only three animals were assigned as true sires namely M2, M3, and M4 of Offspring 1, 2, and 3, respectively, at 95% strict confidence (SC) with zero mismatches as presented in Table 3.

This result demonstrates that VeriSire™ can call true sires despite multiple bull breeding schemes of beef cattle farm A. True parents of the remaining samples were most likely not included in the pool of samples submitted, thus, the Cervus program was unable to assign them.

Additionally, samples from new calves produced were collected and genotyped.

Analysis using 21 probable sires showed that out of 125 calves tested, only 17 have putative sires which are verified as true parents at 95% SC with 0 to 2 mismatches.

Table 2. Overview of parentage result of beef cattle farm A utilizing multiple bulls.

OFFSPRING	BREED	RECORDED PARENTS		ASSIGNED PARENTS	
		Dam ID	Sire ID	Dam ID	Sire ID
Offspring 1	Brahman	F1	MULTIPLE	-	M2
Offspring 2	Brahman	F2	MULTIPLE	-	M3
Offspring 3	Brahman	F3	M1	-	M4

**Animal IDs in green text are verified true parents, red indicates a mismatch*

Table 3. Number of mismatches in loci of parent pairs in beef cattle farm A.

OFFSPRING	SIRE	PAIRED LOCI		RESULT
		MISMATCH	SC	
Offspring 1	M2	0	95%	True Parent
Offspring 2	M3	0	95%	True Parent
Offspring 3	M4	0	95%	True Parent

Artificial insemination

Baruselli, et. al. (2018a) explained that the application of AI is a reliable tool for improving genetic progress and controlling venereal diseases in the herds, but the program should be adequate for the farm conditions to maintain reproductive efficiency.

For this case, parentage verification was applied to validate the pedigree records of the participating dairy cattle farm that applies Fixed-time AI (FTAI) in their breeding program. Thirteen offspring were tested against seven bulls to crosscheck whether the calves were produced via FTAI since semen samples used were limited and not provided for testing. For Calves 1 and 2, the recorded dam matched with the assigned true parents as presented in Table 4, thus, is verified true dam. However, the recorded sire for Calf 1 is the semen used in FTAI, but the assigned true parent upon analysis is the cleanup bull Sire3. After conducting FTAI, a dam subjected to FTAI probably mated with a bull causing the mismatch in the records.

Meanwhile, no true parents were assigned for the remaining offspring, instead, Cervus assigned closely- related samples with 80% strict confidence which does not indicate true parentage. This case is observed on the recorded dam versus the assigned dam which possibly implies that there are errors in the recording system. True parents of the remaining samples below 80% SC were most likely not in the pool of samples submitted. Table 5 presents the paired loci-data as the basis of the result. It should be noted that low mismatch does not always indicate true parentage like in the paired loci data of Calf 3 and 4. Both the mismatch and strict confidence level are considered during the analysis.

Table 4. Overview of parentage result of dairy cattle farm A utilizing AI.

OFFSPRING	BREED	RECORDED PARENTS		ASSIGNED PARENTS	
		Dam ID	Sire ID	Dam ID	Sire ID
Calf 1	Cross Holstein	Dam1	Sire1	Dam1	Sire3
Calf 2	Cross Holstein	Dam2	Sire2	Dam2	Sire4
Calf 3	Cross Holstein	Dam3	Sire1	Dam3	Sire5
Calf 4	Cross Holstein	Dam4	-	Dam8	Sire4
Calf 5	Cross Holstein	Dam5	Sire1	Dam9	Sire6
Calf 6	Cross Holstein	Dam6	Sire1	Dam6	Sire3

**Animal IDs in green text are verified true parents, red indicates a mismatch*

Table 5. Number of mismatches in loci of parent pairs in dairy cattle farm A.

OFF-SPRING	DAM	PAIRED LOCI		RESULT	SIRE	PAIRED LOCI		RESULT
		MIS-MATCH	SC			MIS-MATCH	SC	
Calf 1	Dam1	1	95%	True Parent	Sire3	1	95%	True Parent
Calf 2	Dam2	1	95%	True Parent	Sire4	2	<80%	NAP
Calf 3	Dam3	1	-	NAP	Sire5	1	<80%	NAP
Calf 4	Dam8	1	<80%	NAP	Sire4	1	<80%	NAP
Calf 5	Dam9	1	<80%	NAP	Sire6	2	-	NAP
Calf 6	Dam6	1	80%	NAP	Sire3	4	-	NAP

*NAP- No assigned parent

Simultaneous use of artificial insemination and natural mating

One of the practices observed in some local cattle farms is the simultaneous use of AI and natural mating which is not recommended. This does not only affect the pedigree records but also the AI efficiency. For this case, the participating dairy cattle farm keeps the bulls in after conducting AI. Nineteen offspring and seven probable sires – six cleanup bulls, and one semen sample; were analyzed for parentage. Table 6 presents the results of the representative samples. The recorded parents of Animals 8, and 9 were validated to be true parents based on the paired loci data indicated in Table 7. This case illustrates that validation requires a known and properly sampled trio for establishing the reliability of assigning and

validating the true parents. In the case of Animal 1, two sires were recorded, but Cervus assigned F2 as the true parent, thus pedigree is corrected. Furthermore, the recorded dam for the Animals 2, 3, 10, and 11; and the recorded sire for Animal 7 were called as the true parent.

One case observed in this pool of samples is the sire recorded for Animal 4 is F3 which is via AI, but Cervus assigned the cleanup bull F6 as the true parent. This case shows that the AI efficiency is somehow compromised when bulls are kept in for natural mating. Moreover, the Veri-Set 2 with an additional 12 SSR markers is recommended for analysis of results with 80% and below strict confidence for further analysis since there are still probabilities of identifying the true parent with additional panels. Submission of additional samples is also recommended to properly assign true parents to validate the pedigree records.

Table 6. Overview of parentage result of dairy cattle farm B utilizing AI and natural mating simultaneously.

OFFSPRING	BREED	RECORDED PARENTS		ASSIGNED PARENTS	
		Dam ID	Sire ID	Dam ID	Sire ID
Animal 1	Cross Holstein	M1	F1/F2	M1	F2
Animal 2	Cross Holstein	M2	F3	M2	F5
Animal 3	Cross Holstein	M3	F1/F2	M3	F2
Animal 4	Cross Holstein	M4	F3	M12	F6
Animal 5	Cross Holstein	M5	F3	M6	F2
Animal 6	Cross Holstein	M6	F3	M5	F2
Animal 7	Cross Holstein	M7	F2	M7	F2
Animal 8	Cross Holstein	M8	F2	M8	F2
Animal 9	Cross Holstein	M9	F2	M9	F2
Animal 10	Cross Holstein	M10	F4	M10	F7
Animal 11	Cross Holstein	M11	F3	M11	F2

*****_Animal IDs in green text are verified true parents, red indicates a mismatch_

Alternate use of artificial insemination and natural mating

Proper and correct implementation of biotechnologies such as FTAI generally allows greater reproductive performance than natural breeding (Baruselli et. al., 2018b). Compared with the previous case, this beef cattle farm utilizes alternate use of FTAI and natural mating. Basically, animals were separated from cleanup bulls and subjected to FTAI for a specific season then subjected solely to the cleanup bull the following season. This breeding plan is

more systematic compared with the simultaneous use of AI and natural mating as breeders can easily record information on the pedigree with increased accuracy. Table 8 contains selected samples from a pool of 78 offspring with 30 dams and six sires recorded on the pedigree. Offspring with matching information on the recorded and assigned dam include C6, C8, C9, and C16. The assigned sire for offspring C2, C3, C4, and C13 matched with one of the recorded sires.

The analysis report presented in Table 9 showed that only the offspring C15 have verified true parent pair, however, this result is still not parallel with the recorded pedigree. Overall, there are eight verified true dams and sires among the 15 representative offspring.

Table 7. Number of mismatches in loci of parent pairs in dairy cattle farm B.

OFF-SPRING	DAM	PAIRED LOCI		RESULT	SIRE	PAIRED LOCI		RESULT
		MIS-MATCH	SC			MIS-MATCH	SC	
Animal 1	M1	0	95%	True Parent	F2	1	95%	True Parent
Animal 2	M2	0	95%	True Parent	F5	3	<80%	NAP
Animal 3	M3	1	95%	True Parent	F2	1	<80%	NAP
Animal 4	M12	1	<80%	NAP	F6	0	95%	True Parent
Animal 5	M6	1	80%	NAP	F2	1	<80%	NAP
Animal 6	M5	2	80%	NAP	F2	2	<80%	NAP
Animal 7	M7	0	80%	NAP	F2	0	95%	True Parent
Animal 8	M8	0	95%	True Parent	F2	1	95%	True Parent
Animal 9	M9	1	95%	True Parent	F2	0	95%	True Parent
Animal 10	M10	0	95%	True Parent	F7	3	-	NAP
Animal 11	M11	2	95%	True Parent	F2	5	<80%	NAP

**NAP – No assigned parent*

All in all, the consolidated results for the four different cases presented in this paper demonstrate the practical use of parentage testing in verifying whether the pedigree of each

participating farm is accurately recorded. This can be a stepping stone in improving the recording system in each farm and eventually reestablishing a more efficient and productive breeding program. Also, through parentage verification, the animals with superior genetics will be traceable and can be fully utilized for breeding to establish a more productive breeding herd. The establishment of a database for breeding bulls can be achieved when the farms opt to have the whole herd undergo parentage testing. This will pave the way for a more systematic and efficient breeding scheme.

Moreover, the Veri-Set 2 with an additional 12 markers is recommended for analysis of results with 80% strict confidence for further analysis since there are still probabilities of identifying the true parent with additional panels. Submission of additional samples is also recommended to properly assign true parents and validate the pedigree records.

Table 8. Overview of parentage result of beef cattle farm B utilizing AI and natural mating alternately.

OFFSPRING	BREED	RECORDED PARENTS		ASSIGNED PARENTS	
		Dam ID	Sire ID	Dam ID	Sire ID
C1	Brahman	-	B1/B2/B3	G9	B6
C2	Brahman	G1	B4/B5	G10	B4
C3	Brahman	G2	B4/B5	G2	B4
C4	Brahman	-	B4/B5	G11	B4
C5	Brahman	G3	-	G9	B6
C6	Brahman	G4	-	G4	B6
C7	Brahman	-	-	G12	B6
C8	Brahman	G5	B6	G5	B6
C9	Brahman	G6	-	G6	B7
C10	Brahman	-	B6	G6	B7
C11	Brahman	G7	-	G6	B3
C12	Brahman	-	-	G6	B3
C13	Brahman	-	B1/B2/B3	G13	B3
C14	Brahman	-	B6	G6	B3
C15	Brahman	G8	B4/B5	G8	B3

**Animal IDs in green text are verified true parents, red indicates a mismatch*

Table 9. Number of mismatches in loci of parent pairs in the participating beef cattle farm alternately utilizing AI and natural mating.

OFF-SPRING	DAM	PAIRED LOCI		RESULT	SIRE	PAIRED LOCI		RESULT
		MIS-MATCH	SC			MIS-MATCH	SC	
C1	G9	2	95%	True Parent	B6	5	<80%	NAP
C2	G10	4	<80%	NAP	B4	1	95%	True Parent
C3	G2	4	<80%	NAP	B4	2	95%	True Parent
C4	G11	6	<80%	NAP	B4	2	95%	True Parent
C5	G9	5	80%	NAP	B6	2	95%	True Parent
C6	G4	2	95%	True Parent	B6	4	<80%	NAP
C7	G12	4	<80%	NAP	B6	2	95%	True Parent
C8	G5	2	95%	True Parent	B6	3	<80%	NAP
C9	G6	1	95%	True Parent	B7	7	<80%	NAP
C10	G6	2	95%	True Parent	B7	5	<80%	NAP
C11	G6	4	<80%	NAP	B3	2	95%	True Parent
C12	G6	2	95%	True Parent	B3	6	<80%	NAP
C13	G13	2	80%	NAP	B3	0	95%	True Parent
C14	G6	0	95%	True Parent	B3	3	80%	NAP
C15	G8	1	95%	True Parent	B3	0	95%	True Parent

**NAP – No assigned parent*

CONCLUSION

This study demonstrates the practical application of parentage testing using VeriSire™ in different cases of breeding programs in local cattle farms. The number of samples submitted was only limited, thus some parents were not properly assigned and

pedigrees were not completely corrected. The data suggest that further verification using Veri-Set 2 for animals with 80% and below strict confidence level should be applied to determine whether the assigned parents are just close kins or true parents when additional 12 SSR makers are used. Results showed that details included in the pedigree of participating farms were not keenly recorded as Cervus was not able to assign the true parents. This implies that parentage analysis is an effective method in correcting pedigree records, and identifying and/or validating the true parents of the offspring in a pool of known samples regardless of the breeding scheme applied in farms. Well-kept records are important in preserving superior genetics and for efficiently designing effective breeding programs suitable for different farm conditions, facilities, and management capabilities.

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REFERENCES

Baruselli PS, Ferreira RM, Sá Filho MFD, and Bó GA. 2018a. Using artificial insemination vs. natural service in beef herds. *Animal* 12(1): 45-52.

Baruselli PS, de Souza AH, de Sá Filho MF, Marques MO, and de Sousa Sales JN. 2018b. Genetic market in cattle (Bull, AI, FTAI, MOET and IVP): financial payback based on reproductive efficiency in beef and dairy herds in Brazil. *Anim Repro* 15(3):247.

Bhardwaj R, Sharma N, and Sharma S. 2020. Degradation of DNA samples: Causes and remedies. *Forensic Sci Rev* 32(1): 35-45.

Cochran JR and Talwani, M. 1977. Free-air gravity anomalies in the world's oceans and their relationship to residual elevation. *Geophys J Int* 50(3):495-552.

Hamilton T. 2007. *Maximizing beef bull fertility and reproduction*. OMAFRA. Retrived from http://www.thebeefsite.com/articles/838/maximizing-beef-bull-fertilityand-reproduction.

Hu LR, Li D, Chu Q, Wang YC, Zhou L, Yu Y, and Shi WH. 2021. Selection and implementation of single nucleotide polymorphism markers for parentage analysis in crossbred cattle population. *Animal* 15(1):100066.

Makkar HPS and Viljoen GJ. 2005. *Applications of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries*, Dordrecht, The Netherlands: Springer.

Tan SC and Yiap BC. 2009. DNA, RNA, and protein extraction: The past and the present. *J Biomed Biotechnol* 2009:574398.

Wilfinger WW, Mackey K and Krug DE. 1997. RNA integrity number and spectrophotometric assessment of purity in nucleic acid samples. *Biotechniques* 22(3):556-563.