UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

The genetic mechanisms and inheritance of the polled, scur and horn phenotypes of South African Bonsmara cattle

by

Rulien Grobler

Submitted in fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR (ANIMAL SCIENCE)

With specialization in Animal Breeding and Genetics

In the
Department of Animal Science
Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria
South Africa

February 2022

© University of Pretoria

Supervisory committee

Supervisor

Prof E. van Marle-Köster Department of Animal Science
University of Pretoria
Private Bag X20
Hatfield
0028
South Africa

Co-supervisor

Prof C. Visser Department of Animal Science
University of Pretoria
Private Bag X20
Hatfield
0028
South Africa

© University of Pretoria

To all the women in my life,

who endured and survived so much,

yet conquered unimaginable trials with pride and courage.

You inspired me to always be strong and persevere.

© University of Pretoria

Declaration

I, Rulien Grobler, hereby declare that:

I understand what plagiarism is and I am aware of the University's policy in this regard;

This dissertation is my own original work;

I did not refer to work of current or previous students, textbooks, or any other study material without proper referencing;

Where other people's work has been used this has been properly acknowledged and referenced;

I have not allowed anyone to copy any part of my dissertation;

I have not previously, in its entirety or in part, submitted this dissertation for a degree at any other tertiary institution.

Signature: 

Date: October 2021

© University of Pretoria

Acknowledgements

I would like to thank the following persons who contributed to the success of this dissertation:

Thank you, Lord, for your abundant grace and giving me the strength and ability to complete this degree. Thank you for your provision through every step of this journey.

To my supervisors, Prof Esté van Marle-Köster and Prof Carina Visser, thank you for your expert guidance and patience throughout the completion of this dissertation. I want to thank you for every opportunity and learning experience during my PhD journey. But most of all, thank you for the instrumental mentorship that you provided and for being such inspiring role models.

To my parents, thank you for your unwavering support, unconditional love, for always believing in me and for encouraging me to follow my dreams. Thank you for the sacrifices you made so that I could have opportunities and a better life.

To Charl Uys, thank you for being passionate about research and sharing your knowledge. Thank you for contributing phenotypic records and allowing us to phenotype and sample animals on your farm. Without your enthusiasm, knowledge and willingness to teach me all you know, this research project would not have been possible.

Thank you to every farmer who contributed samples and phenotypic records to the Polled project at the University of Pretoria.

Thank you to Bonsmara SA for granting permission to conduct this research project and thank you for your interest in research. Thank you to SA Stud Book for providing pedigree, phenotypic and genotypic data.

I would like to acknowledge the Beef Genomics Program (BGP) and the Red Meat Research and Development (RMRD) for funding of the project. The financial assistance of THRIP and the National Research Foundation (NRF) towards this research by providing a bursary is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

Thank you Jason Reding and Antoinette van Wyk for assisting with sorting of samples and laboratory work. Thank you for being so eager to learn and assist.

© University of Pretoria

To all my friends and extended family, thank you for your interest in my PhD journey and for your support and love. I would like to especially mention the following people:

Michael, thank you for your friendship, prayers, understanding and never ending support in a time when I needed it the most.
Jani, thank you for always listening, understanding and the encouragement in difficult times. I will always cherish our friendship and the memories we made during this journey.
Simon, thank you for accompanying me on sampling trips, for always being willing to help and mostly for always listening. Thank you for all the good (and bad) times we could share. I will always treasure the countless memories we made during our PhD journeys.

© University of Pretoria

Abstract

Breeding genetically polled animals provide a welfare friendly alternative and a long-term solution to dehorning. The availability of high through-put genomic technology enables the study of the genetic mechanisms of polled and scurs phenotypes with higher precision, compared to microsatellite markers. The majority of research to date on the *POLLED* locus has been performed in European and Australian cattle breeds. The primary aim of this study was to investigate the genetic basis of the polled and scurs phenotypes in the South African Bonsmara breed by using genomic SNP data from phenotyped animals. To validate the Celtic mutation as the causative mutation for polledness in the Bonsmara breed, 386 animals (164 Bonsmara, 133 Drakensberger and 89 Hereford) were screened for the Celtic mutation of the *POLLED* locus. Phenotypically polled and scurred animals were found to carry at least one copy of the Celtic allele (P_C) whereas horned animals were homozygous wildtype. The majority of Bonsmara animals tested were heterozygous polled (P_C = 0.591) with a low frequency of homozygous polled animals observed (P_CP_C = 0.177). A total of 224 heterozygous polled animals were genotyped with the GGP 150K bovine array (139 376 SNPs) to map the *SCURS* locus implementing a case-control study design. The scurs phenotype in the Bonsmara was associated with six different SNPs across three different chromosomes (BTA3, BTA10 and BTA17). The current study identified four candidate genes (*FBN1*, *LRIG2*, *PHGDH*, *GUCY1B1*) across three chromosomes which are involved in various molecular functions and biological processes, including amino acid metabolic processes, regulation of cell signals and processes involved with the central nervous system development. Further characterization of the identified candidate genes from this study may be useful for identifying possible causal mutations for scurs. This research indicated discrepancies and difficulties in recording of the polled phenotype which is exacerbated by the prevalence of the scurs phenotype in the Bonsmara breed. An outcome of this study was the compilation of a phenotyping protocol for Bonsmara farmers for accurate identification of the polled and scurs phenotypes. Accurate phenotypic identification of the polled and scurs phenotypes in the Bonsmara will contribute towards more effective selection and accelerated fixation of the polled allele in Bonsmara herds. Genotypic identification of polledness to distinguish between heterozygous and homozygous polled animals are recommended. Since homozygous polled animals are limited in the Bonsmara breed, breeders are advised to place more selection pressure on polledness to increase the number of polled animals within the breed.

© University of Pretoria

Thesis outputs

Publications

Peer-review journals

Grobler, R., Visser, C., Capitan, A. & van Marle-Köster, E., 2018. Validation of the *POLLED* Celtic variant in South African Bonsmara and Drakensberger beef cattle breeds. Livestock Science. 217, 136-139.

Grobler, R., van Marle-Köster, E. & Visser, C., 2021. Challenges in selection and breeding of polled and scur phenotypes in beef cattle. Livestock Science. 247, 104479.

Popular science articles

Grobler, R., 2017. A DNA based approach to breeding polled South African Drakensberger. Drakensberger Newsletter, pp 12-13.

Grobler, R. & van-Marle-Köster, E., 2018. Moving towards DNA technology for selection of polled beef cattle. Tuli Cattle Breeders' Society, pp 58-60.

Grobler, R., 2019**.** Identifikasie van poena status in die Bonsmara met behulp van DNA tegnologie (*Identification of polled status in the Bonsmara using DNA technology*). Dié Rooi Ras (*The Red Breed),* pp 56-57.

Congresses

National

Grobler, R., Bosman, L., Visser, C. & van Marle-Köster, E., 2015**.** Identification of homozygous polled beef cattle based on the Celtic allele. 48th South African Society for Animal Science (SASAS) Congress, Empangeni, 22-24 September 2015.

Grobler, R., Visser, C. & van Marle-Köster, E., 2017. Accelerating selection for polledness in the South African Bonsmara using DNA technology. 50th South African Society for Animal Science (SASAS) Congress, Port Elizabeth, 18 – 21 September 2017.

Grobler, R., Visser, C. & Van Marle-Köster, E., 2018. Doeltreffende identifikasie van poenskop Bonsmara en Drakensberger-vleisbeeste met behulp van DNS-tegnologie (*Accurate identification of polled Bonsmara and Drakensberger beef cattle breeds using DNA technology*). 18de Studentesimposium in die Natuurwetenskappe/18th Student symposium in the Natural Sciences, Pretoria, 25–26 Oktober 2018.

*Ms Grobler was awarded the 1st prize for best poster award

© University of Pretoria

International

Grobler, R., van-Marle-Köster, E., Visser, C. & Capitan, A., 2018. Haplotype variation at the *POLLED* locus in the South African Bonsmara cattle breed. Proceedings of the 11th World Congress on Genetics Applied to Livestock Production (WCGALP), Auckland, New Zealand, 11-16 February.

Grobler, R., Visser, C. & van-Marle-Köster, E., 2019. Mapping the *SCURS* locus in the South African Bonsmara beef cattle breed. 37th International Society for Animal Genetics (ISAG) Conference, Lleida, Spain, 7-12 July.

*Ms Grobler was awarded an ISAG Travel bursary award

Reports to industry

Grobler, R., C. Visser & E. van Marle-Köster, 2017. The genetic mechanisms and inheritance patterns of the polled and scur phenotypes in local South African beef cattle breeds, BGP Workshop, CSIR, Pretoria, South Africa, 16 – 18 October 2017.

Grobler, R., C. Visser & E. van Marle-Köster, 2018. Oorerwingspatrone van die *Poena* en *Scur* gene in inheemse Suid-Afrikaanse vleisbees rasse (*Inheritance patterns of the Polled and Scurs genes in indigenous South African beef cattle breeds*). Drakensberger Breeder's Society Annual General Meeting, Parys, South Africa, 20 June 2018.

Grobler, R., 2019. Die Poena geen in inheemse Suid-Afrikaanse vleisbees rasse (*The Polled gene in indigenous South African beef cattle breeds*). SA Stud Book Elite awards function, Vryburg, South Africa, 12 April 2019.

© University of Pretoria

Table of contents

© University of Pretoria

© University of Pretoria

List of figures

© University of Pretoria

© University of Pretoria

List of tables

© University of Pretoria

© University of Pretoria

List of abbreviations

ARC	Agricultural Research Council
ARC-BTP	Agricultural Research Council Biotechnology Platform
BAC	Bacterial artificial chromosome
BCE	Before Common (or Current) Era
BGP	Beef Genomics Program
bp	Base pair
BTA	Bovine chromosome
CNS	Central nervous system
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
ECM	Extracellular matrix
GGP	GeneSeek® Genomic Profiler
GWAS	Genome-wide association study
HWE	Hardy-Weinberg Equilibrium
LD	Linkage disequilibrium
MAS	Marker assisted selection
NGS	Next-generation sequencing
NSAID	Non-steroidal-anti-inflammatory analgesic drugs
PCA	Principal component analysis
PCR	Polymerase chain reaction
P	Dominant polled allele
p	Recessive horned allele
PP	Homozygous polled
Pp	Heterozygous polled
pp	Horned
QTL	Quantitative trait loci
RFLPs	Restriction fragment length polymorphisms
RH	Radiation hybrid
ROH	Runs of homozygosity
SA	South Africa
SBS	Sequencing-by-synthesis
SNP	Single nucleotide polymorphism
STRs	Short tandem repeats

© University of Pretoria

Chapter 1
Introduction

1.1 Overview

Bos primigenius, the wild ancestor of domesticated *Bos taurus* and *Bos indicus* cattle, has been described based on archaeological evidence as a large long-horned animal (Schafberg & Swalve, 2015). For male animals, the main advantages of large horns were associated with fighting predators and territorial dominance, as well as for competing for females, while for females the benefits were for protecting offspring against predators and competition for resources (Gifford-Gonzalez & Hanotte, 2011; Knierim *et al.*, 2015). Due to domestication and lower selective pressure for horn size, large horns for fighting became redundant and undesirable in farming environments, which led to short horned and hornless cattle (Ajmone-Marsan *et al.*, 2010; Gifford-Gonzalez & Hanotte, 2011).

According to archaeological findings and ancient art depictions, it was evident that horned and polled cattle existed since prehistoric and ancient times. The oldest known discovery of polled cattle was from bone findings in Germany, with the skull fragments dating back to the period 3400 to 3000 BCE (Schafberg & Swalve, 2015). Evidence of polled cattle also dates to Ancient Egypt, as well as several references to the occurrence of polled breeds during roman times in Europe and in Scandinavia around the 13th century (Lauwerier, 2015; Schafberg & Swalve, 2015). A reason for the existence of polled cattle in ancient times was probably due to the ease of handling and managing polled animals, specifically for herding and milking purposes (Wunderlich *et al.*, 2006; Schafberg & Swalve, 2015). Among the modern beef cattle breeds, the Angus and Galloway are some of the oldest and most prominent polled breeds, while the polled mutation in the Hereford was only reported during the 19th century (Williams & Williams, 1952; Lauwerier, 2015).

Cattle breeders historically showed a preference for horned bulls due to a perception that horned animals were more fertile and exhibited stronger male characteristics (Goonewardene *et al.*, 1999; Knierim *et al.*, 2015; Schafberg & Swalve, 2015). This perception may have led to selection preference for horned bulls in a number of breeds. A recent evaluation of breeding bulls in Holstein cattle reported lower breeding values, as measured with a genetic merit index, for polled bulls compared to horned bulls (Windig *et al.*, 2015), but this could be due to the small number of polled sires available and prolonged selection for production traits in horned counterparts (Petherick, 2005; Thompson *et al.*, 2017). There is no scientific evidence to date that polled beef bulls have a lower libido or fertility, compared to horned bulls.

Modern beef production systems are showing a preference for polled cattle for a number of reasons. Polled cattle have the advantage of being more docile with reduced dominant behaviour, and thus a reduced injury risk to animal handlers (Graf & Senn, 1999). In feedlots more polled animals can be accommodated at the feed troughs. A major advantage is the reduced injury and bruising during

© University of Pretoria

transport to the abattoir (Goonewardene *et al.*, 1999; Petherick, 2005). Although there is the option of dehorning, this practice remains a painful procedure, regardless of the method used (Graf & Senn, 1999; Grøndahl-Nielson *et al.*, 1999). There is worldwide pressure for humane practises in animal handling activities, such as dehorning and castration. In 2009 a formal organization ALCASDE, funded by the European Union, was established (Cozzi *et al.*, 2009). The "Declaration of Dusseldorf" was signed in 2012 in Germany, thereby urging farmers, cattle breeders and welfare organizations to promote the use of polled animals (Schafberg & Swalve, 2015). Certain European countries, including France and Germany, have developed breeding programs to introgress the polled allele in the Charolais and German Fleckvieh beef cattle breeds, respectively (Götz et al., 2015).

In South Africa the polled allele has been introgressed in the Bonsmara breed; an indigenous composite breed consisting of 5/8 Afrikaner and 3/8 exotic *Bos taurus* (Bonsma, 1980). In Bonsmara herds the polled trait was either inherited from the Shorthorn/Hereford ancestors from which the breed was developed or from more recent crosses through the upgrading of Red Poll and Red Angus cows to Bonsmara stud status (Schmulian, 2006). It is also possible that some "polled" strains originated from spontaneous mutations, as it has been described in the Charolais breed (Capitan *et al.*, 2011). Bonsmara cattle play an important role in the red meat industry in South Africa, by contributing 42% registered animals towards the national beef population (SA Stud Book, 2016).

In South Africa, the red meat industry plays a major role in livestock production, with an estimated 22 000 commercial farmers, approximately 100 commercial feedlots and 430 abattoirs (DAFF, 2019). The beef industry is a major contributor of animal protein in South Africa and during the past decade (2008 – 2018), South Africa experienced a 28% increase in both beef production and the number of cattle slaughtered. The total amount of beef produced during this period amounted to nine million tons (DAFF, 2019). Furthermore, South Africa is also a net exporter of beef and exports 30 000 tons of beef annually, mainly to Africa and Asia.

Livestock production systems in South Africa is characterized by a unique combination of extensive and intensive production systems (Webb, 2013), and the livestock industry are further divided into a developed commercial sector versus a developing sector (i.e., smallholder farmers). An estimated 45% of the South African cattle population belongs to smallholder farmers (DAFF, 2019). Intensive beef production systems generally entail feedlots (Van Marle-Köster & Visser, 2018), while approximately 69% of agricultural land in South Africa are used for extensive grazing of livestock (Webb, 2013). The majority of commercial beef cattle farmers sell weaner calves, which are then fattened by production systems that range from intensive to semi-extensive systems (Webb & Erasmus, 2013). More than 70% of all beef cattle slaughtered in the developed sector originates from extensive farms followed by finishing in commercial feedlots (Scholtz *et al.*, 2008; Webb, 2013).

Feedlots in South Africa purchase young weaner calves from extensive commercial farmers (Webb & Erasmus, 2013) and there is an industry preference for Bonsmara animals (Webb *et al.*, 2020). Due to a decreased risk of injuries and ease of handling, the majority of feedlots prefer polled or

© University of Pretoria

dehorned calves and usually discriminate against calves with horns, since this incurs additional labour and costs. Furthermore, certain breed societies require that all horned calves be dehorned, in order to conform to predetermined breed standards, or to ensure sufficient intake of calves into feedlot operations. Calves that are dehorned when entering the feedlot, can have reduced weight gains of up to 23% in the first two weeks (Goonewardene *et al.*, 1999). Therefore, it would be preferred to utilise polled animals in commercial farming operations to ensure a large cohort of polled weaner calves for intake into feedlots.

The number of polled animals can successfully be increased by well-organized breeding programs, but the number of genetically superior polled bulls are limited in the Bonsmara breed (Pers. Comm., C. Uys, November 2017, charluys@vodamail.co.za). Additionally, the accurate identification of polled animals, both on a phenotypic and genetic level, are problematic. Challenges include the prevalence of a third phenotype (scurs), the pattern of development of the scurs phenotype and indiscriminate dehorning.

1.2 Aim of the study

Over the past two decades commercial beef producers and feedlots in South Africa have indicated a preference for polled breeds, due to increased awareness of animal welfare and market preferences. In South Africa there are a number of polled breeds of European descent such as the Hereford, Angus, Charolais and Limousin, as well as a few local breeds that introgressed the polled gene, including the South African Bonsmara and Drakensberger. The first research on polled Bonsmara in South Africa was performed at the Department of Animal and Wildlife Sciences (University of Pretoria) (Schmulian, 2006), on request of the Bonsmara breeders society, based on three Bonsmara families using bovine microsatellite markers. The study by Schmulian (2006) indicated that the inheritance of the polled trait in the South African Bonsmara adhered to the model proposed by Long & Gregory (1978) and found linkage between the polled phenotype in the South African Bonsmara and alleles of nine microsatellite markers located on BTA1. Since the completion of this research project, the Bovine genome sequence was completed in 2009 using high through-put molecular technology (Bovine Genome Sequencing and Analysis Consortium, 2009), providing the foundation for the development of high-density SNP arrays for generation of genomic information.

The availability of DNA and high through-put genomic technology holds the potential to provide insight into the genetic mechanisms of polled and scurred animals with higher precision, compared to microsatellite markers. Characterization of the *POLLED* locus has been more complex than expected and to date, neither the causal mutation(s) nor the molecular basis of scurs or African horn have been resolved. The commercial diagnostic tests available for Taurine breeds cannot identify carriers of the scurs phenotype. Furthermore, the majority of previous research on the *POLLED* locus and polledness has been performed in European and Australian cattle breeds.

© University of Pretoria

This study will focus on the locally developed South African Bonsmara beef cattle breed to gain an understanding of the genetic basis of the polled and scurs phenotypes by using genomic SNP data from phenotyped animals. To achieve this aim, the following objectives were set:

1. To evaluate whether the Celtic variant of the *POLLED* locus is the causative mutation for polledness in Bonsmara and Drakensberger cattle
2. To perform a genome-wide association study (GWAS) of the scurs phenotype to identify genomic regions, potential candidate genes and biological pathways governing scurs in Bonsmara cattle
3. To compile a protocol for the identification of the polled and scurs phenotypes in the Bonsmara breed

1.3 Thesis outline

The dissertation is presented in six chapters, which include a literature review and subsequent chapters according to the objectives of the study. Literature was reviewed on the anatomy and morphology of bovine horns, current knowledge on the inheritance patterns of the polled, scurs and horned phenotypes, as well as the advancements made during the past four decades with regards to the characterization of the *POLLED* and *SCURS* loci by using molecular DNA technology. An extract from the literature review was published as a review article in Livestock Science. Chapter three, which entails the validation of the *POLLED* Celtic variant in the Bonsmara and Drakensberger breeds, was published in Livestock Science. In chapter four, a GWAS analysis was performed to investigate genomic regions associated with the scurs phenotype to identify candidate genes, biological mechanisms and molecular pathways governing scurs in the Bonsmara breed. A protocol for the accurate identification of the polled and scurs phenotypes was presented in chapter five, with the aim to assist breeders with phenotypic identification of horn status and development of appropriate selection strategies. A general discussion and a critical review of the research findings, as well as recommendations for future studies were provided in chapter six.

References

Ajmone-Marsan, P., Garcia, J.F. & Lenstra, J.A., 2010. On the origin of cattle: how aurochs became cattle and colonized the world. Evolutionary Anthropology. 19, 148-157.

Bonsma, J.C., 1980. Cross-breeding, breed creation and the genesis of the Bonsmara. In: Livestock Production - A Global Approach. Tafelberg, Cape Town, South Africa. pp90–110.

Bovine Genome Sequencing and Analysis Consortium, 2009. The genome sequence of taurine cattle: a window to ruminant biology and evolution. Science. 324, 522-528.

Capitan, A., Grohs, C., Weiss, B., Rossignol, M.N., Reversé, P. & Eggen, A., 2011. A newly described bovine type 2 scurs syndrome segregates with a frame-shift mutation in TWIST1. PLoS One. 6, 22242.

© University of Pretoria

Cozzi, G., Prevedello, P., Boukha, A., Winckler, C., Knierim, U, Pentelescu, O., Windig, J., Mirabito, L., Kling-Eveillard, F., Dockes, A.C., Veissier, I., Velarde, A., Fuentes, C. & Dalmau, A., 2009. D.2.1.1. Report on dehorning practices across EU member states. Alternatives to castration and dehorning (ALCASDE; SANCO/2008/D5/018).

DAFF (Department of Agriculture, Forestry and Fisheries), 2019. A profile of the South African beef market value chain. Directorate: Marketing, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.

Gifford-Gonzalez, D. & Hanotte, O., 2011. Domesticating animals in Africa: implications of genetic and archaeological findings. Journal of World Prehistory. 24, 1-23.

Goonewardene, L.A., Pang, H., Berg, R.T. & Price, M.A., 1999. A comparison of reproductive and growth traits of horned and polled cattle in three synthetic beef lines. Canadian Journal of Animal Science. 79, 123-127.

Götz, K.U., Luntz, B., Robeis, J., Edel, C., Emmerling, R., Buitkamp, J., Anzenberger, H. & Duda, J., 2015. Polled Fleckvieh (Simmental) cattle - Current state of the breeding program. Livestock Science. 179, 80-85.

Grøndahl-Nielsen, C., Simonsen, H.B., Lund, J.D. & Hesselholt, M., 1999. Behavioural, endocrine and cardiac responses in young calves undergoing dehorning without and with use of sedation and analgesia. The Veterinary Journal. 158, 14-20.

Graf, B. & Senn, M., 1999. Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. Applied Animal Behaviour Science. 62, 153-171.

Knierim, U., Irrgang, N. & Roth, B.A., 2015. To be or not to be horned – Consequences in cattle. Livestock Science. 179, 29-37.

Lauwerier, R.C., 2015. Polled cattle in the Roman Netherlands. Livestock Science. 179, 71-79.

Long, C.R. & Gregory, K.E., 1978. Inheritance of the horned, scurred, and polled condition in cattle. Journal of Heredity. 69, 395-400.

Petherick, J.C., 2005. Animal welfare issues associated with extensive livestock production: The northern Australian beef cattle industry. Applied Animal Behaviour Science. 92, 211-234.

SA Stud Book, 2016. SA Stud Book Annual Report. Bloemfontein. Available online at: http://www.sastudbook.co.za

Schafberg, R. & Swalve, H.H., 2015. The history of breeding for polled cattle. Livestock Science. 179, 54-70.

Schmulian, A., 2006. Identification of the polled trait in Bonsmara cattle using microsatellite markers. Masters dissertation, University of Pretoria, South Africa.

Scholtz, M.M., Bester, J., Mamabolo, J.M. & Ramsay, K.A., 2008. Results of the national cattle survey undertaken in South Africa, with emphasis on beef. Appl. Anim. Husb. Rural Dev, 1: 1-19.

© University of Pretoria

Thompson, N.M., Widmar, N.O., Schutz, M.M., Cole, J.B. & Wolf, C.A., 2017. Economic considerations of breeding for polled dairy cows versus dehorning in the United States. Journal of Dairy Science. 100, 4941-4952.

Van Marle-Köster, E. & Visser, C., 2018. Genetic improvement in South African livestock: can genomics bridge the gap between the developed and developing sectors? Frontiers in Genetics. 9, 1-12.

Webb, E.C., 2013. The ethics of meat production and quality-a South African perspective. South African Journal of Animal Science. 43, S2-S11.

Webb, E.C. & Erasmus, L.J., 2013. The effect of production system and management practices on the quality of meat products from ruminant livestock. South African Journal of Animal Science. 43, 413-423.

Webb, E.M., Webb, E.C. & Tlhapi, P.T., 2020. Cumulative incidence and causal risk factors of carcass condemnations in a South African high-throughput cattle abattoir. South African Journal of Animal Science. 50, 170-177.

Williams, H.D. & Williams, T., 1952. The inheritance of horns and their modifications in polled Hereford cattle. Journal of Heredity. 43, 267-272.

Windig, J. J., Hoving-Bolink, R. A., & Veerkamp, R. F., 2015. Breeding for polledness in Holstein cattle. Livestock Science. 179, 96–101.

Wunderlich, K.R., Abbey, C.A., Clayton, D.R., Song, Y., Schein, J.E., Georges, M., Coppieters, W., Adelson, D.L., Taylor, J.F., Davis, S.L. & Gill, C.A., 2006. A 2.5-Mb contig constructed from Angus, Longhorn and horned Hereford DNA spanning the polled interval on bovine chromosome 1. Animal Genetics. 37, 592-594.

© University of Pretoria

Chapter 2

Literature review

Extracts of the literature review were published as

Challenges in selection and breeding of polled and scur phenotypes in beef cattle

R. Grobler, E. van Marle-Köster & C. Visser

Department Animal Science, University of Pretoria, Pretoria, 0002, South Africa

Published in: Livestock Science 247 (2021) 104479

https://doi.org/10.1016/j.livsci.2021.104479

© University of Pretoria

2.1 Introduction

The polled phenotype was of interest since the early 19th century with several researchers suggesting a dominant inheritance pattern for polledness. White & Ibsen (1936) reported that polledness is dominant over horns in both sexes and that scurs were epistatic to polled. As research endeavours continued since 1936, knowledge of the polled condition expanded and it became clear that the inheritance patterns of the polled, horned and scurred phenotypes tend to be more complex in Bovine species. The mode of inheritance that involves at least three loci, with interactions between loci such as dominance, epistasis and sex, presents challenges for the selection of the polled phenotype (Prayaga, 2007).

Since the completion of the bovine reference genome in 2009 (Bovine Genome Sequencing and Analysis Consortium, 2009), the development in DNA technology provided additional molecular tools for studying the genetic basis of polledness. Even though four causal mutations of the *POLLED* locus have been identified, characterization of the underlying genetic mechanism of the *POLLED* locus has been more difficult than expected. Furthermore, the *SCURS* and African horn loci interact with the *POLLED* locus in an epistatic manner and both phenotypes are believed to be sex influenced, being primarily expressed in male animals (Long & Gregory, 1978).

In this chapter literature will be reviewed on the anatomy and morphology of bovine horns, current knowledge on the inheritance patterns of the polled, scurs and horned phenotypes, with specific reference to the advancements made using a molecular genetic approach to study the occurrence of polledness and horns in cattle.

2.2 Horn growth in bovines

True horns, defined as horns with a bony core surrounded by a sheath of cornified epithelium, are exclusive to the *Bovidae* family, which includes goats, sheep, cattle, buffalo, bison, and antelope. A characteristic feature of these bovids are their paired frontal horns (Lundrigan, 1996). In horned cattle breeds both male and female animals could have permanent horns (Knierim *et al.*, 2015).

Even though the differentiation of horn buds occurs during embryogenesis, the physical growth of horns only occurs after birth. Horns are fused to the frontal bone, projecting from a caudolateral angle in both sexes (Figure 2.1). This is, however, not the case for polled breeds, which only have a knob-like thickening of the bone (Habel & Budras, 2003). The length and form of horns is species and breed specific, with a high variability between individuals. According to Habel & Budras (2003), horns also show marked differences between sexes; in the cow the horns are slender and long, while in the bull the horns tend to be thick and short. The horn itself consists of dense keratin and the osseous core of the horns in the skin is the cornual process of the frontal bone (Figure 2.1). Until shortly before birth the osseous core is a rounded thickening, which extends after birth to become a massive bony cone. The physical horn buds start to form during the first two months of life and at six months of age the bony cone fuses with the caudal frontal sinus.

© University of Pretoria

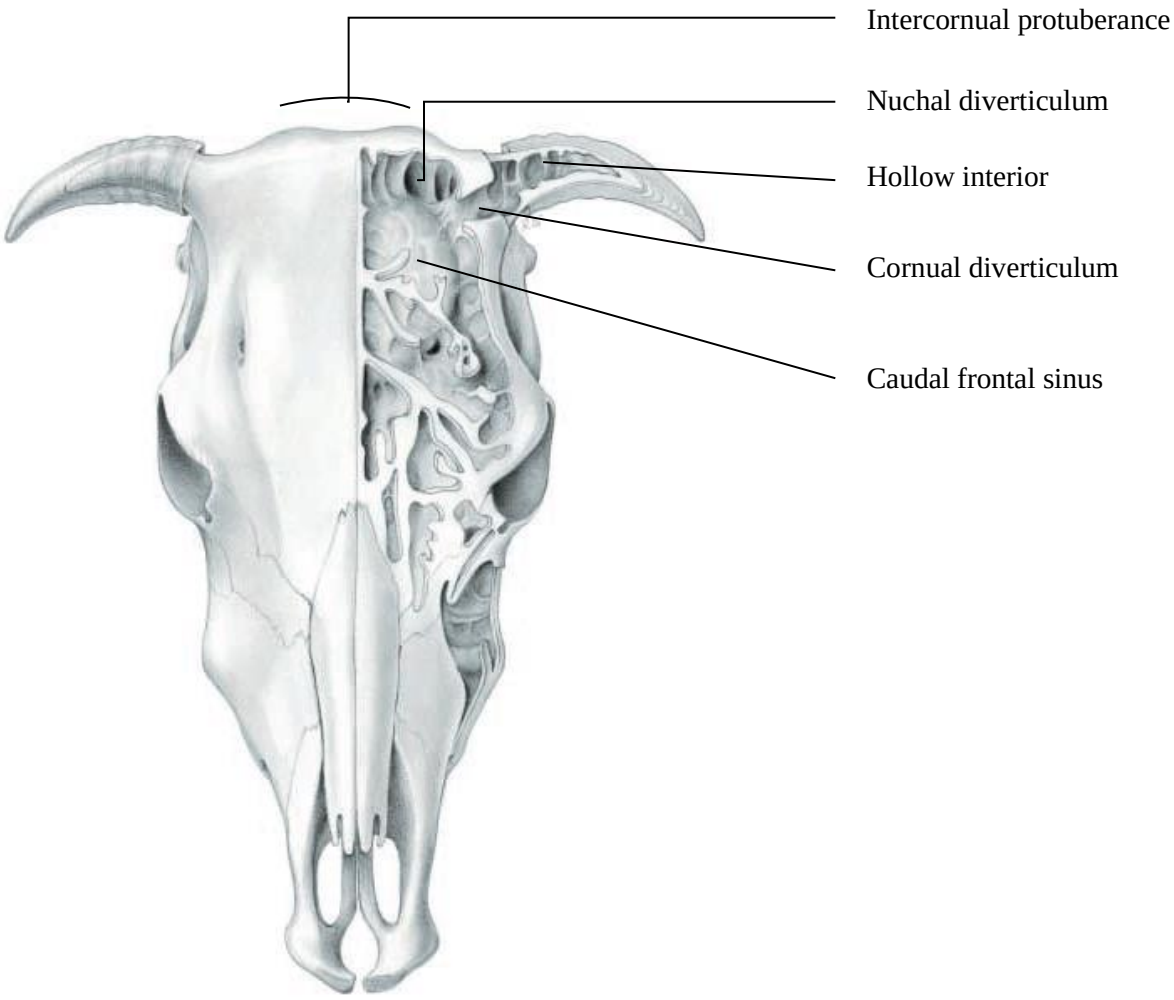


Figure 2.1 The dorsal view of the anatomy of a horned bovine animal (adapted from Habel & Budras, 2003)

Between six and eight months of age, the bony cone are increasingly pneumatised from the caudal frontal sinuses, resulting in the hollow interior of the horn to be directly connected with the frontal sinuses of the skull (Habel & Budras, 2003). This hollow interior of the horns is partially divided into chambers by small bony plates that extend into the frontal sinus of the skull (Figure 2.1) and the formation thereof depends on breed, sex and age (Brenneman *et al.*, 1996; Schafberg & Swalve, 2015). Furthermore, the bony interior of the horns is supplied by blood vessels and nerves and the horns will continue to grow throughout the animal's entire life.

Anomalies of horns occur in bovines and the most frequent variation of horns is that of scurs. Scurs grow in a similar position as horns on the head and even though scurs might look similar to horns in some cases, scurs are loose protuberances that are generally smaller than horns (Asai *et al.*, 2004; Capitan *et al.*, 2011). Similar to horns, scurs also show variation between individuals, breeds and sex,

© University of Pretoria

with a large degree of variation in shape, size and length. They may appear as small scab like bumps up to large horn-like growths of up to fifteen centimetres (Capitan *et al.*, 2011).

Scurs are typically not fused to the frontal bone of the skull (Figure 2.2) (Medugorac *et al.*, 2012), as is the case in horned animals, i.e., these loosely attached appendages have the ability to move (Asai *et al.*, 2004). The bony core at the distal end of scurs originates from a separate ossification centre in the tissues located above the periosteum, and these tissues subsequently attach the scurs to the skull (Medugorac *et al.*, 2012). Skull dissections conducted by Brenneman *et al.* (1996) revealed that the scurs appendages were filled with cartilaginous material which fused to the skull. Radiographic images of scurs confirmed that the space between the bony core of the scurs and the skull is filled with soft tissues (Capitan *et al.*, 2011).

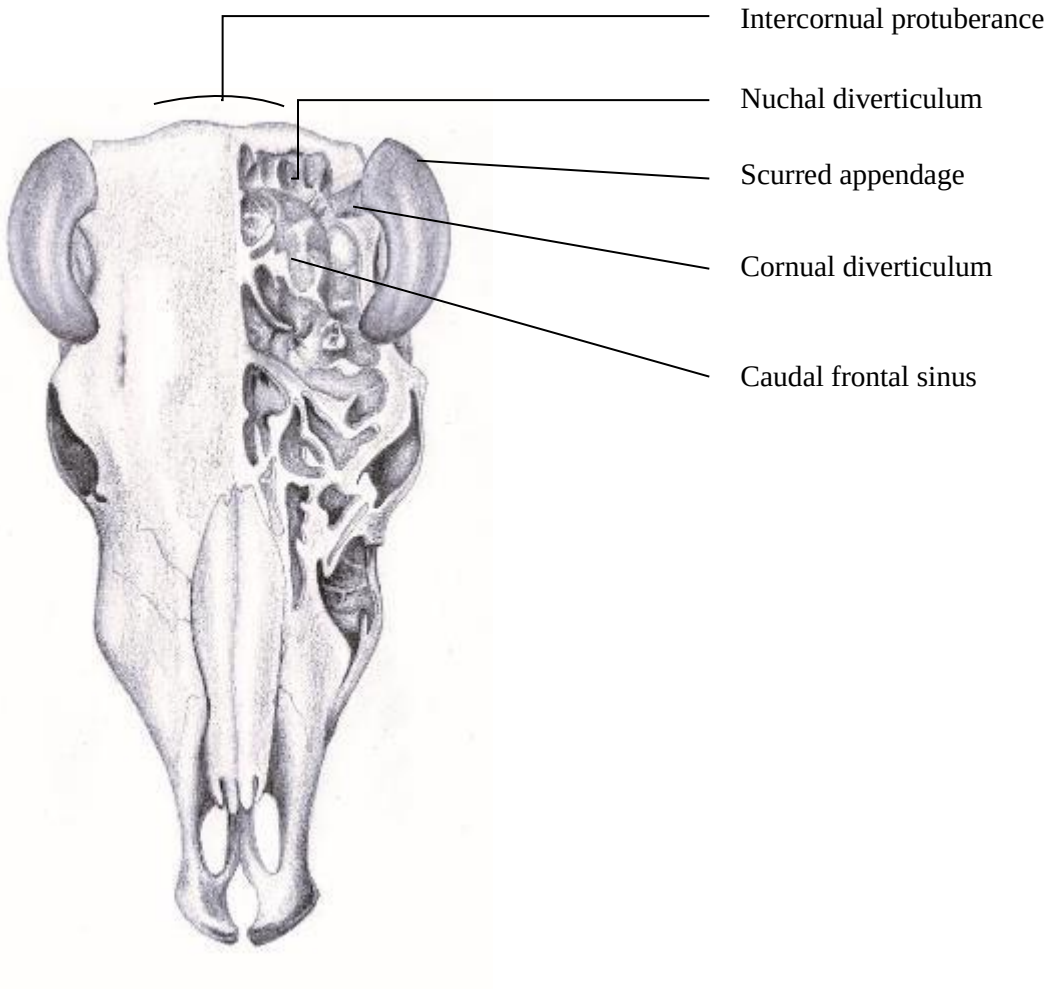


Figure 2.2 The dorsal view of the anatomy of a scurred bovine animal (adapted from Habel & Budras, 2003 and illustrated by Mduduzi Khumalo)

Scurs can be difficult to identify and ambiguities in phenotypic characterisation occur, especially between the horned and scurred phenotypes, since these phenotypes look similar in some cases (Brenneman *et al.*, 1996). Horns are usually visible within a few weeks after birth, whereas scurs only appear after four months of age (Asai *et al.*, 2004). In some cases, scurs can also occur asymmetrically,

© University of Pretoria

with one side being scurred and the other side either being polled or horned (Prayaga, 2007). Generally, scurs grow earlier in males and can be detected from between four and six months of age, whereas in females scurs are observed later in life and can be detected between nine and eighteen months of age (Capitan *et al.*, 2011).

Classification of scurs are further complicated by the fact that some animals may be identified as having scurs at weaning (six to nine months of age) but may develop horns at a later stage in life (Brenneman *et al.*, 1996). The complete absence of horns is observed in cattle and breeds exist that are naturally hornless, i.e., polled. Polledness is an observable phenotype that can be identified at a relatively young age and which does not change with age. Cattle that are polled, have a different head shape compared to horned animals and generally have a narrower more rounded poll (the central prominence of the head) (Brenneman *et al.*, 1996; Habel & Budras, 2003), compared to horned animals that present with a broad, flat poll (i.e., horn crown).

In most polled cattle breeds, polledness is governed by a comparable genetic basis and breeding polled cattle is preferable to dehorning, especially in terms of management and welfare (Windig *et al.*, 2015). Although polled dairy and beef cattle breeds exist, the majority of cattle breeds are horned. Even though breeding programs have been designed to introgress the polled allele into purebred horned breeds, this has been restricted due to the inability to discriminate between heterozygous and homozygous polled animals. Horned offspring will still be prevalent in the polled introgression breeding programs and backcrossing is time consuming. Therefore, disbudding and dehorning are standard practices for most cattle operations to have hornless cattle and are even recommended to prevent injuries to both cattle and the handlers (Graf & Senn, 1999; Aubry, 2005; Knierim *et al*., 2015; Cardoso *et al.*, 2016).

Disbudding is the destruction of the horn-producing cells before the horn bud attaches to the skull (Robbins *et al.*, 2015; Cardoso *et al.*, 2016) and is usually carried out when the horn bud is easily palpable and approximately 5 – 10 mm long (Stafford & Mellor 2005). In contrast, dehorning entails the removal of horns after they have attached to the skull and are already formed (Robbins *et al.*, 2015; Cardoso *et al.*, 2016). The time when the horn bud attaches to the skull varies between breeds and individuals (Robbins *et al.*, 2015) and it is difficult to set a definite age for disbudding or dehorning. Firstly, the onset of development of horns differs between beef and dairy breeds and usually occur later in beef breeds, and secondly, calves born in extensive production systems may, in some instances, not be handled until weaning at approximately six months of age (Stafford & Mellor, 2011).

Disbudding is usually carried out in the first four to six weeks of life by destroying the horn bud and surrounding tissue with a heated disbudding iron (Stafford & Mellor, 2011). Cautery disbudding results in physiological and behavioural changes, such as increased plasma cortisol levels and head shaking, which are indicative of pain; these changes may last up to four hours after disbudding (Graf & Senn, 1999; Stafford & Mellor, 2011). During chemical disbudding or dehorning a caustic agent, such as sodium hydroxide or calcium hydroxide paste, is applied to the horn bud to burn the tissues and this

© University of Pretoria

chemical burning continues as long as the chemical that is applied remains on the horn bud (Stafford & Mellor, 2011). Chemical disbudding has various side effects and are not frequently used.

The most common method used to dehorn cattle involves cauterization through a heated iron (Cozzi *et al.*, 2015; Kling-Eveillard *et al.*, 2015). All methods of dehorning involve tissue destruction and dehorning causes acute pain (Graf & Senn, 1999; Stafford & Mellor, 2011). Various studies observed an immediate rise in plasma cortisol levels, an indication of stress, which can last up to six hours after dehorning (Stafford & Mellor, 2005; Stafford & Mellor, 2011; Stock *et al.*, 2013). Even though the chronic pain associated with dehorning is difficult to identify and assess, the wound caused by dehorning might take several weeks or even a few months to heal properly (Prayaga, 2007; Stafford & Mellor, 2011). It has also been reported that dehorning influences weight gains negatively, especially during the first two to six weeks after dehorning and it may be potentially more significant in warmer climates (Winks *et al.*, 1977; Goonewardene *et al.*, 1999; Stafford & Mellor, 2011).

It is standard practice in most dairy and beef cattle operations to dehorn cattle at a young age by means of physical dehorning but in most cases very few farmers use any form of medication before and/or after the procedure (Cozzi *et al.*, 2015; Kling-Eveillard *et al.*, 2015; Cardoso *et al.*, 2016). Aubry (2005) recommends that calves should be dehorned as young as possible (with the use of a hot iron), because the horn buds of younger calves are smaller compared to older calves, which will then minimize the amount of tissue damage and inflammatory pain. Several studies showed that the use of a local anaesthetic markedly reduces the stress and pain associated with disbudding and dehorning at least up to two hours after dehorning (Graf & Senn, 1999; Stafford *et al.*, 2003; Sylvester *et al.*, 2004). Studies also recommended that stress and pain can be alleviated by using a combined treatment approach of local anaesthesia and non-steroidal-anti-inflammatory analgesic drugs (NSAID) (Petrie *et al.*, 1996; McMeekan *et al.*, 1998; McMeekan *et al.*, 1999; Stafford & Mellor, 2011).

The fact that very few farmers implement the use of local anaesthesia and/or inflammatory analgesic medications further emphasizes the need to address welfare concerns. Various studies advocated the breeding of genetically polled cattle which would thereby eliminate the need for dehorning (Brenneman *et al.*, 1996; Rushen *et al.*, 2007; Windig *et al.*, 2015). Breeding for genetically polled cattle would be a long-term solution and welfare friendly alternative to dehorning (Prayaga, 2007; Rushen *et al.*, 2007; Stafford & Mellor, 2011).

2.3 Inheritance patterns of the polled, scurs and horned phenotypes

The inheritance of horns is one of the first traits that has been studied in cattle and reported to have a Mendelian inheritance pattern in cattle (Prayaga, 2007). At first it was thought that the polled condition can be attributed to a simple Mendelian inheritance of a single gene mutation (from p to P) (Prayaga, 2007). The polled condition was believed to be dominant and several early studies in the 1900's confirmed this observation, thereby supporting the single gene hypothesis initially postulated (Bateson & Saunders, 1902; Boyd, 1906; Spillman, 1906; Lloyd-Jones & Evvard, 1916). These studies

© University of Pretoria

did, however, not take sex and the possible effect thereof into account while studying horned and polled animals.

As research efforts contributed to the knowledge of the polled condition, it became clear that the inheritance patterns of the polled, horned and scurred phenotypes tend to be more complex in Bovine species. Gowen (1918) was the first to provide evidence to suggest that the simple and dominant inheritance hypothesis might not fully explain the inheritance of horns. In his study using crosses between polled Angus cattle and horned breeds, more horned males than females were observed, which led Gowen (1918) to hypothesise a sex-influenced inheritance pattern. Thus, the single gene mutation theory (from p to P) was expanded to explain the inheritance as follow: a homozygous dominant genotype (PP) will result in polled males and females, while a heterozygous polled genotype will result in horned males and polled females; the homozygous recessive genotype (pp) will result in horned males and females (Gowen 1918; Prayaga, 2007). A sex-influenced inheritance was also proposed by Watson (1921); by using crosses between Aberdeen-Angus and West Highland Cattle, he observed that the polled condition is completely dominant in female animals, but for heterozygous males, the horn development is inhibited and not always expressed. Further evidence to support this sex-influenced hypothesis were provided by studies from Churchill (1927) and Smith (1927).

The first complex model that was proposed to explain the inheritance of the polled, horned and scurred phenotypes, was suggested by White & Ibsen (1936). According to these authors four genes, that segregate independently, were involved in the inheritance of polledness. The first gene, *P*, represented the completely dominant gene for the polled condition, while *p* symbolised the absence of *P*, and this *POLLED* gene was believed to be epistatic to horns in both sexes. *H*, symbolised the gene for horns, and was always present in both sexes in the homozygous state; this gene was also epistatic to the gene for scurs (*Sc*). In addition, White & Ibsen (1936) further postulated that *h* does not exist in domestic cattle and this locus only represented the genetic complexity of cattle.

A third locus was postulated by White & Ibsen (1936) where *Ha* symbolised the African horn gene, whereas *ha* merely symbolised the absence of *Ha*. Furthermore, *Ha* had no modifying effect in an otherwise horned animal. This African horn gene was believed to be epistatic to the *POLLED* gene (P) in males, but the effect of *Ha* was not certain in females. White & Ibsen (1936) alleged that although *Ha* is present in most breeds, it occurred at a low frequency in many breeds. The fourth locus, *Sc*, symbolised the gene for the scurred condition and the expression of the *SCURS* gene was hypothesised to be sex influenced. In males, the heterozygote (*Scsc*) was usually scurred, while in females only the homozygote (*ScSc*) was scurred (White & Ibsen, 1936)

Gowen (1918) suggested separate loci to explain the inheritance of horns and scurs and proposed a sex-influenced inheritance pattern for scurs, since he observed a higher frequency of scurred males compared to females from his studies. Williams & Williams (1952) proposed a sex-influenced pattern for the inheritance of scurs in polled Hereford. Their study further provided evidence to support the inheritance model postulated by White & Ibsen (1936), expect that the *SCURS* gene was observed to be

© University of Pretoria

recessive instead of dominant (Williams & Williams, 1952). Blackwell & Knox (1958) investigated the inheritance pattern of scurs in an Aberdeen-Angus herd and observed that scurs were inherited as a sex-influenced trait. This initial polled inheritance model (White & Ibsen, 1936) was confirmed by Long & Gregory (1978), who stated that their results were generally consistent with the inheritance model of four separate loci controlling polled, horned, African horn and scurs.

Subsequently, Long & Gregory (1978) proposed a modification of the hypothesis by White & Ibsen (1936), in that males that were heterozygous for scurs (*Scsc*) must also be heterozygous for the *POLLED* locus (*Pp*) for scurs to be expressed (Table 2.1). Furthermore, the frequency of females showing scurs were less than the percentage of males having a scurred phenotype (Gowen, 1918; Williams & Williams, 1952; Blackwell & Knox, 1958). This led Long & Gregory (1978) to confirm the observations from these previous studies, that the scurs phenotype was inherited as a sex-influenced trait, which showed incomplete penetrance in male animals (Table 2.1). Long & Gregory (1978) also suggested that males that do not have scurs but sire scurred females, must have a genotype *PPScsc*.

© University of Pretoria

Table 2.1 Inheritance model proposed for the polled, horned and scurs phenotypes (adapted from Long & Gregory (1978), Georges *et al.* (1993) and Prayaga (2007))

Genotype	Phenotype	
	Males	**Females**
Inheritance of the polled phenotype		
PP	Polled	Polled
Pp	Polled	Polled
pp	Horned	Horned
Inheritance of the scurred phenotype		
PP ScSc	Scurred	Scurred
PP Scsc	Polled	Polled
PP scsc	Polled	Polled
Pp ScSc	Scurred	Scurred
Pp Scsc	Scurred*	Polled
Pp scsc	Polled	Polled
pp -/- **	Horned	Horned
Epistatic effect of the African Horn gene on the Polled locus		
PP HaHa	Horned	Horned
PP Haha	Horned	Polled
PP haha	Polled	Polled
Pp HaHa	Horned	Horned
Pp Haha	Horned	Polled
Pp haha	Polled	Polled
pp -/- ***	Horned	Horned

* According to Long & Gregory (1978), Sc/sc males express the scurred phenotype only when heterozygous Pp
** -/- = either ScSc, Scsc or scsc
*** -/- = either HaHa, Haha or haha

In addition to the *POLLED* and *SCURS* loci, Georges *et al.* (1993) also suggested that an African Horn locus influence the inheritance of polledness and horns in cattle. This locus would explain the high frequency of horned offspring from crosses between polled *Bos taurus* breeds and horned *Bos indicus* breeds, as well as the occurrence of polled offspring from horned parents (Georges *et al.*, 1993). It is hypothesised that the gene for African horns (*Ha*) is epistatic to the *POLLED* gene in males, but this gene is probably not epistatic to the *POLLED* gene in females (Long & Gregory, 1978; Georges *et al.*, 1993). Furthermore, the presence of horns masks the expression of the scurs phenotype, as indicated in Table 2.1 above.

It is evident that the genetic determination, and the variation in size and shape of horns, may be under the influence of more than one gene. Due to the complex mode of inheritance that involves three loci, together with interactions such as dominance, epistasis and sex, selection for the favourable polled phenotype is difficult. Phenotypic selection for polledness was further complicated by the lack of

© University of Pretoria

accurate identification of polled individuals, especially when considering the interaction with the *SCURS* locus and potential confusion between the horned and scurred phenotypes (Brenneman *et al.*, 1996; Prayaga, 2007). An additional problem is introduced by the dominant inheritance pattern of polledness, since both the heterozygous and homozygous polled genotype will be phenotypically polled, making it impossible to distinguish between homozygous and heterozygous polled animals solely based on phenotype.

To identify polled individuals, and more specifically in an attempt to identify homozygous polled animals, test matings were utilised. Test matings would usually entail the mating of a phenotypic polled bull with horned cows. The phenotype of the offspring of these matings were recorded to trace the polled phenotype through the pedigree (Williams & Williams, 1952). If a bull had ten consecutive polled calves, it was considered as homozygous polled. This method of identifying polled individuals and the subsequent selection based on phenotype was time consuming, expensive and inefficient to incorporate polled animals into the breeding herd. Selection based on phenotype would also result in slow genetic progress. The development of molecular genetic approaches was imperative to the characterization of the polled and scurs phenotypes on a genetic level.

2.4 Molecular characterization of the bovine genome

As molecular technology developed over the past four decades, various molecular tools became available to analyse the bovine genome and different strategies have been followed by researchers to study the genetic determination and molecular basis of the polled and scurs phenotypes (Figure 2.3). A primary goal of bovine genome research has been to identify highly polymorphic DNA markers with known chromosomal locations that can be systematically applied to map and characterize traits of economic importance (Womack & Kata, 1995; Andersson, 2001).

Genomic research in livestock species, including cattle, developed successfully following the technical advances and scientific accomplishments of the human genome initiative. A new era of animal genetics started with the development of DNA-based genetic markers, which allowed for the investigation of the genome instead of only focusing on a few individual genes (Dodgson *et al.*, 1997; Davey *et al.*, 2011; Blasco & Toro, 2014). Before this time, the available genetic markers were limited to a few morphological, biochemical and blood group polymorphisms for domestic animal species (Womack, 2012).

The discovery of restriction fragment length polymorphisms (RFLPs) in the 1980's opened up the possibility to identify and map genetic loci affecting quantitative economic traits of importance in livestock species (Botstein *et al.*, 1980; Soller & Beckmann, 1983). For the first time, it was possible to analyse non-coding sequences, silent changes in a protein coding sequence and changes in coding sequences (Schlötterer, 2004). The development of the polymerase chain reaction (PCR) by Mullis during the 1980's created a breakthrough in DNA-based molecular research and drastically changed how molecular studies were conducted (Mullis *et al.*, 1986; Schlötterer, 2004). PCR enabled the

© University of Pretoria

amplification and analysis of any genomic region in multiple individuals for the first time, without using recombinant DNA technology or isolating large amounts of pure genomic DNA (Schlötterer, 2004).

The subsequent discovery of short tandem repeats (STRs) lead to the development of microsatellite DNA markers, an abundant source of highly polymorphic markers that are widely distributed throughout the genome and generally specific in closely related species (Tautz, 1989; Bishop *et al.*, 1994; Georges & Andersson, 1996). These characteristics have made microsatellites one of the most popular autosomal genetic markers for mapping quantitative traits, parentage identification and studying population genetics and diversity at that time (Schlötterer, 2004). The relative ease of developing and genotyping microsatellite markers using PCR technology advanced the rapid development of saturated genetic linkage maps in livestock species (Bishop *et al.*, 1994).

© University of Pretoria

Development of molecular genetic technology	Characterization of the *POLLED* and *SCURS* loci
1983 Discovery of polymerase chain reaction (PCR) (Mullis et al., 1986)	
1993 Cytogenetic bovine genome map (Fries *et al.*, 1993)	**1993** ***POLLED*** locus mapped to BTA 1 (Georges *et al.*, 1993)
1994 1st Generation microsatellite based bovine linkage maps (Barendse *et al.*, 1994; Bishop *et al.*, 1994)	
	1995 ***POLLED*** locus mapped to BTA 1 in Charolais (Schmutz *et al.*, 1995)
	1996 Linkage map of BTA1 in a *Bos taurus* x *Bos indicus* cross (Brenneman *et al.*, 1996)
1997 2nd Generation bovine linkage maps (Kappes *et al.*, 1997) Radiation hybrid panel (5000 rad) (Womack *et al.*, 1997)	
	2004 ***SCURS*** locus mapped to BTA19 (Asai *et al.*, 2004)
2005 Next-generation sequencing (NGS)	**2005** ***POLLED*** locus fine mapped to a 1.31Mb interval on BTA1 (Drögemüller *et al.*, 2005a, b)
2009 Bovine refence genome sequence(s) released (Bovine Genome Seq. & Analysis Consortium, 2009; Zimin *et al.*, 2009) Development of Bovine SNP50 chip (Matukumalli *et al.*, 2009)	**2009** ***SCURS*** locus studied in Charolais could not be mapped to BTA19; different inheritance pattern of scurs (Capitan *et al.*, 2009)
	2011 Type II **Scurs**, *TWIST* mutation (Capitan *et al.*, 2011)
	2012 ***POLLED*** interval - 381kb on centromeric region of BTA1 (Seichter *et al.*, 2012) Identification of the Celtic (P_C) and Friesian (P_F) mutations of the ***POLLED*** locus (Medugorac *et al.*, 2012)
	2013 Confirmed the Celtic and Friesian mutations of the ***POLLED*** locus; Horn ontogenesis study (Allais-Bonnet *et al.*, 2013)
	2015 GWAS of ***SCURS*** locus in polled Simmental cattle (Tetens *et al.*, 2015)
	2017 Identification of the Mongolian (P_M) variant of the ***POLLED*** locus (Medugorac et al., 2017)
	2019 Identification of the Guarani variant (P_G) of the ***POLLED*** locus (Utsunomiya et al., 2019)

Figure 2.3 A timeline depicting the development of molecular genetic technology and the subsequent characterization of the *POLLED* and *SCURS* loci in cattle

© University of Pretoria

The initial bovine genome map consolidated by Fries *et al.* (1993) consisted primarily of cytogenetic markers with very few microsatellite markers assigned. The first comprehensive microsatellite-based linkage maps of the bovine genome were published in 1994 and covered approximately 90% of the bovine genome (Barendse *et al.*, 1994; Bishop *et al.*, 1994). The second-generation linkage maps that were published in 1997 increased marker number and reduced the average interval size (Barendse *et al.*, 1997; Kappes *et al.*, 1997). The second-generation linkage map produced by Barendse *et al.* (1997) covered more than 95 percent of the bovine genome. These second-generation linkage maps provided a basis for the high-resolution mapping of the bovine genome and provided sufficient marker density for QTL detection and subsequent implementation of marker-assisted selection (MAS) programs (Barendse *et al.*, 1997; Kappes *et al.*, 1997).

High-resolution maps of the bovine genome were made possible by the development of panels of radiation hybrid (RH) clones, with the first comprehensive RH panel developed by Womack *et al.* (1997). This 5000 rad panel were used as a foundation to develop high density comparative maps of individual bovine chromosomes, as well as subsequent higher resolution maps. Rexroad *et al.* (2000) constructed a 12 000 rad panel, specifically characterizing the chromosome that harbours the *POLLED* interval (BTA1). RH panels lead to the development of a high-resolution map of the bovine genome and together with the subsequent elaboration of the bovine linkage and physical genome maps, it provided the first resources for comparative mapping of the bovine genome against the human genome (Womack, 2012).

The advent of sequencing technology has rapidly changed the way causative variants were discovered. In 1977 the Sanger sequencing method were developed, which were a sequencing-by-synthesis (SBS) method. Improvements to the Sanger Sequencing method led to the development of automated DNA sequencing (Heather & Chain, 2016), which evolved into the next-generation sequencing (NGS) era. This development led to the increased sequencing of DNA in an automated fashion, by parallel sequencing reactions (Heather & Chain, 2016). NGS is a valuable tool for the discovery, validation, and assessment of single nucleotide polymorphism (SNP) markers in populations (Davey *et al.*, 2011; Kumar *et al.*, 2012).

Microsatellite markers are increasingly being replaced by SNPs as the genetic DNA marker of choice. SNPs are bi-allelic DNA markers that are widely distributed throughout the genome and may present more potential markers closely linked to a specific locus or mutation of interest (Beuzen *et al.*, 2000; Fan *et al.*, 2010). Even though SNPs are less polymorph than microsatellites, they are usually located in coding regions and therefore directly affect the function of the protein that is being expressed (Beuzen *et al.*, 2000). It is estimated that three to eight biallelic SNPs are as informative as one microsatellite marker (Rosenberg *et al.*, 2003; Schopen *et al.*, 2008).

The bovine reference genome sequence resulted from the sequencing of an inbred horned Hereford cow and her sire, by using a combination of both whole-genome shotgun and bacterial artificial chromosome (BAC)-to-BAC sequencing (Bovine Genome Sequencing and Analysis Consortium, 2009;

© University of Pretoria

Fan *et al.*, 2010). A second study, which was conducted concurrently with the Bovine Genome Sequencing and Analysis Consortium project, identified approximately 2.86 million SNPs by using independent mapping data and conserved synteny between the bovine and human genomes (Zimin *et al.*, 2009). These authors mapped approximately 91% of the genome on the 30 *Bos taurus* chromosomes and identified a portion of the *Bos taurus* Y-chromosome (Zimin *et al.*, 2009).

For the development of SNP arrays, it is important to identify a large number of SNPs to design and construct the array which should be suitable and applicable for an assortment of breeds, to decrease the occurrence of ascertainment bias (Fan *et al.*, 2010; Lenstra *et al.*, 2012). The completion of the bovine reference genome sequence uncovered a large number of variants available for this purpose and the first commercial SNP array were developed by Matukumalli *et al.* (2009), which included a selection of 54 001 SNPs on this BovineSNP50 assay. The development of SNP genotyping arrays allows for the detection of hundreds of thousands of loci simultaneously in a high throughput platform (Matukumalli *et al.*, 2009; Lenstra *et al.*, 2012). Due to the development in SNP bead chip technology and market or research requirements over the past few years, several different commercial SNP arrays have been developed with varying marker densities (Table 2.2).

The information provided by automated SNP genotyping assays can be utilised for studies on a variety of factors relating to livestock genome functioning and diversity. SNP markers enables improved investigation of linkage disequilibrium (LD) between markers, which in turn is useful for investigating population history, admixture, and breeding systems, as well as the history of natural selection and mutations within specific genomic regions (Schlötterer, 2004; Gurgul *et al.*, 2014). LD is also important to detect possible allelic associations between a QTL and a marker to localise genes affecting quantitative traits (Gurgul *et al.*, 2014). Furthermore, the LD across the genome can be investigated to detect regions, associated with favourable traits to improve animal production traits (Fan *et al.*, 2010), that are under selection by analysing the allele frequency of genome-wide SNPs (Gurgul *et al.*, 2014). High-density SNP arrays can also be used to identify runs of homozygosity (ROH) in the genomes of livestock. ROHs are adjoining continuous homozygous segments of a DNA sequence where the two haplotypes inherited from the parents are identical. The extent and frequency of ROH provides information on the ancestry and inbreeding of an individual animal and its population (Gurgul *et al.*, 2014; Peripolli *et al.*, 2016).

© University of Pretoria

Table 2.2 A non-comprehensive list of commercially available bovine SNP chips (adapted from Illumina and Geneseek)

Platform	SNP Chip	Size (number of SNPs)		
		Low	Medium	High
Affymetrix®	Axiom® Genome-wide BOS1			648 875
Illumina®	Golden Gate Bovine 3k	2 900		
	Bovine LD v2	7 931		
	BovineSNP50 v2		54 609	
	BovineSNP50 v3		53 714	
	Bovine HD			777 962
Geneseek®	GGP Bovine LD v3	26 151		
	GGP Bovine LD v4	30 125		
	GGP *Indicus*	35 090		
	GGP *Bos indicus* HD		74 000	
	GGP Bovine 50K		48 268	
	GGP Bovine 100K			100 000
	GGP Bovine 150K v2			139 480
	GGP F250			220 000
Weatherbys Scientific	IDB v3 54K		54 000	
	Bovine VersaSNP 50K		49 629	
	IDB 770K HD			770 000

The availability of high-density SNP arrays enabled genetic association studies with the potential for mapping causal genes underlying common diseases and quantitative traits (Hirschhorn & Daly, 2005; Sharma *et al.*, 2015). Genome-wide association studies (GWAS) systematically investigate hundreds of thousands of SNPs in the genome, without any prior knowledge on the exact location of candidate genes or mutations (Ziegler *et al.*, 2008). GWAS ultimately enables the identification of candidate genomic regions, which will require fine mapping to search for causative SNPs in candidate genes and identify the underlying genetic mechanism of the trait of interest (Hayes & Goddard, 2010). Hence, GWAS studies now allow a more direct approach to studying phenotypic variation and to unravel the genetic architecture of complex traits (McCarthy *et al.*, 2008).

GWAS were first successfully performed in humans in 2005 and mainly focused on identifying QTLs and genomic regions associated with numerous complex diseases (McCarthy *et al.*, 2008). The primary aim was to gain insight into the biology of diseases, with the assumption that a better understanding of the biological mechanisms of disease will result in prevention or better treatment (Visscher *et al.*, 2017). This research approach was extended in livestock species to identify markers in LD with mutations affecting the disease or trait of interest, to be incorporated into MAS breeding

© University of Pretoria

programs to manage recessive defects in livestock populations (Charlier *et al.*, 2008; Hayes & Goddard, 2010). The majority of initial GWAS studies in cattle were on disease related traits, since a case-control design could easily be implemented in mapping the causal variants (Charlier *et al.*, 2008; Fan *et al.*, 2010). In beef and dairy cattle, various GWAS studies followed to identify markers associated with traits of economic importance with a primary focus on production and reproduction traits (Table 2.3), including traits such as meat quality, milk yield, fat and protein percentage, calving ease and fertility traits (Sharma *et al.*, 2015). Association studies for other traits of interest in cattle, such as coat colour and the horned/polled condition, are limited (Table 2.3).

Table 2.3 A non-comprehensive summary of GWAS for major traits of economic importance reported in beef cattle

Trait	Breed	SNP chip	BTA	Candidate genes	Reference
Production					
Residual feed intake, Average daily gain, Hip height	Angus, Murray Grey, Shorthorn, Hereford, Brahman, Santa Gertrudis, Belmont Red	BovineSNP50	3, 5, 7, 8		Bolormaa *et al.* (2011)
Carcass weight, Eye muscle area, Backfat thickness, Marbling score	Hanwoo (Korean cattle)	BovineSNP50	3, 6, 11, 13, 16	*HIVEP3*	Kim *et al.* (2011)
Body weight	Angus, Charolais, Gelbvieh, Hereford, Limousin, Simmental	BovineSNP50	Multiple	*SPP1*, *NCAPG*	Snelling *et al.* (2010)
Feed efficiency	Angus	BovineSNP50	Multiple		Rolf *et al.* (2012)
Feed intake, Residual feed intake	Nellore	BovineSNP50 Bovine HD777k	4, 8, 14, 21	*STMN2*, *CCDC171*, *PSIP1*, *SNAPC3*, *ZNF804B*, *ANXA10*, *DDX60*, *GPR132*, *CDCA4*, *AHNAK2*, *BRF1*	Santana *et al.* (2014)
Birth weight	Nellore	Bovine HD777k	14	*PLAG1*, *CHCHD7*, *MOS*, *RPS20*, *LYN*, *RDHE2*, *PENK*	Utsunomiya *et al.* (2013)

© University of Pretoria

Trait	Breed	SNP chip	BTA	Candidate genes	Reference
Meat quality traits	Simmental	Bovine HD777k Infinium II Assay	3, 6, 7, 9, 13, 15, 16, 18	*TMEM236, SORL1, TRDN, S100A10, AP2S1, KCTD16, LOC506594, HX15, LAMA4, PREX1, BRINP3*	Xia *et al.* (2016)
Meat tenderness	Nellore	Bovine HD777k GGP Indicus HD	3, 13, 17, 20, 21, 25		Castro *et al.* (2017)
Internal organ weights	Simmental	Bovine HD777k		*NDUFAF4, LCORL, BT.94996, SLIT2, FAM184B, LAP3, BBS12, MECOM, D300LF, HSD17B3, TLR4, MXI1, MB21D2.*	An *et al.* (2018)
Reproduction					
Age at puberty, Anoestrous interval, First postpartum ovulation	Brahman Tropical Composite	BovineSNP50	Multiple		Hawken *et al.* (2012)
Age at first calving	Nellore	Bovine HD777k	Multiple	27 protein coding genes	Costa *et al.* (2015)
Other					
Coat colour	Black Angus, Red Angus	BovineSNP50	18	*MC1R*	Matukumalli *et al.* (2009)
Polled	Limousin, Hereford	BovineSNP50	1		Matukumalli *et al.* (2009)
Scurs	Simmental		19		Tetens *et al.* (2015)
Flight speed (temperament)	Nellore	Bovine HD777k	2, 9, 11, 15, 17, 26	*NCKAP5, PARK2, ANTXR1, GUCY1A2, CPE, DOCK1*	Valente *et al.* (2016)

The majority of published GWAS studies for simply inherited Mendelian traits features case-control study designs, due to the relative ease of mapping causative mutations of these monogenic traits. A case-control design for an association study comprises the comparison between a group of individuals (cases) ascertained for the specific phenotype of interest, with the assumption that these individuals have a high prevalence of alleles associated with the phenotype, and a second group (controls) not ascertained for the phenotype of interest and with a lower prevalence of associated alleles (McCarthy *et al.*, 2008).

© University of Pretoria

Case-control association studies have the risk of potential bias due to population substructure and reduced power, however, these issues can be overcome by implementing family-based association studies or by proper study design, which include accurate definition of the case-control phenotypes and optimal selection of cases and controls (Zondervan & Cardon, 2007; McCarthy *et al.*, 2008). When cases and controls are well matched for genetic ancestry, the impact of confounding effects on type I errors due to population substructure is limited.

2.4.1 *POLLED* locus

Microsatellite markers from the bovine syntenic group U10 were used by Georges *et al.* (1993) to first map the *POLLED* locus to bovine chromosome 1 (BTA1) in close proximity to the centromere. Georges *et al.* (1993) mapped this locus based on paternal half-sib pedigrees of three *Bos taurus* beef breeds: Hereford x Shorthorn, South Devon and Saler. Due to low resolution and a limited number of markers used, the precise position of this locus could not be determined (Georges *et al.*, 1993). This breakthrough study by Georges *et al.* (1993) sparked interest from various research groups to link additional microsatellite markers to the *POLLED* locus and to determine the relative position of this locus on BTA1.

Subsequent studies in Charolais, Simmentaler, Pinzgauer and an Angus x Brahman cross, associated additional microsatellite markers from the available bovine linkage maps (Barendse *et al.*, 1994; Bishop *et al.*, 1994) with polledness and confirmed the position of the *POLLED* locus towards the centromere of BTA1 (Schmutz *et al.*, 1995; Brenneman *et al.*, 1996; Harlizius *et al.*, 1997). Even though the subsequent studies from Schmutz *et al.* (1995), Brenneman *et al.* (1996) and Harlizius *et al.* (1997) increased the number of microsatellite markers associated with the polled phenotype, the exact order of the *POLLED* locus relative to these markers still could not be determined. This was mainly due to a low number of recombinants in the families that were studied, incorrect phenotyping and the fact that different markers were used across studies. Even though the genomic region for the *POLLED* gene was narrowed down to a location proximal to the centromere (Brenneman *et al.*, 1996), a more precise location and additional highly polymorphic markers was needed to enable MAS of the polled trait.

The fine mapping studies conducted by Drögemüller *et al.* (2005a and 2005b) in six German cattle breeds enabled the physical ordering of the microsatellite markers from the various available bovine linkage maps which linked the *POLLED* region with the polled phenotype. This allowed the exact determination of the size of the *POLLED* interval on BTA1 (Drögemüller *et al.*, 2005b). These authors narrowed the critical region for the *POLLED* locus to a 1-Mb segment (c. 1.36 Mb: 0.661 – 2.025 Mb UMD3.1) close to the centromere of BTA1 (Drögemüller *et al.*, 2005b). This location was confirmed by Wunderlich *et al.* (2006) who constructed a larger 2.5 Mb contig spanning the *POLLED* interval on BTA1, by using BAC clones from horned and polled breeds. The identification of this specific region for the markers linked to the *POLLED* interval would subsequently enable future research efforts to precisely identify and map causal mutations for the bovine *POLLED* locus.

© University of Pretoria

Even though the genomic region for the *POLLED* locus were narrowed down and various advancements in molecular technologies were made since the *POLLED* locus was first mapped in 1993, the characterization and identification of causative mutations proved to be more difficult than expected. The major constraints included the difficulty to identify functional candidate genes by comparative mapping with human or mouse due to a lack of a similar phenotype (i.e. horned model species); the lack of functional or positional candidate genes in the localized genomic region of the *POLLED* locus; the possible allelic heterogeneity of the phenotype between different breeds investigated due to the breeding history of cattle; and the variation in genes that were differentially expressed between horn buds from polled and horned new born calves (Medugorac *et al.*, 2012; Allais-Bonnet *et al.*, 2013).

The completion of the bovine reference genome in 2009 (Bovine Genome Sequencing and Analysis Consortium, 2009) contributed novel genomic information in cattle and resulted in the development of the Bovine SNP50 Beadchip, a high-density genome-wide tool to study phenotypic variation (Matukumalli *et al.*, 2009). To investigate the effectiveness of the newly developed Bovine SNP50 Beadchip for genome-wide association applications, Matukumalli *et al.* (2009) used black/red coat colour and the presence or absence of horns as known Mendelian phenotypes to identify the genomic locations for these traits. A strong genome-wide signal detected at the known location for the *POLLED* locus at the centromeric region of BTA1, indicated association with the polled phenotype and the Bovine SNP50 Beadchip was thereby validated for implementation for GWAS studies (Matukumalli *et al.*, 2009).

A SNP association study in a sample of divergent cattle breeds conducted by Seichter *et al.* (2012) identified one shared homozygous haplotype block, consisting of nine neighbouring SNPs, in all phenotypically polled animals included in the study. This haplotype was, however, also observed in the horned animals and could therefore not be used for population wide testing. This study by Seichter *et al.* (2012) was the first step towards the confinement of a genomic region for high throughput sequencing and the candidate mutation for the *POLLED* locus was narrowed down to a 381kb segment on BTA1. Seichter *et al.* (2012) recommended the use of higher SNP density arrays and the application of high throughput sequencing for the further characterization of the *POLLED* locus and identification of causative mutations.

One of the most comprehensive studies yet by Medugorac *et al.* (2012) provided evidence for the existence of at least two different alleles at the *POLLED* locus in *Bos taurus* breeds, namely the Celtic (P_C) and Friesian (P_F) alleles, and identified one and five candidate mutations, respectively, for each. Using whole genome sequencing , these results were confirmed by Allais-Bonnet *et al.* (2013), which identified four possible candidate mutations for the Friesian allele, namely three SNPs (g.1764239T>C, g.1768587C>A, g.1855898G>A) and a large duplication of an 80kb fragment presenting with two sequence variations compared to the original fragment. For the Celtic allele only one polymorphism showed complete concordance with the polled phenotype and a duplication of 212bp were identified

© University of Pretoria

(Allais-Bonnet *et al.*, 2013). The causative mutations that were identified in this study were not located in known genes or coding regions.

The causative mutation for polledness in Holstein-Friesian populations were subsequently narrowed down to a single causative mutation, that corresponds to the 80 kb DNA duplication identified for the Friesian (P_F) variant of the *POLLED* locus (Allais-Bonnet *et al.*, 2013; Rothammer *et al.*, 2014). More recently two additional variants associated with the polled phenotype were identified. Medugorac *et al.* (2017) identified a third allele at the Polled locus, namely the Mongolian allele (P_M). However, this allele has only been described in East Asian *Bos taurus* and *Bos grunniens* breeds. Utsunomiya *et al.* (2019) identified a novel polled variant, denoted as the Guarani variant (P_G), in Brazilian Nelore cattle.

With regards to polledness in *Bos indicus* cattle breeds, Mariasegaram *et al.* (2012) was the first to confirm the location of the *POLLED* locus on BTA1 in Zebu cattle. Furthermore, these authors identified allele 303 of the CSAFG29 marker to be predictive of the polled phenotype in Brahman cattle (Mariasegaram et al., 2012), but could not identify the causative mutation in Brahman cattle. A recent GWAS study by Stafuzza *et al.* (2018) in Nelore beef cattle (*Bos indicus*) mapped the *POLLED* locus to a 3.11 Mb centromeric region on BTA1. The authors identified a total of 28 protein-coding genes in this interval, which might be related to horn development, but requires further investigation.

Investigation and characterization of the polled phenotype is limited in indigenous African and Sanga cattle breeds, which are a hybrid between *Bos taurus* and *Bos indicus* (Rege & Tawah, 1999). A study by Schmulian (2006) indicated that the inheritance of the polled trait in the South African Bonsmara a composite beef breed, fit the model proposed by Long & Gregory (1978) and found linkage between the polled phenotype in the South African Bonsmara and alleles of nine microsatellite markers located on BTA1. The causative mutation for polledness has not been investigated in African cattle breeds.

Characterization of the *POLLED* locus has been more difficult than expected and after more than two decades of research related to the *POLLED* locus (Figure 2.3), the exact causal gene or molecular basis governing polledness has not been identified yet and neither has the genetic mechanism of the *POLLED* gene been completely explained. None of the mutations that were identified in cattle (P_C, P_F, P_M and P_G) were located in known protein coding or regulatory regions, thus adding to the complexity of the molecular basis of polledness.

2.4.2 *SCURS* locus

Since the expression of scurs are linked to the polled phenotype (scurs are expressed in heterozygous polled animals), these two loci were postulated to interact in an epistatic fashion (Long & Gregory, 1978). Even though the inheritance and characterization of the polled phenotype has been studied for almost a century, scurs were not researched to the same extent.

© University of Pretoria

The first study that characterized scurs on a molecular genetic level used 162 autosomal microsatellite markers across the 29 bovine autosomes to conduct a genome scan across three full-sib families (Asai *et al.*, 2004). Fine mapping of the scurs phenotype with 16 microsatellite markers mapped the *SCURS* locus 4 cM distal of the BMS2142 microsatellite marker on BTA19 (Asai *et al.*, 2004). These authors did not find linkage between markers on the X chromosome and the scurs phenotype, thereby demonstrating that this phenotype is probably not sex linked (Asai *et al.*, 2004). Since the *POLLED* locus was mapped to the centromeric end of BTA1 and the *SCURS* locus was mapped to BTA19, the polled and scurs phenotypes were found not to be linked in *Bos taurus* (Asai *et al.*, 2004).

The inheritance pattern of the scurs phenotype in French Charolais cattle were investigated by Capitan *et al.* (2009) and these researchers investigated whether the localisation of the *SCURS* locus on BTA19 could be confirmed in the French Charolais. Capitan *et al.* (2009) concluded that in the French Charolais breed the inheritance of the scurs phenotype is autosomal and that the *sc* allele of the *SCURS* locus is completely dominant over the *Sc* allele in both sexes. These authors reported that males with the genotype *PpScsc* are polled and not scurred, which contradicted the inheritance pattern proposed by Long & Gregory (1978). Furthermore, Capitan *et al.* (2009) could not find any significant linkage between the scurs phenotype and eleven microsatellite markers on BTA19 in the French Charolais breed.

Capitan *et al.* (2011) described a new type of scurs in the French Charolais breed, which were specifically observed in animals that were homozygous wildtype at the *POLLED* locus. This secondary type of scurs was named Type 2 scurs, due to its similarity to the already known scurs phenotype. All affected animals with this syndrome, showed both horn abnormalities that are similar to classical scurs, as well as interfrontal suture synostosis, which is characterized by acrocephaly and extra bone deposition along the interfrontal suture. These authors mapped the Type 2 scurs syndrome to a 1.7 Mb region on BTA4 using a genome-wide linkage analysis with the Illumina Bovine SNP50 chip and identified a frame shift mutation in the *TWIST1* gene as a causative mutation of this syndrome (Capitan *et al.*, 2011). This study demonstrated the complexity of horn ontogenesis and the genetic variability of the scurs phenotype.

A study conducted by Mariasegaram *et al.* (2012) indicated that scurs generally occur in animals that are heterozygous for the *POLLED* locus and suggested that epistasis may be involved. The heterozygosity for the *POLLED* locus associated with scurs was also confirmed by Wiedemar *et al.* (2014). A variation of the incidence of scurs between breeds were also observed, which is consistent with the proposed epistatic nature of the *SCURS* locus (Mariasegaram *et al.*, 2012).

A SNP based association study in polled Simmental cattle confirmed the challenges in identification of the *SCURS* locus (Tetens *et al.*, 2015), indicating a possible association with one SNP on BTA19 close to the previously identified region by Asai *et al.* (2004). This study also observed suspected spurious associations on BTA1, BTA4 and BTA7, and associations that failed to achieve genome-wide significance on BTA2, BTA9 and BTA10 (Tetens *et al.*, 2015). Tetens *et al.* (2015) supported the localization of the *SCURS* locus on BTA19 and reported genetic heterogeneity of this

© University of Pretoria

locus and demonstrated that the scurs phenotype in the Simmental breed cannot be explained by a single locus. These authors also noted that there was no evidence for association of the scurs phenotype with the X-chromosome.

To date, the exact location of the *SCURS* locus and the molecular basis governing the scurs phenotype, has not been resolved (Figure 2.3). Combined research findings indicate that scurs is a complex phenotype that shows genetic heterogeneity in various breeds (Asai *et al.*, 2004; Capitan *et al.*, 2011; Tetens *et al.*, 2015) and that more than one gene might be involved in the molecular basis of scurs.

2.5 Conclusion

The inheritance of polledness, horns and scurs were of interest since the early 1900's, primarily due to the economic importance of the polled trait and increased ease of management of hornless cattle. Prior to the development of molecular technology, selection of the polled, scurs and horned phenotypes, however, was limited due to a lack of suitable DNA markers for MAS, and the unresolved molecular basis of these phenotypes. Genomics now provides more advanced and powerful tools for unravelling the molecular basis and genetic architecture of phenotypic diversity in livestock.

References

Allais-Bonnet, A., Grohs, C., Medugorac, I., Krebs, S., Djari, A., Graf, A., Fritz, S., Seichter, D. *et al.*, 2013. Novel insights into the bovine polled phenotype and horn ontogenesis in Bovidae. PLoS One. 8, 63512.

Andersson, L., 2001. Genetic dissection of phenotypic diversity in farm animals. Nature Reviews Genetics. 2, 130-138.

An, B., Xia, J., Chang, T., Wang, X., Miao, J., Xu, L., Zhang, L., Gao, X., Chen, Y., Li, J. & Gao, H., 2018. Genome-wide association study identifies loci and candidate genes for internal organ weights in Simmental beef cattle. Physiological Genomics. 50, 523-531.

Asai, M., Berryere, T.G. & Schmutz, S.M., 2004. The scurs locus in cattle maps to bovine chromosome 19. Animal Genetics. 35, 34-39.

Aubry, P., 2005. Routine surgical procedures in dairy cattle under field conditions: Abomasal surgery, dehorning, and tail docking. Veterinary Clinics: Food Animal Practice. 21, 55-72.

Barendse, W., Armitage, S.M., Kossarek, L.M., Shalom, A., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li, L. *et al.*, 1994. A genetic linkage map of the bovine genome. Nature Genetics. 6, 227.

Barendse, W., Vaiman, D., Kemp, S.J., Sugimoto, Y., Armitage, S.M., Williams, J.L., Sun, H.S., Eggen, A. *et al.*, 1997. A medium-density genetic linkage map of the bovine genome. Mammalian Genome. 8, 21-28.

Bateson, W. & Saunders, E.R., 1902. The facts of heredity in the light of Mendel's discovery. Reports to the Evolution Committee of the Royal Society. 1, 125-160.

© University of Pretoria

Beuzen, N.D., Stear, M.J. & Chang, K.C., 2000. Molecular markers and their use in animal breeding. The Veterinary Journal. 160, 42-52.

Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D. & Yoo, J. & Beattie, C.W., 1994. A genetic linkage map for cattle. Genetics. 136, 619-639.

Blackwell, R.L. & Knox, J.H., 1958. Scurs in a herd of Aberdeen-Angus cattle. Journal of Heredity. 49, 117-119.

Blasco, A. & Toro, M.A., 2014. A short critical history of the application of genomics to animal breeding. Livestock Science. 166, 4-9.

Bolormaa, S., Hayes, B.J., Savin, K., Hawken, R., Barendse, W., Arthur, P.F., Herd, R.M. & Goddard, M.E., 2011. Genome-wide association studies for feedlot and growth traits in cattle. Journal of Animal Science. 89, 1684-1697.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 32, 314.

Bovine Genome Sequencing and Analysis Consortium, 2009. The genome sequence of taurine cattle: a window to ruminant biology and evolution. Science. 324, 522-528.

Boyd, M.M., 1906. Breeding of Polled Herefords. Proceedings of the American Breeders Association. 2, 198–201.

Brenneman, R.A., Davis, S.K., Sanders, J.O., Burns, B.M., Wheeler, T.C., Turner, J.W. & Taylor, J.F., 1996. The polled locus maps to BTA1 in a Bos indicus× Bos taurus cross. Journal of Heredity. 87, 156-161.

Capitan, A., Grohs, C., Gautier, M. & Eggen, A., 2009. The scurs inheritance: new insights from the French Charolais breed. BMC Genetics. 10, 1-11.

Capitan, A., Grohs, C., Weiss, B., Rossignol, M.N., Reversé, P. & Eggen, A., 2011. A newly described bovine type 2 scurs syndrome segregates with a frame-shift mutation in TWIST1. PLoS One. 6, 22242.

Cardoso, C.S., von Keyserlingk, M.A. & Hötzel, M.J., 2016. Trading off animal welfare and production goals: Brazilian dairy farmers' perspectives on calf dehorning. Livestock Science. 187, 102-108.

Castro, L.M., Rosa, G.J.M., Lopes, F.B., Regitano, L.C.A., Rosa, A.J.M. & Magnabosco, C.U., 2017. Genomewide association mapping and pathway analysis of meat tenderness in Polled Nellore cattle. Journal of Animal Science. 95, 1945-1956.

Charlier, C., Coppieters, W., Rollin, F., Desmecht, D., Agerholm, J.S., Cambisano, N., Carta, E., Dardano, S., *et al.*, 2008. Highly effective SNP-based association mapping and management of recessive defects in livestock. Nature Genetics. 40, 449-454.

Churchill, O.O., 1927. Sex and horns in cattle, a note on an exceptional mode of inheritance. Journal of Heredity. 18, 279–280.

© University of Pretoria

Costa, R.B., Camargo, G.M., Diaz, I.D., Irano, N., Dias, M.M., Carvalheiro, R., Boligon, A.A., Baldi, F., Oliveira, H.N., Tonhati, H. & Albuquerque, L.G., 2015. Genome-wide association study of reproductive traits in Nellore heifers using Bayesian inference. Genetics Selection Evolution, 47, 1-9.

Cozzi, G., Gottardo, F., Brscic, M., Contiero, B., Irrgang, N., Knierim, U., Pentelescu, O., Windig, J.J., Mirabito, L., Eveillard, F.K. & Dockès, A.C., 2015. Dehorning of cattle in the EU Member States: A quantitative survey of the current practices. Livestock Science. 179, 4-11.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M. & Blaxter, M.L., 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics. 12, 499-510.

Dodgson, J.B., Cheng, H.H. & Okimoto, R., 1997. DNA marker technology: a revolution in animal genetics. Poultry Science. 76, 1108-1114.

Drögemüller, C., Wöhlke, A., Leeb, T. & Distl, O., 2005a. A 4 Mb high resolution BAC contig on bovine chromosome 1q12 and comparative analysis with human chromosome 21q22. Comparative and functional genomics. 6, 194-203.

Drögemüller, C., Wöhlke, A., Mömke, S. & Distl, O., 2005b. Fine mapping of the polled locus to a 1-Mb region on bovine chromosome 1q12. Mammalian Genome. 16, 613-620.

Fan, B., Du, Z.Q., Gorbach, D.M. & Rothschild, M.F., 2010. Development and application of high-density SNP arrays in genomic studies of domestic animals. Asian-Australasian Journal of Animal Sciences. 23, 833-847.

Fries, R., Eggen, A. & Womack, J.E., 1993. The bovine genome map. Mammalian Genome. 4, 405-428.

Georges, M., Drinkwater, R., King, T., Mishra, A., Moore, S.S., Nielsen, D., Sargeant, L.S., Sorensen, A., *et al.*, 1993. Microsatellite mapping of a gene affecting horn development in *Bos taurus*. Nature Genetics. 4, 206-210.

Georges, M. & Andersson, L., 1996. Livestock genomics comes of age. Genome Research. 6, 907-921.

Goonewardene, L.A., Pang, H., Berg, R.T. & Price, M.A., 1999. A comparison of reproductive and growth traits of horned and polled cattle in three synthetic beef lines. Canadian Journal of Animal Science. 79, 123-127.

Gowen, J.W., 1918. Studies in inheritance of certain characters of crosses between dairy and beef breeds of cattle. Journal of Agricultural Research. 15, 1–58.

Graf, B. & Senn, M., 1999. Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. Applied Animal Behaviour Science. 62, 153-171.

Gurgul, A., Semik, E., Pawlina, K., Szmatoła, T., Jasielczuk, I. & Bugno-Poniewierska, M., 2014. The application of genome-wide SNP genotyping methods in studies on livestock genomes. Journal of Applied Genetics. 55, 197-208.

Habel, R. & Budras, K.H., 2003. Skull with paranasal sinuses and horns. In: Bovine Anatomy. Budras, K. H. & Habel, R. (Eds.), Schlütersche, Hannover, Germany, pp.34–35.

© University of Pretoria

Harlizius, B., Tammen, I., Eichler, K., Eggen, A. & Hetzel, D.J.S., 1997. New markers on bovine chromosome 1 are closely linked to the polled gene in Simmental and Pinzgauer cattle. Mammalian genome. 8, 255-257.

Hawken, R.J., Zhang, Y.D., Fortes, M.R.S., Collis, E., Barris, W.C., Corbet, N.J., Williams, P.J., Fordyce, G., *et al.*, 2012. Genome-wide association studies of female reproduction in tropically adapted beef cattle. Journal of Animal Science. 90, 1398-1410.

Hayes, B. & Goddard, M., 2010. Genome-wide association and genomic selection in animal breeding. Genome. 53, 876-883.

Heather, J.M. & Chain, B., 2016. The sequence of sequencers: The history of sequencing DNA. Genomics. 107, 1-8.

Hirschhorn, J.N. & Daly, M.J., 2005. Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics. 6, 95-108.

Kappes, S.M., Keele, J.W., Stone, R.T., McGraw, R.A., Sonstegard, T.S., Smith, T.P., Lopez-Corrales, N.L. & Beattie, C.W., 1997. A second-generation linkage map of the bovine genome. Genome Research. 7, 235-249.

Kim, Y., Ryu, J., Woo, J., Kim, J.B., Kim, C.Y. & Lee, C., 2011. Genome-wide association study reveals five nucleotide sequence variants for carcass traits in beef cattle. Animal Genetics. 42, 361-365.

Kling-Eveillard, F., Knierim, U., Irrgang, N., Gottardo, F., Ricci, R. & Dockès, A.C., 2015. Attitudes of farmers towards cattle dehorning. Livestock Science. 179, 12-21.

Knierim, U., Irrgang, N. & Roth, B.A., 2015. To be or not to be horned – Consequences in cattle. Livestock Science. 179, 29-37.

Kumar, S., Banks, T.W. & Cloutier, S., 2012. SNP discovery through next-generation sequencing and its applications. International Journal of Plant Genomics. 831460, 1-15.

Lenstra, J.A., Groeneveld, L.F., Eding, H., Kantanen, J., Williams, J.L., Taberlet, P., Nicolazzi, E.L., Sölkner, *et al.*, 2012. Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. Animal Genetics. 43, 483-502.

Long, C.R. & Gregory, K.E., 1978. Inheritance of the horned, scurred, and polled condition in cattle. Journal of Heredity. 69, 395-400.

Lloyd-Jones, O. & Evvard, J.M., 1916. Inheritance of color and horns in blue-gray cattle. Iowa agricultural experimental station. Research Bulletin of Iowa State College. 30, 67–106.

Lundrigan, B., 1996. Morphology of horns and fighting behavior in the family Bovidae. Journal of Mammalogy. 77, 462-475.

Mariasegaram, M., Harrison, B.E., Bolton, J.A., Tier, B., Henshall, J.M., Barendse, W. & Prayaga, K.C., 2012. Fine-mapping the POLL locus in Brahman cattle yields the diagnostic marker CSAFG29. Animal Genetics. 43, 683-688.

© University of Pretoria

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'Connell, J., Moore, S.S., Smith, T.P., Sonstegard, T.S. & Van Tassell, C.P., 2009. Development and characterization of a high density SNP genotyping assay for cattle. PloS One. 4, e5350.

McCarthy, M.I., Abecasis, G.R., Cardon, L.R., Goldstein, D.B., Little, J., Ioannidis, J.P. & Hirschhorn, J.N., 2008. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nature Reviews Genetics. 9, 356-369.

McMeekan, C.M., Stafford, K.J., Mellor, D.J., Bruce, R.A., Ward, R.N. & Gregory, N.G., 1998. Effects of regional analgesia and/or a non-steroidal anti-inflammatory analgesic on the acute cortisol response to dehorning in calves. Research in Veterinary Science. 64, 147-150.

McMeekan, C.M., Stafford, K.J., Mellor, D.J., Bruce, R.A., Ward, R.N. & Gregory, N.G., 1999. Effects of a local anaesthetic and a nonsteroidal anti-inflammatory analgesic on the behavioural responses of calves to dehorning. New Zealand Veterinary Journal. 47, 92–96.

Medugorac, I., Seichter, D., Graf, A., Russ, I., Blum, H., Göpel, K.H., Rothammer, S., Förster, M. & Krebs, S., 2012. Bovine polledness–an autosomal dominant trait with allelic heterogeneity. PLoS One, 7, 39477.

Medugorac, I., Graf, A., Grohs, C., Rothammer, S., Zagdsuren, Y., Gladyr, E., Zinovieva, N., Barbieri, J., Seichter, D., Russ, I. & Eggen, A., 2017. Whole-genome analysis of introgressive hybridization and characterization of the bovine legacy of Mongolian yaks. Nature Genetics. 49, 470-475.

Mullis, K., Faloona, F., Scharf, S., Saiki, R.K., Horn, G.T. & Erlich, H., 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. In: Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor Laboratory Press. 51, 263-273.

Peripolli, E., Munari, D.P., Silva, M.V.G.B., Lima, A.L.F., Irgang, R. & Baldi, F., 2016. Runs of homozygosity: current knowledge and applications in livestock. Animal Genetics. 48, 255-271.

Petrie, N., Mellor, D.J., Stafford, K.J., Bruce, R.A. & Ward, R.N., 1996. Cortisol responses of calves to two methods of disbudding used with or without local anaesthesia. New Zealand Veterinary Journal. 44, 9–14.

Prayaga, K. C., 2007. Genetic options to replace dehorning in beef cattle – a review. Crop and Pasture Science. 58, 1-8.

Rege, J.E.O. & Tawah, C.L., 1999. The state of African cattle genetic resources II. Geographical distribution, characteristics and uses of present-day breeds and strains. Animal Genetic Research Information. 26, 1‑25.

Rexroad, C.E. III, Owens, E.K., Johnson, J.S. & Womack, J.E., 2000. A 12 000 rad whole genome radiation hybrid panel for high resolution mapping in cattle: characterization of the centromeric end of chromosome 1. Animal Genetics. 31, 262-265.

Robbins, J.A., Weary, D.M., Schuppli, C.A. & Von Keyserlingk, M.A.G., 2015. Stakeholder views on treating pain due to dehorning dairy calves. Animal Welfare. 24, 399-406.

© University of Pretoria

Rolf, M.M., Taylor, J.F., Schnabel, R.D., McKay, S.D., McClure, M.C., Northcutt, S.L., Kerley, M.S. & Weaber, R.L., 2012. Genome-wide association analysis for feed efficiency in Angus cattle. Animal Genetics. 43, 367-374.

Rosenberg, N.A., Li, L.M., Ward, R. & Pritchard J.K., 2003. Informativeness of genetic markers for inference of ancestry. American Journal of Human Genetics. 73, 1402–1422.

Rushen, J., De Passillé, A.M., Keyserlingk, M.A. & Weary, D.M., 2007. Acute or short-term challenges to animal welfare. In: The welfare of cattle. Volume 5, Springer, Dordrecht. pp115-141.

Rothammer, S., Capitan, A., Mullaart, E., Seichter, D., Russ, I. & Medugorac, I., 2014. The 80-kb DNA duplication on BTA1 is the only remaining candidate mutation for the polled phenotype of Friesian origin. Genetics Selection Evolution. 46, 1-5.

Santana, M.H., Utsunomiya, Y.T., Neves, H.H., Gomes, R.C., Garcia, J.F., Fukumasu, H., Silva, S.L., Junior, G.A.O., *et al.*, 2014. Genome-wide association analysis of feed intake and residual feed intake in Nellore cattle. BMC Genetics. 15, 21.

Schafberg, R. & Swalve, H.H., 2015. The history of breeding for polled cattle. Livestock Science. 179, 54-70.

Schlötterer, C., 2004. The evolution of molecular markers – just a matter of fashion? Nature Reviews Genetics. 5, 63.

Schmutz, S.M., Marquess, F.L.S., Berryere, T.G. & Moker, J.S., 1995. DNA marker assisted selection of the polled condition in Charolais cattle. Mammalian Genome. 6, 710–713.

Schopen, G.C., Bovenhuis, H., Visker, M.H. & van Arendonk, J.A., 2008. Comparison of information content for microsatellites and SNPs in poultry and cattle. Animal Genetics. 39, 451–453.

Seichter, D., Russ, I., Rothammer, S., Eder, J., Förster, M. & Medugorac, I., 2012. SNP-based association mapping of the polled gene in divergent cattle breeds. Animal Genetics. 43, 595-598.

Sharma, A., Lee, J.S., Dang, C.G., Sudrajad, P., Kim, H.C., Yeon, S.H., Kang, H.S. & Lee, S.H., 2015. Stories and challenges of genome wide association studies in livestock - a review. Asian-Australasian Journal of Animal Sciences. 28, 1371-1379.

Smith, A.D.B., 1927. The inheritance of horns in cattle. Some further data. Journal of Genetics. 18, 365–374.

Snelling, W.M., Allan, M.F., Keele, J.W., Kuehn, L.A., Mcdaneld, T., Smith, T.P.L., Sonstegard, T.S., Thallman, R.M. & Bennett, G.L., 2010. Genome-wide association study of growth in crossbred beef cattle. Journal of Animal Science. 88, 837-848.

Soller, M. & Beckmann, J.S., 1983. Genetic polymorphism in varietal identification and genetic improvement. Theoretical and Applied Genetics. 67, 25-33.

Spillman, W.J., 1906. A Mendelian character in cattle. Science. 23, 549-551.

Stafford, K.J., Mellor, D.J., Todd, S.E., Ward, R.N. & McMeekan, C.M., 2003. Effects of different combinations of lignocaine, ketoprofen, xylazine and tolazoline on the acute cortisol response to dehorning in calves. New Zealand Veterinary Journal. 51, 219–226.

© University of Pretoria

Stafford, K.J. & Mellor, D.J., 2005. Dehorning and disbudding distress and its alleviation in calves. The Veterinary Journal. 169, 337-349.

Stafford, K.J. & Mellor, D.J., 2011. Addressing the pain associated with disbudding and dehorning in cattle. Applied Animal Behaviour Science. 135, 226-231.

Stafuzza, N.B., de Oliveira Silva, R.M., Peripolli, E., Bezerra, L.A.F., Lôbo, R.B., de Ulhoa Magnabosco, C., Di Croce, F.A., Osterstock, J.B. *et al.*, 2018. Genome-wide association study provides insights into genes related with horn development in Nelore beef cattle. PloS One. 13, 0202978.

Stock, M.L., Baldridge, S.L., Griffin, D. & Coetzee, J.F., 2013. Bovine dehorning: Assessing pain and providing analgesic management. Veterinary Clinics: Food Animal Practice. 29, 103-133.

Sylvester, S.P., Stafford, K.J., Mellor, D.J., Bruce, R.A. & Ward, R.N., 2004. Behavioural responses of calves to amputation dehorning with and without local anaesthesia. Australian Veterinary Journal. 82, 697-700.

Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research. 17, 6463-6471.

Tetens, J., Wiedemar, N., Menoud, A., Thaller, G. & Drögemüller, C., 2015. Association mapping of the scurs locus in polled Simmental cattle – evidence for genetic heterogeneity. Animal Genetics. 46, 224-225.

Utsunomiya, Y.T., Do Carmo, A.S., Carvalheiro, R., Neves, H.H., Matos, M.C., Zavarez, L.B., O'Brien, A.M.P., Sölkner, J., *et al.*, 2013. Genome-wide association study for birth weight in Nellore cattle points to previously described orthologous genes affecting human and bovine height. BMC Genetics. 14, 1-12.

Utsunomiya, Y.T., Torrecilha, R.B.P., Milanesi, M., Paulan, S.D.C., Utsunomiya, A.T.H. & Garcia, J.F., 2019. Hornless Nellore cattle (*Bos indicus*) carrying a novel 110 kbp duplication variant of the polled locus. Animal Genetics. 50, 187-188.

Valente, T.S., Baldi, F., Sant'Anna, A.C., Albuquerque, L.G. & Paranhos da Costa, M.J.R., 2016. Genome-wide association study between single nucleotide polymorphisms and flight speed in Nellore cattle. PloS One. 11, e0156956.

Visscher, P.M., Wray, N.R., Zhang, Q., Sklar, P., McCarthy, M.I., Brown, M.A. & Yang, J., 2017. 10 years of GWAS discovery: biology, function, and translation. The American Journal of Human Genetics. 101, 5-22.

Watson, J.A.S., 1921. A Mendelian experiment with Aberdeen-Angus and West Highland cattle. Journal of Genetics. 11, 59–67.

White, W.T. & Ibsen, H.L., 1936. Horn inheritance in Galloway-Holstein cattle-crosses. Journal of Genetics. 32, 33–49.

© University of Pretoria

Wiedemar, N., Tetens, J., Jagannathan, V., Menoud, A., Neuenschwander, S., Bruggmann, R., Thaller, G. & Drögemüller, C., 2014. Independent polled mutations leading to complex gene expression differences in cattle. PloS One. 9, e93435.

Williams, H.D. & Williams, T., 1952. The inheritance of horns and their modifications in polled Hereford cattle. Journal of Heredity. 43, 267-272.

Windig, J. J., Hoving-Bolink, R. A., & Veerkamp, R. F., 2015. Breeding for polledness in Holstein cattle. Livestock Science. 179, 96–101

Winks, L., Holmes, A.E. & O'Rourke, P.K., 1977. Effect of dehorning and tipping on liveweight gain of mature Brahman crossbred steers. Australian Journal of Experimental Agriculture and Animal Husbandry. 17, 16–19.

Womack, J.E. & Kata, S.R., 1995. Bovine genome mapping: evolutionary inference and the power of comparative genomics. Current Opinion in Genetics & Development. 5, 725-733.

Womack, J.E., Johnson, J.S., Owens, E.K., Rexroad, C.E., Schläpfer, J. & Yang, Y.P., 1997. A whole-genome radiation hybrid panel for bovine gene mapping. Mammalian Genome. 8, 854-856.

Womack, J.E., 2012. First steps: bovine genomics in historical perspective. Animal Genetics. 43, 2-8.

Wunderlich, K.R., Abbey, C.A., Clayton, D.R., Song, Y., Schein, J.E., Georges, M., Coppieters, W., Adelson, D.L., Taylor, J.F., Davis, S.L. & Gill, C.A., 2006. A 2.5-Mb contig constructed from Angus, Longhorn and horned Hereford DNA spanning the polled interval on bovine chromosome 1. Animal Genetics. 37, 592-594.

Xia, J., Qi, X., Wu, Y., Zhu, B., Xu, L., Zhang, L., Gao, X., Chen, Y., Li, J. & Gao, H., 2016. Genome-wide association study identifies loci and candidate genes for meat quality traits in Simmental beef cattle. Mammalian Genome. 27, 246-255.

Ziegler, A., König, I.R. & Thompson, J.R., 2008. Biostatistical aspects of genome-wide association studies. Biometrical Journal: Journal of Mathematical Methods in Biosciences. 50, 8-28.

Zimin, A.V., Delcher, A.L., Florea, L., Kelley, D.R., Schatz, M.C., Puiu, D., Hanrahan, F., Pertea, G., Van Tassell, C.P., Sonstegard, T.S. & Marçais, G., 2009. A whole-genome assembly of the domestic cow, Bos taurus. Genome Biology. 10, R42.

Zondervan, K.T. & Cardon, L.R., 2007. Designing candidate gene and genome-wide case–control association studies. Nature Protocols. 2, 2492-2501.

© University of Pretoria

Chapter 3

Validation of the *POLLED* Celtic variant in South African Bonsmara and Drakensberger beef cattle breeds

R. Grobler[a, #], C. Visser[a], A. Capitan[b, c] & E. van Marle-Köster[a]

[a] Department Animal and Wildlife Sciences, University of Pretoria, Pretoria, 0002, South Africa

[b] UMR GABI, INRA, AgroParisTech, Université Paris Saclay, 78350 Jouy en Josas, France

[c] Allice, 75595 Paris, France

[#] Corresponding author: rulieng@hotmail.com

Published in: Livestock Science 217 (2018) 136–139

https://doi.org/10.1016/j.livsci.2018.10.003

© University of Pretoria

Short communication

Validation of the *POLLED* Celtic variant in South African Bonsmara and Drakensberger beef cattle breeds

Abstract

An increased awareness of animal welfare necessitates the breeding of genetically polled animals, especially since more than 70% of South African beef cattle are rounded off in commercial feedlots. The Bonsmara and the Drakensberger, two locally developed breeds, play a major role in beef production in South Africa. The causative mutation for polledness in these breeds has not been confirmed, therefore, this study aimed to validate the *POLLED* Celtic variant as the causative mutation of polledness in the South African Bonsmara and Drakensberger beef cattle breeds. A total of 386 animals, consisting of Bonsmara, Drakensberger and Herefords (included as a *Bos taurus* control), were tested for the Celtic mutation by PCR-based screening. Phenotypically polled and scurred animals were found to carry at least one copy of the Celtic allele (P_C) whereas horned animals were homozygous wild type. The highest frequency of homozygous polled animals (P_C/P_C = 0.337) was observed in the *Bos taurus* control (Hereford breed) while the majority of the Bonsmara animals were heterozygous polled (P_C/p = 0.591). For the Drakensberger, a heterozygous (P_C/p) genotypic frequency of 0.346 was observed, with the majority of animals being horned (p/p = 0.639). In the Bonsmara and Hereford breeds, a high proportion of heterozygous polled animals were phenotypically scurred, emphasizing the importance of correct phenotyping at farm level. This research validates the Celtic mutation as causative mutation for polledness in indigenous South African beef cattle breeds. It also demonstrates the current challenges with regards to both phenotypic and genetic verification of the scurs phenotype and requires further investigation in South African beef cattle breeds.

Keywords: Polledness, Heterozygous polled, Sanga cattle, Scurs

3.1 Introduction

Since the domestication of cattle, selection practises were focused on adapted animals and aesthetic traits, and horned cattle were favoured by selection. Horns, especially in male animals, were associated with fertility (Knierim et al., 2015; Schafberg and Swalve, 2015). However, over the past few decades the focus has shifted towards sustainable animal production, with an increased awareness of animal welfare. Horns in cattle are a major cause of bruising, hide and carcass damage, as well as other injuries, but the practice of dehorning cattle has serious welfare implications (Graff and Senn, 1999; Windig et al., 2015). Breeding genetically polled animals would provide a long-term solution and welfare friendly alternative to dehorning.

The *POLLED* locus has been mapped to the centromeric region of BTA1 in a number of cattle breeds (Georges et al., 1993; Drögemüller et al., 2005; Seichter et al., 2012) and three distinct causative

© University of Pretoria

variants have recently been identified at this locus, namely the Celtic, Friesian and Mongolian alleles (Medugorac et al., 2012; Allais-Bonnet et al., 2013; Medugorac et al., 2017). The Celtic allele (P_C) is responsible for polledness in most of the European *Bos taurus* breeds, while the Friesian allele (P_F) predominantly governs the polled phenotype in the Holstein Friesian breed (Medugorac et al., 2012). The Mongolian allele (P_M) has been described only in East Asian *Bos taurus* and *Bos grunniens* breeds (Medugorac et al., 2017). None of these mutations are located in known coding or regulatory regions, thus adding to the complexity of the molecular basis of polledness. The genetic basis of polledness is further complicated by the presence of scurs, which develop as small horn-like growths in the same area as horns on the skull, but these abnormal horns are loosely attached to the skull (Capitan et al., 2009). The *POLLED* locus has been found to be epistatic to scurs in both sexes.

In South Africa, the red meat industry plays a major role in livestock production, with more than 70% of all beef cattle slaughtered in the formal sector originating from commercial feedlots (Scholtz et al., 2008). In Bonsmara herds, the polled trait occurred either spontaneously due to the Shorthorn/Hereford ancestors from which the breed was developed or by infusion through the upgrading of Red Poll and Red Angus cows to Bonsmara stud status (Schmulian, 2006). The Drakensberger breed is naturally horned, with the assumption that polledness was introgressed in the breed by upgrading with naturally polled breeds, such as the Black Angus. In South Africa, polledness was historically not a trait selected for by beef cattle breeders, mainly due to the belief that polled animals are inferior compared to horned animals (Schmulian, 2006). However, over the past two decades, South African breeders realized the advantages of polled cattle and showed an increased interest in breeding polled animals, primarily due to welfare and market preferences.

The majority of previous research on the *POLLED* locus and polledness has been performed in European breeds (Allais-Bonnet et al., 2013), and South African indigenous breeds are genetically distinct from the European *Bos taurus* breeds (Makina et al., 2014). Besides the two main types of cattle, *Bos taurus* and *Bos indicus*, indigenous African cattle, such as the Sanga, are also found in South Africa. The Drakensberger is classified as a Sanga breed, which are a hybrid between *Bos taurus* and *Bos indicus* (Rege and Tawah, 1999).

A preliminary study on polledness in Bonsmara cattle indicated association between the polled phenotype and nine microsatellite markers on BTA1 (Schmulian, 2006). The causative mutation for polledness is still unknown for indigenous South African cattle. This study forms part of a larger research project to investigate the inheritance patterns of the *POLLED* and *SCURS* loci in indigenous South African beef cattle breeds. This study investigated polledness in the South African Bonsmara and Drakensberger beef cattle breeds, with the aim of validating the Celtic variant as the causative mutation of polledness in these breeds.

© University of Pretoria

3.2 Materials and methods

3.2.1 Animals and phenotypes

The study was performed with consent from the respective Breeders' Associations, as well as individual breeder consent, and ethical approval from the University of Pretoria (EC170424-110). Hair samples and phenotypic records of the horn status of mature registered purebred animals were provided by four Bonsmara breeders, six Drakensberger breeders and three Hereford breeders. The samples included animals with polled, horned and scurred phenotypes. Samples with an unknown phenotype and sex were excluded from analyses and a total of 386 animals were included in this study, consisting of 164 Bonsmara and 133 Drakensberger. 89 Hereford were included as a *Bos taurus* control.

3.2.2 Celtic genotyping

Genomic DNA were extracted from the hair samples with a Zymogen Tissue kit (www.zymoresearch.com) in the Animal Breeding and Genetics laboratory at the Department of Animal and Wildlife Sciences, University of Pretoria. The polled, horned and scurred animals were screened for their status for the Celtic mutation at the *POLLED* locus using a microsatellite marker-based diagnostic test. To identify the Celtic mutation, the CELT primer (CELT-Fw: GAAGTGTGGCCGGTAGAAAA and CELT-Rv: ATCAAGGACACCTCCCACAC) was used (Allais-Bonnet et al., 2013). This screening allows the identification of carriers of the Celtic mutation, as well as the identification of genotypic status (P_C/P_C, P_C/p or p/p).

The PCR reaction was performed with a final volume of 15 µl. The amplification reaction contained 8 µl Bioline MyTaq Red Mix® enzyme (www.bioline.com), 1.4 µl molecular grade water, 0.3 µl each of both forward and reverse primer [10 pmol/µl] and 5 µl genomic bovine DNA. The PCR conditions were performed as follow: 94 °C for 5 min, 39 cycles of 94 °C for 30s, 55 °C for 30s and 72 °C for 30s, with a final extension step of 72 °C for 5 min. The PCR products were visualized on a 3% agarose gel with a 100bp size ladder to determine the fragment size of the products. There is a 202 bp difference between the Celtic and wildtype allele.

3.2.3 Statistical analysis

Genotype frequencies were calculated for the three possible genotypes of the Celtic allele (P_C/P_C, P_C/p and p/p) by direct counting. A Hardy-Weinberg Equilibrium (HWE) p-value was calculated for the genotype frequencies using a Chi-square test and the significance threshold was set at 0.05. Pearson correlation coefficients between the phenotypes recorded on farm and the Celtic genotypes obtained from the PCR-based Celtic screening of the samples, were calculated by R software v3.3.1 (R Core Team, 2013). Correlation coefficients were calculated to validate the accuracy of the Celtic allele to indicate the polled status of an animal, as well as to determine the accuracy of the phenotypic recording of each sample group. The Pearson correlation (r) measures a linear dependence between two variables,

© University of Pretoria

x and y, where in this case x is equal to the on farm recorded phenotype of the horn status of each animal, and y equals the observed Celtic genotype obtained from the PCR-based screening.

3.3 Results and discussion

The South African Bonsmara, Drakensberger and Hereford beef cattle breeds were screened for the Celtic allele (P_C) (Medugorac et al., 2012) and both homozygous and heterozygous polled animals were observed. It was possible to distinguish between horned, homozygous polled and heterozygous polled animals at a genotypic level.

The frequency of the observed genotypes for the Celtic allele in the three breeds are shown in Table 3.1. All the phenotypically polled and scurred animals were found to carry at least one copy of the Celtic allele (P_C) whereas horned animals were homozygous wild type. Based on the HWE p-values ($p < 0.0001$, Table 3.1), the genotypic frequencies observed were significantly different and deviated from Hardy-Weinberg Equilibrium ($p < 0.0001$). The deviation from HWE was expected, due to indirect selection for the *POLLED* locus in these breeds. The Hereford had the highest frequency of homozygous polled animals ($P_C/P_C = 0.337$), as expected due to the selection preference for polled Hereford in South Africa. In the Bonsmara the majority of the animals tested were heterozygous ($P_C/p = 0.591$) for the polled phenotype, with a lower frequency of homozygous polled animals ($P_C/P_C = 0.177$). The majority of phenotypic polled Drakensberger animals were heterozygous polled ($P_C/p = 0.346$) with very few animals being homozygous polled ($P_C/P_C = 0.015$). The lower frequency of the polled allele in the Drakensberger can be attributed to the fact that this breed is historically horned and very few breeders have introgressed the polled allele into their breeding herds. Some Drakensberger breeders also are of the opinion that polled Drakensberger bulls are inferior compared to horned bulls, with polled bulls exhibiting lower masculinity and a higher incidence of penile prolapses (Pers. Comm., D. Orsmond, May 2017). In general, cattle breeders historically showed a preference for horned bulls due to a perception that horned animals were more fertile and exhibited stronger male characteristics. This perception may have led to selection preference for horned bulls in a number of breeds (Götz et al., 2015).

© University of Pretoria

Table 3.1 The total observed genotypes and genotypic frequencies for the Celtic variant observed in three South African beef cattle breeds

Breed	Genotype						Total	HWE* p-value
	Pc/Pc		Pc/p		p/p			
	Total	Frequency	Total	Frequency	Total	Frequency		
Bonsmara	29	0.177	97	0.591	38	0.232	164	< 0.0001
Drakensberger	2	0.015	46	0.346	85	0.639	133	< 0.0001
Hereford	30	0.337	46	0.517	13	0.146	89	< 0.0001
Total	61		189		136		386	

*HWE = Hardy-Weinberg equilibrium

It can be concluded that the polled phenotype in the South African Bonsmara, Drakensberger and Hereford are genetically determined by the Celtic allele (P_C). This corresponds to the ancestry of the breeds, as introgression and upgrading with *Bos taurus* breeds occurred in both Bonsmara and Drakensberger breeds, and the Hereford is an exotic *Bos taurus* breed. The Celtic allele have been identified in most European cattle *Bos taurus* breeds (Allais-Bonnet et al., 2013) and more recently in a synthetic Chinese cattle breed, Shuxuan, which was crossbred with both Simmental and Holstein semen. Chen et al. (2017) observed the Celtic allele in the Shuxuan cattle at a frequency of 0.437.

The *SCURS* locus is epistatic to the *POLLED* locus and according to the model of Long and Gregory (1978), a sex-influenced expression pattern is assumed for the scurs phenotype and in males, the heterozygote (*Scsc*) is usually scurred, while in females only the homozygote (*ScSc*) is scurred. Furthermore, males that are heterozygous for scurs must also be heterozygous at the *POLLED* locus in order for the scurs phenotype to be expressed. Thus, animals with a scurs phenotype should show a heterozygous polled genotype (Long and Gregory, 1978) for the Celtic allele (P_C/p), which further suggests that animals with a heterozygous polled genotype can either be phenotypically polled or scurred. However, scurs cannot be identified on a genotypic level based on the Celtic allele (P_C). In all three breeds phenotypically scurred animals were genotyped as heterozygous polled carriers of the P_C allele. The proportion of animals with a heterozygous polled genotype (P_C/p) for the Celtic mutation, that are phenotypically identified as scurred are shown in Table 3.2. In the Bonsmara breed, 50% of the male heterozygous genotypes (P_C/p) corresponded with a scurred phenotype, while in the Hereford breed, 42% of female animals that were genotyped as heterozygous polled (P_C/p) were phenotypically scurred. None of the homozygous P_C/P_C animals have a scurred phenotype and therefore the effect of the P_C allele is additive.

© University of Pretoria

Table 3.2 The horn status phenotypes for each breed as recorded on farm per sex, with the Celtic genotype observed for each breed per phenotype for male and female animals (the corresponding genotypic frequencies are indicated in brackets)

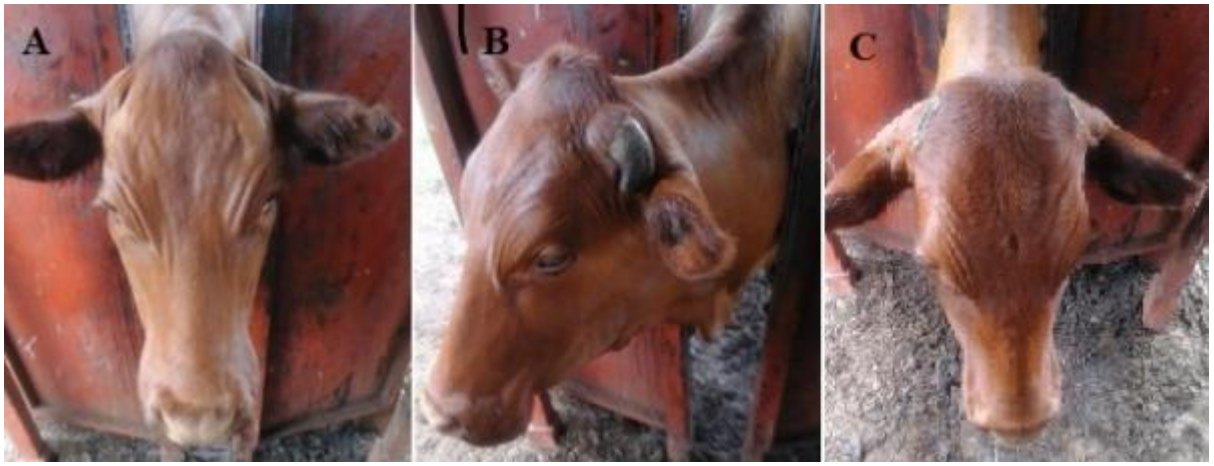
Breed	Sex	On farm phenotype				Genotype*				
		Polled	Scurred	Horned	Total	Pc/Pc Polled	Pc/p polled	Pc/p Scurs	p/p horned	Total
Bonsmara	Male	38	27	8	73	14 (0.192)	24 (0.329)	24 (0.329)	11 (0.151)	73
	Female	59	13	19	91	15 (0.165)	43 (0.473)	6 (0.066)	27 (0.297)	91
Drakensberger	Male	36	2	9	47	1 (0.021)	25 (0.532)	2 (0.043)	19 (0.404)	47
	Female	49	0	37	86	1 (0.012)	19 (0.221)	0 (0.000)	66 (0.767)	86
Hereford	Male	47	11	5	63	27 (0.429)	21 (0.333)	9 (0.143)	6 (0.095)	63
	Female	8	15	3	26	3 (0.115)	5 (0.192)	11 (0.423)	7 (0.269)	26

* P_C – dominant polled allele for the Celtic variant, p – recessive horned allele

© University of Pretoria

Correlation coefficients calculated to determine the accuracy of phenotypic recording amongst the breeds and the results of the Celtic PCR-based screening, indicated that there is a strong positive correlation between the phenotypes of the horn status of animals identified on farm level and the Celtic genotype. The high positive correlation in the Bonsmara breed (0.84; $p < 0.001$), indicates more accurate phenotypic recording of the polled status in the Bonsmara herds included in this study. The low and moderate correlation coefficients for the Drakensberger (0.55; $p < 0.001$) and Hereford (0.62; $p < 0.001$) breeds, respectively, can be attributed to the occurrence of a few discrepancies between the phenotype identified and the genotype obtained from the Celtic screening. These discrepancies can be explained by incorrect phenotypic identification of animals, since the inconsistencies were found between horned and scurred phenotypes for the Hereford breed. In the Drakensberger herds, animals were even incorrectly phenotyped as polled. Table 3.2 further demonstrates the poor phenotypic recording of some animals, by indicating the differences in the on farm recorded phenotype and the actual genotype that were observed.

These inconsistencies emphasize the importance of visual inspection of animals both at a young age, as well as between 18 to 24 months of age. It is also important that farmers and farm workers be trained to be able to distinguish the difference between a horned and scurs phenotype. Polledness is an observable phenotype that can be identified at a relatively young age and which does not change with age. Scurs develop as small horn-like growths in the same area as horns on the skull, but these protuberances are loosely attached to the skull (Capitan et al., 2009). The scurs phenotype, however, develops approximately after four months of age and needs to be confirmed between 18 and 24 months of age (Capitan et al., 2009). The different phenotypes, with regards to the horn status in Bonsmara cattle observed in this study, are illustrated in Figure 3.1. It can be clearly seen that there is a marked difference in the head shape of the polled versus dehorned animals.



*A horned animal was not included, since all animals must be dehorned according to the Bonsmara breed standard.

Figure 3.1 The polled (A), scurs (B) and dehorned (C) phenotype in Pc/p polled, Pc/p scurred and p/p horned South African Bonsmara cows, respectively.

© University of Pretoria

It is standard practice to dehorn cattle at a young age by means of physical dehorning, but in most cases without the appropriate pain relief (Knierim et al., 2015). The practice of dehorning has increasingly become a welfare concern and alternatives to dehorning are advocated worldwide. Breeding genetically polled cattle is a long-term, non-invasive and welfare friendly alternative to dehorning. Identification of genetically polled animals through a diagnostic test would therefore be advantageous, but a specific commercial diagnostic test for the polled phenotype is not currently available in South Africa. The DNA tests that are available internationally are applicable to European *Bos taurus* breeds, which can give inconclusive results for indigenous South African and Sanga cattle breeds. Developing a commercial diagnostic test in South Africa for indigenous cattle breeds based on the Celtic mutation will contribute to accurate testing in these breeds, that will enable breeders to market certified polled bulls.

3.4 Conclusion

PCR-based screening of the Celtic mutation concluded that the polled phenotype in the South African Bonsmara, Drakensberger and Hereford are genetically determined by the Celtic allele (P_C). Therefore, the *POLLED* Celtic variant is validated as the causative mutation of polledness in three South African beef cattle breeds and can be used as an efficient diagnostic test for polledness. This study highlighted the current limitations of accurate phenotypic recording of the horn status. Current limitations include the difficulty in recording scurs accurately, due to the development of scurs, indiscriminate dehorning and extensive farming systems that make phenotypic recording difficult. It also confirmed that scurs cannot be identified on a genotypic level with the Celtic screening, and the *SCURS* locus requires further investigation in Sanga beef cattle breeds.

Conflict of interest

All authors declare that they do not have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, the current work.

Acknowledgements

The authors would like to acknowledge the Bonsmara, Drakensberger and Hereford breeders who contributed samples and phenotypic records. The financial support of the National Research Foundation (NRF) and Red Meat Research and Development Trust (RMRDT) is hereby acknowledged. The funders had no role in the study design, data collection and analysis, decision where to publish or preparation of the manuscript.

© University of Pretoria

References

Allais-Bonnet, A., Grohs, C., Medugorac, I., Krebs, S., Djari, A., Graf, A., Fritz, S., Seichter, D. et al., 2013. Novel insights into the bovine polled phenotype and horn ontogenesis in Bovidae. PLoS One. 8, 63512.

Capitan, A., Grohs, C., Gautier, M., Eggen, A., 2009. The scurs inheritance: new insights from the French Charolais breed. BMC Genet. 10,1.

Chen, S.Y., Liu, L., Fu, M., Zhang, G.W., Yi, J., Lai, S.J., Wang, W., 2017. Simultaneous introgression of three POLLED mutations into a synthetic breed of Chinese cattle. PloS one. 12, p.e0186862.

Drögemüller, C., Wöhlke, A., Mömke, S., Distl, O., 2005. Fine mapping of the polled locus to a 1-Mb region on bovine chromosome 1q12. Mamm. Genome. 16, 613-620.

Georges, M., Drinkwaterz, R., Kingz, T., Mishra, A., Moorez, S.S., 1993. Microsatellite mapping of a gene affecting horn development in. Nat. Genet. 4

Götz, K.U., Luntz, B., Robeis, J., Edel, C., Emmerling, R., Buitkamp, J., Anzenberger, H., Duda, J., 2015. Polled Fleckvieh (Simmental) cattle – Current state of the breeding program. Livest. Sci. 179, 80-85.

Graf, B., Senn, M., 1999. Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. Appl. Anim. Behav. Sci. 62, 153-171.

Knierim, U., Irrgang, N., Roth, B.A., 2015. To be or not to be horned – Consequences in cattle. Livest. Sci. 179, 29-37.

Long, C.R., Gregory, K.E., 1978. Inheritance of the horned, scurred and polled condition in cattle. J. Hered. 69, 395-400.

Makina, S.O., Muchadeyi, F.C., van Marle-Köster, E., MacNeil, M.D., Maiwashe, A., 2014. Genetic diversity and population structure among six cattle breeds in South Africa using a whole genome SNP panel. Front. Gen. 5, 1-7.

Medugorac, I., Seichter, D., Graf, A., Russ, I., Blum, H., Göpel, K.H., Rothammer, S., Förster, M., Krebs, S., 2012. Bovine polledness – an autosomal dominant trait with allelic heterogeneity. PLoS One, 7, 39477.

Medugorac, I., Graf, A., Grohs, C., Rothammer, S., Zagdsuren, Y., Gladyr, E., Zinovieva, N., Barbieri, J., Seichter, D., Russ, I., Eggen, A., 2017. Whole-genome analysis of introgressive hybridization and characterization of the bovine legacy of Mongolian yaks. Nat. Genet. 49, 470-475.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rege, J.E. O., C. L. Tawah, 1999. The state of African cattle genetic resources II. Geographical distribution, characteristics and uses of present-day breeds and strains. Anim. Genet. Res. Inf. 26:1-25.

Schafberg, R., Swalve, H.H., 2015. The history of breeding for polled cattle. Livest. Sci. 179, 54-70.

© University of Pretoria

Scholtz, M.M., Bester, J., Mamabolo, J.M., Ramsay, K.A., 2008. Results of the national cattle survey undertaken in South Africa, with emphasis on beef. Appl. Anim. Husb. Rural Dev. 1, 1-19.

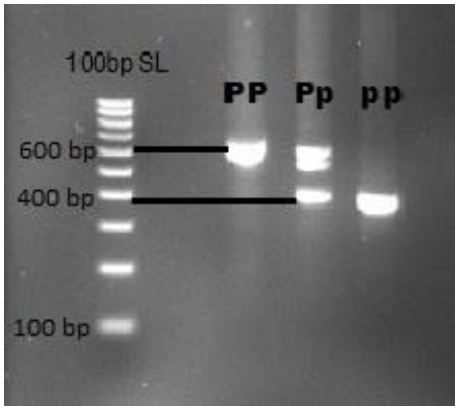
Schmulian, A., 2006. Identification of the polled trait in Bonsmara cattle using microsatellite markers (Masters dissertation, University of Pretoria).

Seichter, D., Russ, I., Rothammer, S., Eder, J., Förster, M., Medugorac, I., 2012. SNP-based association mapping of the polled gene in divergent cattle breeds. Anim. Gen. 43, 595-598.

Windig, J. J., Hoving-Bolink, R. A., Veerkamp, R. F., 2015. Breeding for polledness in Holstein cattle. Livest. Sci. 179, 96–101.

© University of Pretoria

Supplementary figure: Agarose gel image indicating the genotyping results for the CELT primer and the corresponding Celtic genotype



P:	dominant polled allele
p:	recessive horned allele, therefore
PP:	Homozygous polled
Pp:	Heterozygous polled
pp:	Homozygous horned

© University of Pretoria

Chapter 4

Genome-wide association mapping of the scurs phenotype in South African Bonsmara beef cattle

Extracts of this chapter will be prepared as a manuscript to be submitted for publication

© University of Pretoria

4.1 Introduction

Horns are observed in all members of the *Bovidae* family, which includes small stock, cattle and buffalo (Mariasegaram *et al.*, 2010). Horn ontogenesis in cattle remains poorly understood with no candidate genes identified for horn development in cattle. A major constraint for identification of candidate genes includes the difficulty in locating functional genes by using comparative mapping with other species due to a non-existent similar phenotype. The challenge in elucidating horn development is complicated by the presence of scurs; a complex phenotype expressed in polled cattle, as well as difficulty in distinguishing between horned and scurred phenotypes, which leads to the inaccurate recording of horn status (Brenneman *et al.*, 1996). All these factors impede the identification of candidate genes for horn and scurs development in cattle (Mariasegaram *et al.*, 2010).

The phenotypic and genotypic identification of scurs remains a challenge in several polled beef cattle breeds. The scurs phenotype is not easy to identify due to the variation in the size, shape and length of scurs observed among males and females, individuals, and breeds. Scurs may vary from a small scab-like bump to large growths, similar to a short horn of up to fifteen centimetres (Capitan *et al.*, 2011).

Despite a study by Asai *et al.* (2004) where the *SCURS* locus was mapped to BTA19 based on microsatellite markers in crossbred pedigrees (Asai *et al.*, 2004), this mapping could not be confirmed in a subsequent study by Capitan *et al.* (2009) in French Charolais cattle. A new Type 2 scurs, associated with skull interfrontal suture synostosis in animals that are homozygous wildtype at the *POLLED* locus was discovered and mapped to BTA4, with a causative mutation identified in the *TWIST1* gene (Capitan *et al.*, 2011). More recently Tetens *et al.* (2015) reported genetic heterogeneity for the scurs phenotype in Simmental cattle after performing a genome-wide association study (GWAS) in 150 heterozygous polled males and females. A recent study by Ketel (2020) confirmed the mapping of the *SCURS* locus to BTA19 in Canadian beef cattle.

The composite South African Bonsmara breed is the most numerous beef cattle breed in South Africa and contributes approximately 42% to the national beef cattle population registered with SA Stud Book (SA Stud Book, 2016). Approximately 70% of South African beef cattle slaughtered for the formal market are finished off in commercial feedlots (Webb, 2013), which highlights the importance of breeding genetically polled animals to improve welfare of animals during the feedlot period and transportation. The polled trait has been emphasised in selection programs of the Bonsmara for the past two decades. The Celtic test has been used to determine the genotype at the *POLLED* locus (Grobler *et al.*, 2018), but the scurs and horned phenotypes remain prevalent due to upgrading allowed in the breed (Addendum A). Identification of the causal mutation for the scurs phenotype would assist in the management of scurs and selecting against the scurs phenotype.

In GWAS studies significant associations is based on the assumption that the SNP is in linkage disequilibrium (LD), and thus close to, a causative mutation affecting the phenotypic trait of interest (Zondervan & Cardon, 2007). The genomic regions that have been identified, can further be fine mapped

© University of Pretoria

to allow the efficient identification of candidate genes (Matukumalli *et al.*, 2009). In this study, a GWAS was performed to identify genomic regions and potential candidate genes associated with the scurs phenotype in South African Bonsmara beef cattle.

4.2 Materials and Methods

Animal selection and phenotypes

Ethical approval was granted by the ethics committee of the University of Pretoria (EC170424-110) and consent was provided by the respective breeders. A total of 600 animals were sampled from two polled Bonsmara breeders in South Africa, located in the Northern Cape Province (504 animals) and the Limpopo Province (96 animals), respectively. The phenotyping of horn status was done in collaboration with the respective breeders. The 600 purebred Bonsmara animals comprised 206 intact males and 394 females and were between five months and 10 years of age. The phenotyped animals consisted of unrelated individuals sampled based on horn status (polled, scurred or horned) and sex. The total number of animals that were sampled per phenotype and sex are presented in Table 4.1.

Table 4.1 Polled, horned and scurred phenotypes (divided per sex) recorded on farm for the 600 Bonsmara animals phenotyped in this study

Sex	Phenotype				Total
	Polled	Scurs	Horned	Uncertain*	
Male	96	93	17	0	206
Female	238	122	23	11	394
Total	334	215	40	11	600

* A clear distinction between scurs and horns could not be made

With regards to the phenotypes, animals were identified as smooth polled with the absence of horns or any hornlike structures. Additionally, polled animals presented with a rounded peaked poll, i.e. the central prominence on the head (Figure 4.1 A). For the Bonsmara breed, it is compulsory that all animals be dehorned to comply with specific breed standards, therefore no animals with physical horns were observed but dehorned animals were classified as horned animals. The scurs phenotype (Figure 4.1 B and C) was identified based on the following criteria: i) appendages that grew in a similar place on the head as horns, ii) for animals of the same age, these appendages were smaller than horns and iii) were loosely attached to the head and were moveable.

© University of Pretoria

Figure 4.1 The polled (A) and scurs (B and C) phenotypes in the Bonsmara beef cattle breed

Polled genotype based on the Celtic variant

For the expression of the scurs phenotype, scurred animals are heterozygous polled at the *POLLED* locus (Grobler *et al.*, 2018; Chapter three of this dissertation). The 600 animals were tested for genotypic status at the *POLLED* locus using PCR-based genotyping for the Celtic variant following the method as explained in Grobler *et al.* (2018). The Celtic test is applied to identify the genotypic status, to confirm that animals phenotyped as scurred were genetically polled (P_Cp) (and not possibly horned) and to distinguish between polled animals that are heterozygous (P_Cp) versus homozygous polled (P_CP_C). Direct counting was used to calculate the genotype frequencies for the three possible genotypes, while a Hardy-Weinberg Equilibrium (HWE) p-value was estimated for the genotype frequencies using a Chi-square test with a significance threshold of 0.05.

SNP Genotyping and quality control

Based on the genotypic status for the Celtic variant, a subset of 224 heterozygous polled animals were selected for SNP genotyping. The 224 animals consisted of 113 males and 111 females and were grouped, based on phenotype, into 116 scurred cases and 108 heterozygous polled controls. Hair samples of the 224 animals were sent for DNA extraction and genotyping with the GeneSeek® Genomic Profiler (GGP) 150K Bovine SNP array, which contains 139 376 single nucleotide polymorphism (SNP) markers. SNP genotyping was done at the Agricultural Research Council Biotechnology Platform (ARC-BTP) and at Geneseek (USA), respectively.

For the SNP genotype data, the base pair positions for each SNP marker were updated according to the UMD3.1 reference genome assembly (Zimin *et al.*, 2009) and were subjected to quality control using PLINK v1.9 (Purcell *et al.*, 2007). Markers mapped to non-autosomal (X, Y and mitochondrial SNPs) and unmapped regions and those with duplicated genomic coordinates were removed. A total of 12 245 SNP markers were removed based on genotype efficiency (SNP call rate) < 95% and minor allele frequency of < 0.02. All animals with an individual call rate below 85% were excluded from analysis,

© University of Pretoria

thereby removing four animals from the dataset. After quality control 120 353 SNP markers and 220 animals (115 scurred cases and 105 polled controls) remained in the dataset for downstream analysis.

The genetic structure and potential within-breed population stratification was assessed by principal component analysis (PCA) using GCTA software (Genome-wide complex trait analysis; Yang *et al.*, 2011). For PCA analysis, the SNP markers in the dataset were additionally pruned based on r^2 value of 0.05 using PLINK v1.9 software (Purcell *et al.*, 2007). The pruned dataset consisted of 61 731 SNPs and was used to construct a genomic relationship matrix for the PCA analysis.

Genome-wide association analysis

Since the scurs phenotype was recorded as categorical, the GWAS analysis for the scurs phenotype was performed by implementing a case-control analysis with the post quality control dataset, including 220 heterozygous polled animals, that consists of 115 scurred cases and 105 polled controls, respectively. Case-control association studies have the risk of potential bias due to population substructure and reduced power, however, these issues were accounted for by using a well-defined definition of the case-control phenotypes and ensuring optimal selection of cases and controls.

To test for a possible sex-effect, a case-control GWAS was performed for males and females separately. In the second GWAS analysis, the group of males consisted of 59 scurred cases versus 52 polled controls, and the female group consisted of 56 scurred cases versus 53 polled controls. The GWAS analyses was also performed by including the X chromosome in the analysis (Supplemental figure S2) and by fitting the first two principal components from the PCA analysis as covariates, but no differences in the results were observed. The GWAS analyses was performed with PLINK v1.9 (Purcell *et al.*, 2007) using the --ASSOC function, which applies a chi-square test and calculates a false discovery rate.

For the GWAS analyses conducted, a Bonferroni correction was applied to correct for multiple testing and the corrected genome-wide significance level was $P_{\text{nominal value}} < 4.15\text{e-}07$ ($P < 0.05 / 120\ 353$ markers to be tested) (Clarke *et al.*, 2011). The results of the GWAS analyses were visualized by creating Manhattan plots with the qqman package (Turner, 2014) in R Studio (https://cran.r-project.org/). Potential candidate genes were identified from the genome-wide significant SNPs using the NCBI database (https://www.ncbi.nlm.nih.gov/genome/gdv/?org=bos-taurus). Panther v14 (Mi *et al.*, 2019) was used to classify and functionally annotate the potential candidate genes.

4.3 Results

Frequencies for Celtic variant of the POLLED locus

Before GWAS analysis, the 600 phenotyped animals were tested for the Celtic variant to identify animals for the case and control groups. The frequencies of the Celtic genotypes are shown in Table 4.2. Based on the Celtic screening, only 164 of the 215 animals phenotyped as scurred were heterozygous polled (P_Cp), with a higher frequency of scurred males (0.398) being heterozygous polled compared to

© University of Pretoria

scurred females (0.208). One case was documented of a homozygous polled bull with small scurs (Figure S1). Fifty one of the animals that were phenotyped as scurred showed a horned genotype (pp). A higher frequency of scurred animals that had a horned genotype were observed in females (0.102) compared to males (0.053) (Table 4.2). When comparing the horn status phenotypes recorded (Table 4.1) with the observed genotypic frequencies for the Celtic variant (Table 4.2), it is clear that some animals might have been incorrectly phenotyped. Based on the results from the screening for the Celtic variant, a subset of 116 scurred animals ($P_C p$) and 108 heterozygous polled animals ($P_C p$) were selected as cases and controls, respectively.

Table 4.2 The total observed genotypes and genotypic frequencies for the *POLLED* Celtic variant observed in male and female Bonsmara cattle (HWE* p-value < 0.0001)

Genotype	Sex				Total
	Male		Female		
	Total	Frequency	Total	Frequency	
Pc/Pc Polled	38	0.184	63	0.160	101
Pc/p polled	57	0.277	163	0.414	220
Pc/Pc Scurs	1	0.005	0	0.000	1
Pc/p Scurs	**82**	**0.398**	**82**	**0.208**	**164**
p/p Scurs**	11	0.053	40	0.102	51
p/p horned	17	0.083	46	0.117	63
Total	206		394		600

* HWE = Hardy-Weinberg equilibrium

** Animals phenotyped as scurred, but have a p/p horned genotype

Genome-wide association analysis

After quality control, 220 animals (115 scurred cases versus 105 polled controls) remained in the dataset for downstream analysis. The population structure of the 220 animals genotyped was assessed with a PCA to determine potential population stratification, which can occur when cases and controls for a GWAS have been sampled disproportionately from groups with different genetic ancestry. As expected, some minor substructure was present in the dataset (Figure 4.2), and two separate clusters based on herd of origin were observed (Figure 4.2 A). No particular population structure was observed between case and control groups (Figure 4.2 B) since care was taken to collect both case and control animals from both herds during sampling.

© University of Pretoria

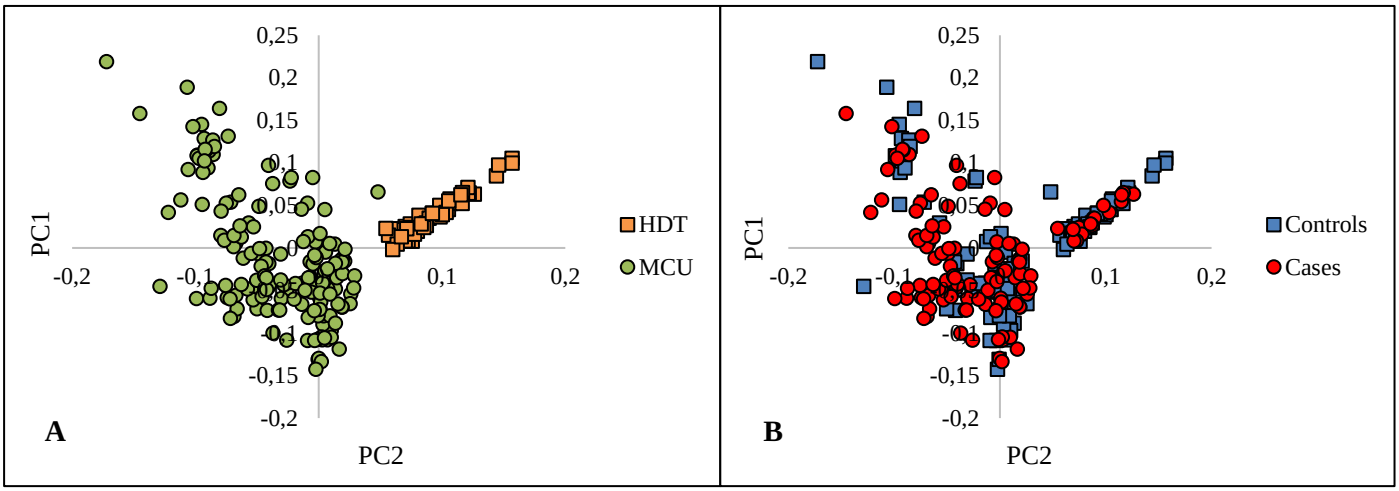


Figure 4.2 Principal component analysis indicating the genetic structure of the sampled population, with graph A depicting the population structure of the two different herds (HDT – Limpopo Province, MCU – Northern Cape Province) and graph B indicating the cases (P_{CP} Scurs) versus the controls (P_{CP} Polled) for this study

The case-control GWAS analysis identified six genome-wide significant SNPs associated with the scurs phenotype on three respective chromosomes (BTA3, BTA10 and BTA17) (Figure 4.3). Several SNPs that approached genome-wide significance ($P_{nominal\ value}$ < 4.15e-07), and identified as regions of interest, were observed on chromosomes BTA3, BTA5, BTA10, BTA15, BTA21 and BTA25 (Figure 4.3).

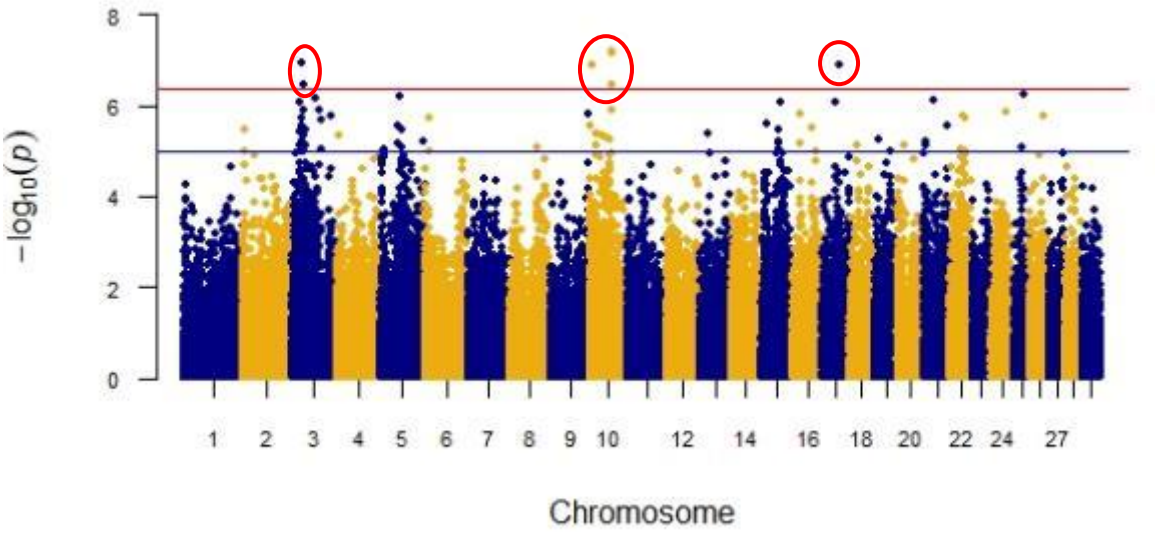


Figure 4.3 Manhattan plot of the GWAS results for the scurs phenotype in the South African Bonsmara breed (blue line: Bonferroni threshold $P_{nominal\ value}$ < 1e-05, red line: Bonferroni corrected genome-wide significance level $P_{nominal\ value}$ < 4.15e-07)

© University of Pretoria

Of the six SNPs significantly associated with the scurs phenotype in the Bonsmara breed, four of these SNPs were located directly within a protein coding gene (Table 4.3). The list of SNPs that were observed in the regions of interest near genome-wide significance and the potential candidate genes are included in the supplementary material (Table S1).

Table 4.3 Genome-wide significant SNPs and potential candidate genes associated with the scurs phenotype in the South African Bonsmara breed

BTA	SNP marker	bp Position	Unadjusted p-value	Candidate gene	Gene name
3	ARS-BFGL-NGS-14193	23710738	1.226e-07	7209 bp from *PHGDH*	Phosphoglycerate dehydrogenase
3	BovineHD0300009574	30404144	3.544e-07	*LRIG2*	Leucine rich repeats and immunoglobulin like domains 2
10	BovineHD1000002255	7193076	1.282e-07	-	-
10	BovineHD1000017913	62036795	6.459e-08	*FBN1*	Fibrillin 1
10	BTB-00432352	62038632	7.398e-08	*FBN1*	Fibrillin 1
17	BovineHD1700012300	44526807	1.325e-07	*GUCY1B1*	Guanylate cyclase 1 soluble subunit beta 1

The four candidate genes associated with the scurs phenotype are involved in a number of molecular functions and biological processes (Table 4.4). These processes primarily involve various amino acid metabolic processes, regulation of cell signals (i.e., communication between different cell molecules) and processes involved with the central nervous system development.

Table 4.4 Classification and functional annotation of the candidate genes associated with the scurs phenotype based on the PANTHER database (Mi *et al.*, 2019)

BTA	Candidate gene	Molecular function	Biological processes	Cellular component
3	*PHGDH*	Oxidoreductase activity Acting on the CH-OH group of donors NAD or NADP as acceptor NAD binding Phosphoglycerate dehydrogenase activity	Neural tube development Neuron projection development Spinal cord development Glial cell development Cellular amino acid metabolic process Glutamine metabolic process Glycine metabolic process Taurine metabolic process Threonine metabolic process L-serine biosynthetic process Gamma-aminobutyric acid metabolic process Regulation of gene expression Oxidation-reduction process	

© University of Pretoria

BTA	Candidate gene	Molecular function	Biological processes	Cellular component
3	*LRIG2*	Signalling receptor binding	Negative regulation of axon regeneration Positive regulation of protein localization to cell surface Negative regulation of membrane protein ectodomain proteolysis Innervation (nerve supply) Regulation of platelet-derived growth factor receptor signalling pathway Regulation of neuron migration	Extracellular matrix Extracellular space Integral component of membrane Neuronal cell body Intracellular vesicle
10	*FBN1*	Extracellular matrix structural constituent; chitin binding, identical protein binding, calcium ion binding, integrin binding, heparin binding, hormone activity	Anatomical structure morphogenesis Sequestering of TGFbeta in extracellular matrix Signal transduction Activation of protein kinase A activity Sequestering of BMP in extracellular matrix Glucose metabolic process Heart development Cell adhesion facilitated by integrin Skeletal system development Embryonic eye morphogenesis Post-embryonic eye morphogenesis Protein kinase A signalling Camera-type eye development Glucose homeostasis Negative regulation of osteoclast development	Extracellular matrix Extracellular space Basement membrane Microfibril Extracellular region
17	*GUCY1B1*	GTP binding Metal ion binding Guanylate cyclase activity Heme binding	cGMP-mediated signalling cGMP biosynthetic process Trans-synaptic signalling by nitric oxide Modulating synaptic transmission Cellular response to nitric oxide Nitric oxide-cGMP-mediated signalling pathway	Presynaptic active zone Guanylate cyclase complex Soluble Presynaptic active zone Cytoplasm

In the second GWAS analysis, based on separation of sex, only one genome-wide significant SNP was observed on BTA21 in female animals (Figure 4.4 B). In the GWAS analysis for females, regions of interest were observed on BTA10, BTA15 and BTA24. For males, one SNP approaching genome-wide significance was observed on BTA16 (Figure 4.4 A). This SNP (Hapmap26502-BTA-39343) is located 28 277 bp from the closest gene (*RALGPS2*), which is related to enzyme regulator activity and guanyl-nucleotide exchange factor activity. For both males and females no significant associations or regions of interest were observed on the X chromosome thereby confirming that the scurs phenotype is not sex-linked.

© University of Pretoria

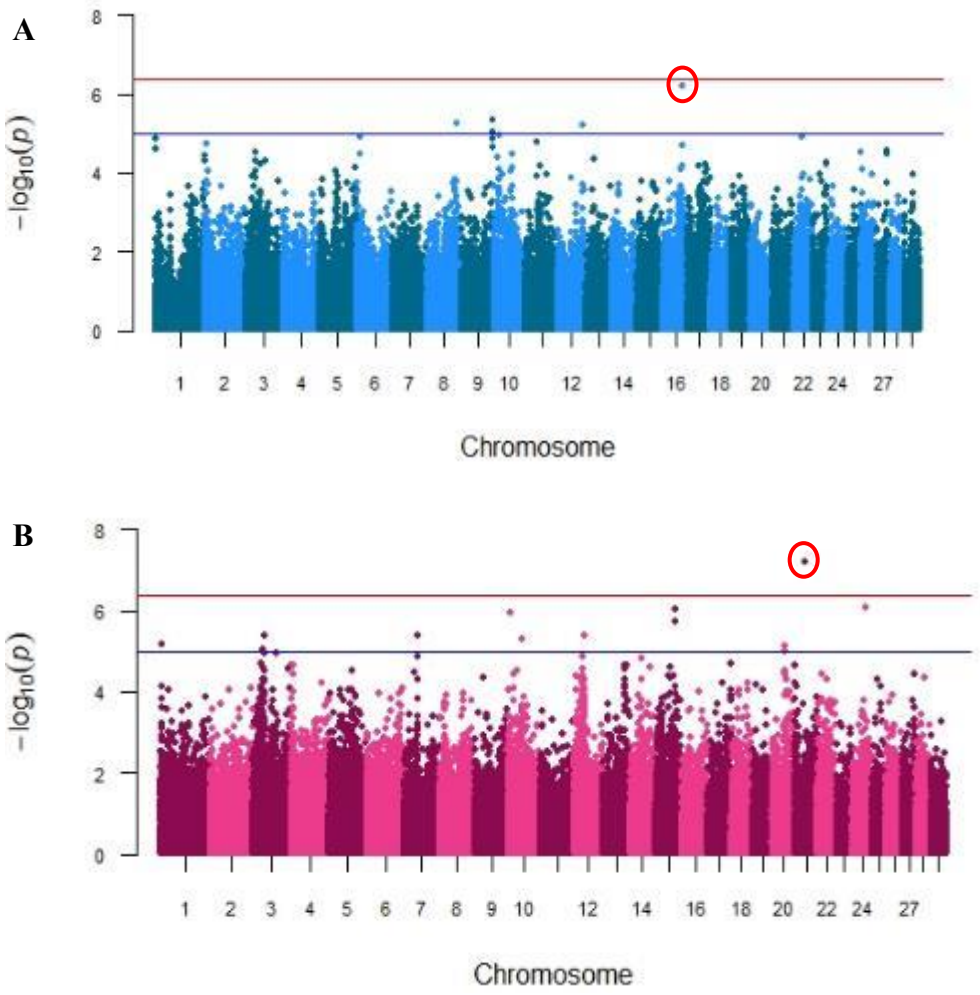


Figure 4.4 Manhattan plot of the GWAS results for the scurs phenotype for male (plot A) versus female (plot B) animals of the South African Bonsmara breed (blue line: Bonferroni threshold $P_{nominal\ value}$ < 1e-05, red line: Bonferroni corrected genome-wide significance level $P_{nominal\ value}$ < 4.15e-07)

In females, the SNP marker approaching genome-wide significance on BTA24 were located within the *RAB31* gene, which plays a role in regulated exocytosis, vesicle-mediated transport, and the biological pathway of post-translation protein modification. The genome-wide significant SNP on BTA21 was located within a cluster of neuronal nicotinic acetylcholine subunit receptors (Table 4.5). These receptors are part of a superfamily of neurotransmitter-gated ion channels that mediate intercellular communication

© University of Pretoria

Table 4.5 Genome-wide significant SNP on BTA21 in female Bonsmara cattle and potential candidate genes associated with the scurs phenotype

SNP marker	bp Position	Unadjusted p-value	Distance from gene	Candidate gene	Gene name
BovineHD2100009073	31513901	6.554e-08	974 bp	*CHRNA3*	Cholinergic receptor nicotinic alpha 3 subunit
			4 469 bp	*CHRNB4*	Cholinergic receptor nicotinic beta 4 subunit
			17 944 bp	*CHRNA5*	Cholinergic receptor nicotinic alpha 5 subunit

The molecular function of these nicotinic acetylcholine subunit receptors primarily includes acetylcholine-gated cation-selective channel activity, acetylcholine receptor activity and transmembrane signalling receptor activity. Acetylcholine functions as the primary neurotransmitter of the autonomic nervous system. These receptors are a molecular component of the postsynaptic membrane, i.e., the membrane that receives a signal (binds neurotransmitter), a vital component required for the functioning of the central nervous system (CNS).

4.4 Discussion

In South Africa, the Bonsmara is the most numerous beef cattle breed, contributing approximately 118 758 registered animals to the national population (SA Stud Book, 2016). Within the Bonsmara breed polledness are not necessarily advocated and horns, scurs and polled phenotypes are present in the breed. In this study, most of the animals phenotyped as polled had a heterozygous polled genotype (P_Cp = 0.685). Animals that were phenotypically scurred and tested heterozygous polled based on the Celtic screening, were observed at a frequency of 0.273. Variation in the size and shape of scurs, as well as variation between sex (Addendum B), were observed in this study. Even though the scurs phenotype were observed in both sexes in this study, scurs were observed at a higher frequency of 0,398 in Bonsmara males, compared to a lower frequency of 0,208 in Bonsmara females. Results from the screening for the Celtic variant indicated that not all animals phenotyped as scurred were genetically polled, implying that some of the animals were in fact horned. The scurs phenotype is expressed, if the animal is heterozygous polled at the *POLLED* locus (Wiedemar *et al.*, 2014; Grobler *et al.*, 2018).

To map the *SCURS* locus in the Bonsmara breed, a case-control association analysis was implemented. In this study, six SNPs were significantly associated with the scurs phenotype in the Bonsmara breed, located on three different chromosomes, i.e., BTA3, BTA10 and BTA17. Several studies have attempted to map the *SCURS* locus, with initial mapping to BTA19 in Canadian beef cattle using three full-sib families (Asai *et al.*, 2004). An association study in German Simmental cattle, reported a single SNP associated with scurs on BTA19 (Tetens *et al.*, 2015), near the identified region previously reported by Asai *et al.* (2004). The mapping of scurs to BTA19 was also corroborated by Ketel (2020) in Canadian beef cattle, while Type 2 Scurs syndrome was mapped to BTA4 in French

© University of Pretoria

Charolais cattle (Capitan *et al.*, 2011). Animals with Type 2 scurs were homozygous wildtype at the *POLLED* locus with horn growths similar to typical scurs. In addition, interfrontal suture synostosis was noted, which results in an abnormal peaked cone shaped skull with additional bone deposition along the interfrontal suture. A similar condition was not observed in the Bonsmara breed.

Previous research findings indicate genetic heterogeneity for the *SCURS* locus (Asai *et al.*, 2004; Capitan *et al.*, 2009; Capitan *et al.*, 2011; Tetens *et al.*, 2015) and therefore it is likely that the causative mutation and expression of scurs differ between breeds, especially between breeds of different origin, i.e *Taurus* versus *Indicus*. The Bonsmara is a composite beef breed, developed from the Afrikaner (Sanga), Hereford and Shorthorn breeds (Bonsma, 1980). The genetic heterogeneity for the *SCURS* locus between *Bos taurus* and the Bonsmara, could be attributed to different ancestral origins and different selection strategies between breeds (Liang *et al.*, 2016). Since the *POLLED* locus is believed to be epistatic to the *SCURS* locus (Long & Gregory, 1978), selection emphasis on the polled trait may also contribute to the phenotypic expression of scurs.

Four putative candidate genes (*FBN1*, *LRIG2*, *PHGDH*, *GUCY1B1*), which may contribute to the molecular regulation of scurs, were identified in the current study. These candidate genes are involved in a variety of molecular functions and biological processes, which primarily involve various amino acid metabolic processes, regulation of cell signals (i.e., communication between different cell molecules) and processes involved with the central nervous system development (Mi *et al.*, 2019). Both the *LRIG2* and *FBN1* genes are cellular components of the extracellular matrix and extracellular space. Previous research did not identify any candidate genes associated with the scurs phenotype (Asai *et al.*, 2004; Tetens *et al.*, 2015; Ketel, 2020). However, for the Type 2 scurs syndrome, a causative mutation was identified as a frame shift mutation postulated to inactivate the *TWIST1* gene, which might be linked with horn bud development during embryogenesis (Capitan *et al.*, 2011).

FBN1 encodes for the extracellular matrix (ECM) component fibrillin-1. The fibrillin-1 protein functions as a structural component of microfibrils that binds calcium, which provide long-term force-bearing structural support in non-elastic and elastic connective tissue (Jensen & Handford, 2016). In the reticular dermis of skin, fibrillin-1 is organized into dense horizontally positioned elastic fibres, connected to the dermal-epidermal junction through vertically arranged bundles of elaunin and oxytalan fibre microfibrils (Godwin *et al.*, 2019). *FBN1* is also involved in sequestering transforming growth factor (TGF) beta and bone morphogenetic protein (BMP) in the ECM, which is crucial for homeostasis and tissue remodelling (Godwin *et al.*, 2019). Unlike horns which consist of keratinized epithelium, scurs primarily consist of cartilaginous tissues (Brennemam *et al.*, 1996). Cartilage is a tough, elastic, flexible connective tissue with no nerves or blood vessels.

It has been reported that horn development is primarily controlled by the skin (Mariasegaram *et al.*, 2010), but the exact molecular regulation of horn growth in ruminants remains poorly understood. Unlike horns, which are fused to the frontal bone of the skull, scurs are not fused to the skull but is rather attached to the skull by soft tissue. The bony interior at the distal end of scurs is derived from a separate

© University of Pretoria

ossification centre in the tissues situated above the periosteum, and scurs are attached to the skull by these tissues (Medugorac *et al.*, 2012). Dissections of cattle skulls performed by Brenneman *et al.* (1996) indicated that scurs appendages consisted of cartilaginous material and were fused to the skull.

Differential gene expression studies between polled and scurred cattle indicated a lower expression of cytokeratin components in scurred animals and an elevated expression of fibrillar collagen genes which codes for components of the ECM (Mariasegaram *et al.*, 2010).

Histological analysis indicated that during the development of bovine foetal horn buds, there are characteristic differences in the development of nervous tissue and hair follicles between horn buds and polled frontal skin (Wiener *et al.*, 2015).

The *LRIG2* gene encodes a protein that is a cellular component of the ECM. The ECM interacts with cell surface receptors and binds growth factors to regulate transcription of genes, which directs essential morphological organization and physiological functions (Frantz et al., 2010). The LRIG2 protein is a transmembrane signal receptor containing leucine-rich repeats and immunoglobulin-like domains and is implicated in the regulation of growth factor receptors (Simion *et al.*, 2014). *LRIG* genes show homology in their ECM domains, but the specific molecular function of *LRIG2* in cattle has not yet been resolved (Simion *et al.*, 2014).

The *PHGDH* gene encodes for the enzyme 3-phosphoglycerate dehydrogenase, which is involved in the initial steps of the serine biosynthesis pathway (Possemato *et al.*, 2011). Serine is vital for the synthesis of proteins and other biomolecules required for cell proliferation (Possemato *et al.*, 2011). The SNP identified on BTA17 is located within the *GUCY1B1* gene, encoding the beta subunit of soluble guanylate cyclase. *GUCY1B1* is a part of the intracellular cell component and is involved in the gonadotropin-releasing hormone receptor pathway, as well as the endothelin signalling pathway (Mi *et al.*, 2019). Soluble guanylate cyclase probably functions as an intercellular messenger molecule; however, limited information is available on the role of *GUCY1B1* in bovine tissue.

To test for a possible sex effect involved in the scurs phenotype, the association analysis was conducted for males and females separately. Significant associations were not observed for males with scurs. In contrast, in female Bonsmara animals several markers across multiple chromosomes were observed near genome-wide significance and a significant association with one SNP was observed on BTA21. This SNP is linked to a cluster of neuronal nicotinic acetylcholine subunit receptors (nAChRs), which are part of a superfamily of neurotransmitter-gated ion channels that mediate intercellular communication. These nAChRs receptors are expressed in the peripheral and central nervous systems, where they facilitate fast synaptic transmission. Neuronal nAChRs are also expressed in non-neuronal tissues, but their functions are not completely understood (Sala *et al.*, 2008). Similarly to the candidate genes identified in the total sample group, the candidate gene observed in females also play a role in regulation of intercellular communication. It appears that females display greater genetic heterogeneity for the scurs phenotype compared to males. However, the sample size of each gender group was small, and these findings should be validated in a larger sample group.

© University of Pretoria

The results in this study indicate genetic heterogeneity and suggests the involvement of more than one gene in the expression of the scurs phenotype in the SA Bonsmara breed. It is, however, not certain how the genes interact to express the scurs phenotype. Since the exact genetic mechanism and molecular regulation of horns and scurs are still unknown, functional studies in horned, polled and scurred cattle are required to resolve the exact pathways involved. Gene regulation of horn development should be studied along with the regulatory sequences of the loci associated with polled and scurs phenotypes (Liang *et al.*, 2016) . Future studies should focus on sequencing regions of interest and investigating pathways for gene expression, which may aid in an improved understanding of horn ontogenesis.

4.5 Conclusion

This was the first attempt to characterize the scurs phenotype and study the *SCURS* locus in a South African composite beef breed on a genomic level. This study indicates the genetic heterogeneity and the complexity of resolving the molecular basis of the scurs phenotype. Current results suggest that the development of scurs in the Bonsmara breed might be influenced by more than one gene which are located on different chromosomes. GWAS analysis has revealed four candidate genes for the *SCURS* loci on various chromosomes, which requires further investigation and validation in a larger population.

References

Asai, M., Berryere, T.G. & Schmutz, S.M., 2004. The scurs locus in cattle maps to bovine chromosome 19. Animal genetics. 35, 34-39.

Bonsma, J.C., 1980. Cross-breeding, breed creation and the genesis of the Bonsmara. In: Livestock Production - a Global Approach. Tafelberg, Cape Town, South Africa. pp90–110.

Brenneman, R.A., Davis, S.K., Sanders, J.O., Burns, B.M., Wheeler, T.C., Turner, J.W. & Taylor, J.F., 1996. The polled locus maps to BTA1 in a Bos indicus× Bos taurus cross. Journal of Heredity. 87, 156-161.

Capitan, A., Grohs, C., Gautier, M. & Eggen, A., 2009. The scurs inheritance: new insights from the French Charolais breed. BMC Genetics. 10,1-11.

Capitan, A., Grohs, C., Weiss, B., Rossignol, M.N., Reversé, P. & Eggen, A., 2011. A newly described bovine type 2 scurs syndrome segregates with a frame-shift mutation in TWIST1. PLoS One. 6, 22242.

Clarke, G.M., Anderson, C.A., Pettersson, F.H., Cardon, L.R., Morris, A.P. & Zondervan, K.T., 2011. Basic statistical analysis in genetic case-control studies. Nature Protocols. 6, 121-133.

Frantz, C., Stewart, K.M. & Weaver, V.M., 2010. The extracellular matrix at a glance. Journal of Cell Science. 123, 4195-4200.

Godwin, A.R., Singh, M., Lockhart-Cairns, M.P., Alanazi, Y.F., Cain, S.A. & Baldock, C., 2019. The role of fibrillin and microfibril binding proteins in elastin and elastic fibre assembly. Matrix Biology. 84, 17-30.

© University of Pretoria

Grobler, R., Visser, C., Capitan, A. & van Marle-Köster, E., 2018. Validation of the *POLLED* Celtic variant in South African Bonsmara and Drakensberger beef cattle breeds. Livestock Science. 217, 136-139.

Jensen, S.A. & Handford, P.A., 2016. New insights into the structure, assembly and biological roles of 10–12 nm connective tissue microfibrils from fibrillin-1 studies. Biochemical Journal. 473, 827-838.

Ketel, C.R., 2020. Investigating Scur Candidate Genes in *Bos taurus* cattle. MSc dissertation, Department of Animal and Poultry Science, University of Saskatchewan.

Liang, C., Wang, L., Wu, X., Wang, K., Ding, X., Wang, M., Chu, M., Xie, X., Qiu, Q., & Yan, P., 2016. Genome-wide Association Study Identifies Loci for the Polled Phenotype in Yak. PLoS One. 11, e0158642.

Long, C.R. & Gregory, K.E., 1978. Inheritance of the horned, scurred, and polled condition in cattle. Journal of Heredity. 69, 395-400.

Mariasegaram, M., Reverter, A., Barris, W., Lehnert, S.A., Dalrymple, B. & Prayaga, K., 2010. Transcription profiling provides insights into gene pathways involved in horn and scurs development in cattle. BMC Genomics. 11, 1-14.

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'Connell, J., Moore, S.S., Smith, T.P., Sonstegard, T.S. & Van Tassell, C.P., 2009. Development and characterization of a high density SNP genotyping assay for cattle. PloS One. 4, e5350.

Medugorac, I., Seichter, D., Graf, A., Russ, I., Blum, H., Göpel, K.H., Rothammer, S., Förster, M. & Krebs, S., 2012. Bovine polledness–an autosomal dominant trait with allelic heterogeneity. PLoS One, 7, 39477.

Mi, H., Muruganujan, A., Ebert, D., Huang, X. & Thomas, P.D., 2019. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Research. 47, D419-D426.

Possemato, R., Marks, K.M., Shaul, Y.D., Pacold, M.E., Kim, D., Birsoy, K., Sethumadhavan, S., Woo, H.K., Jang, H.G., Jha, A.K. & Chen, W.W., 2011. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature. 476, 346-350.

Purcell, S., Neale, B. & Todd-Brown, K., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics. 81, 559–575.

SA Stud Book, 2016. SA Stud Book Annual Report. Bloemfontein. Available online at: http:// www.sastudbook.co.za

Sala, F., Nistri, A. & Criado, M., 2008. Nicotinic acetylcholine receptors of adrenal chromaffin cells. Acta Physiologica. 192, 203-212.

Simion, C., Cedano-Prieto, M.E. & Sweeney, C., 2014. The LRIG family: enigmatic regulators of growth factor receptor signaling. Endocrine-related cancer. 21, R431-R443.

© University of Pretoria

Tetens, J., Wiedemar, N., Menoud, A., Thaller, G. & Drögemüller, C., 2015. Association mapping of the scurs locus in polled Simmental cattle – evidence for genetic heterogeneity. Animal Genetics. 46, 224-225.

Turner, S.D., 2014. qqman: an R package for visualizing GWAS results using QQ and manhattan plots. Biorxiv. 005165, 1-2.

Webb, E.C., 2013. The ethics of meat production and quality-a South African perspective. South African Journal of Animal Science. 43, S2-S11.

Wiedemar, N., Tetens, J., Jagannathan, V., Menoud, A., Neuenschwander, S., Bruggmann, R., Thaller, G. & Drögemüller, C., 2014. Independent polled mutations leading to complex gene expression differences in cattle. PloS One. 9, e93435.

Wiener, D.J., Wiedemar, N., Welle, M.M. & Drögemüller, C., 2015. Novel Features of the Prenatal Horn Bud Development in Cattle (Bos taurus). PloS One. 10, 0127691.

Yang, J., Hong Lee, S., Goddard, M.E. & Visscher, P.M., 2011. GCTA: A Tool for Genome-wide Complex Trait Analysis. The American Journal of Human Genetics. 88, 76-82.

Zimin, A.V., Delcher, A.L., Florea, L., Kelley, D.R., Schatz, M.C., Puiu, D., Hanrahan, F., Pertea, G., Van Tassell, C.P., Sonstegard, T.S. & Marçais, G., 2009. A whole-genome assembly of the domestic cow, Bos taurus. Genome Biology. 10, R42.

Zondervan, K.T. & Cardon, L.R., 2007. Designing candidate gene and genome-wide case–control association studies. Nature Protocols. 2, 2492-2501.

© University of Pretoria

Supplemental material

A

B

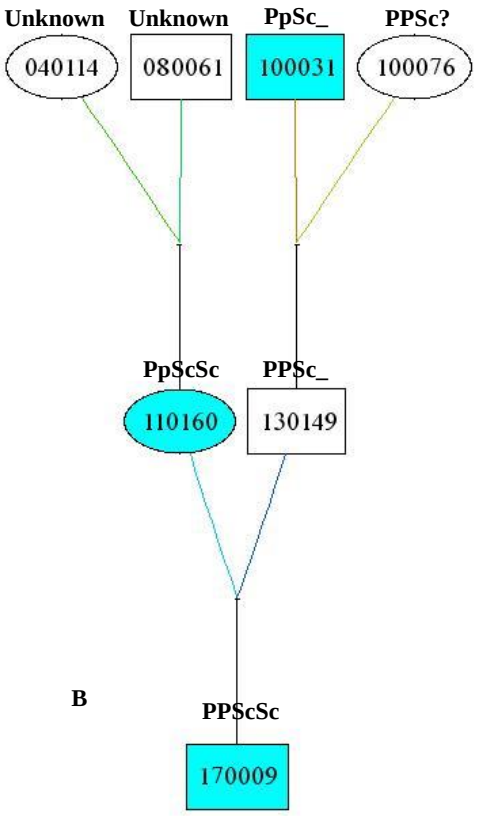


Figure S1 The phenotype (A) and pedigree (B) of a homozygous polled (P_CP_C) Bonsmara male with small scurs (Scurred animals are indicated in blue; Genotypes were assigned based on proposed inheritance model)

© University of Pretoria

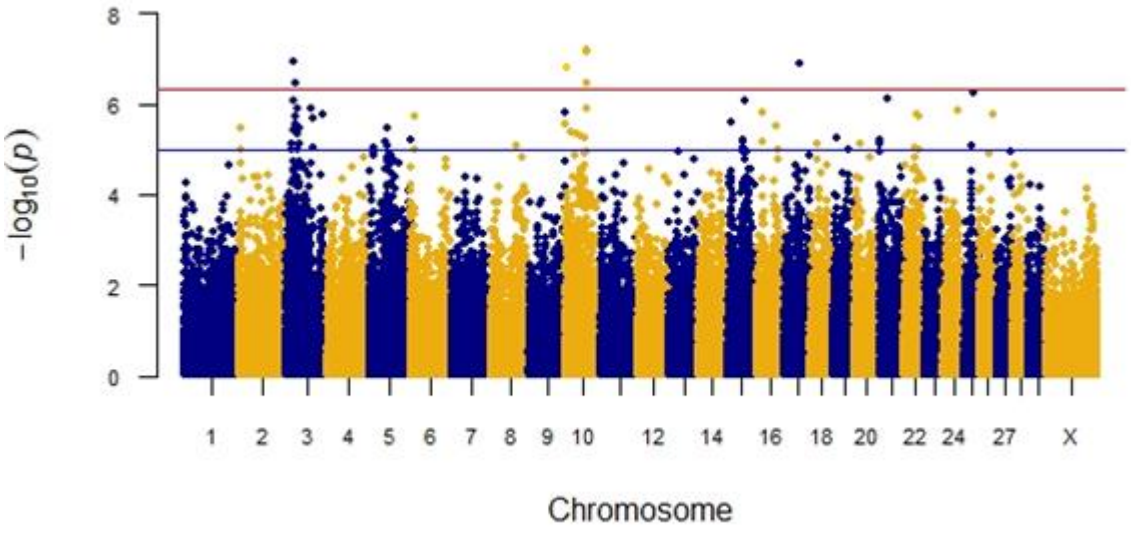


Figure S2 Manhattan plot of the GWAS results, including the X chromosome in the analysis, for the scurs phenotype in the SA Bonsmara breed (blue line: Bonferroni threshold $P_{nominal\ value}$ < 1e-05, red line: Bonferroni corrected genome-wide significance level $P_{nominal\ value}$ < 4.7e-07).

© University of Pretoria

Table S1 SNPs in regions of interest between the Bonferroni and genome-wide significance threshold and the potential candidate genes associated with the scurs phenotype (Rows in bold represent genome-wide significant SNPs)

BTA	SNP marker	bp Position	Unadjusted p-value	Candidate gene	Gene name
2	ARS-BFGL-BAC-2817	7562127	3.318e-06	-	-
3	ARS-BFGL-NGS-39443	22944367	8.152e-07	*PDE4DIP*	Phosphodiesterase 4D interacting protein
3	BovineHD0300007246	23166333	3.704e-06	*PDE4DIP*	Phosphodiesterase 4D interacting protein
3	**ARS-BFGL-NGS-14193**	**23710738**	**1.226e-07**	**7209 bp from *PHGDH***	**Phosphoglycerate dehydrogenase**
3	ARS-BFGL-NGS-118132	24288407	3.396e-06	*TBX15*	T-box transcription factor 15
3	BovineHD0300008927	27926470	1.855e-06	-	-
3	ARS-BFGL-NGS-32344	27963904	2.874e-06	-	-
3	**BovineHD0300009574**	**30404144**	**3.544e-07**	***LRIG2***	**Leucine rich repeats and immunoglobulin like domains 2**
3	BovineHD0300010338	33168941	1.288e-06	*SLC16A4*	Solute carrier family 16 member 4 (monocarboxylic acid transporter 5)
3	BovineHD0300012053	39387920	3.502e-06	-	-
3	BovineHD0300019093	63894339	7.366e-07	-	-
3	ARS-BFGL-NGS-67327	72519744	1.332e-06	-	-
3	ARS-BFGL-NGS-34570	80562496	2.157e-06	*AK4*	Adenylate kinase 4
3	ARS-BFGL-NGS-116561	105576161	1.743e-06	46 271bp from *SCMH1*	Scm polycomb group protein homolog 1
5	Hapmap39507-BTA-73544	52300318	6.484e-07	-	-
5	BovineHD0500013676	47342153	2.716e-06	*GRIP1*	Glutamate receptor interacting protein 1
5	BovineHD0500016018	56364658	3.548e-06	14 239bp from *INHBC*	Inhibin subunit beta C
6	BovineHD0600003858	14416929	1.868e-06	*TIFA*	TRAF interacting protein with forkhead associated domain
9	BovineHD0900030173	103084220	1.523e-06	*RPS6KA2*	Ribosomal protein S6 kinase A2
10	BovineHD1000000800	2629026	2.688e-06	-	-
10	**BovineHD1000002255**	**7193076**	**1.282e-07**	**-**	-
10	**BovineHD1000017913**	**62036795**	**6.459e-08**	***FBN1***	**Fibrillin 1**
10	**BTB-00432352**	**62038632**	**7.398e-08**	***FBN1***	**Fibrillin 1**
10	BovineHD1000017915	62039297	3.609e-07	*FBN1*	Fibrillin 1
10	Hapmap47084-BTA-72369	62081998	1.252e-06	*FBN1*	Fibrillin 1
15	BovineHD1500003133	12282171	2.567e-06	-	-
15	ARS-BFGL-NGS-67071	48688111	3.454e-06	*OR52V2P*	Olfactory receptor family 52 subfamily V member 2, pseudogene
15	Hapmap41245-BTA-36880	52982531	8.934e-07	*PDE2A*	Phosphodiesterase 2A
16	BovineHD1600005807	20857364	1.492e-06	*ESRRG*	Estrogen related receptor gamma
16	BovineHD1600015397	55310176	3.275e-06	27 543bp from *KDM5B*	Lysine demethylase 5B
17	ARS-BFGL-NGS-54215	34315609	8.833e-07	-	-

© University of Pretoria

BTA	SNP marker	bp Position	Unadjusted p-value	Candidate gene	Gene name
17	**BovineHD1700012300**	**44526807**	**1.325e-07**	***GUCY1B1***	**Guanylate cyclase 1 soluble subunit beta 1**
21	BovineHD2100007520	26123921	7.894e-07	*MINAR1*	Membrane integral NOTCH2 associated receptor 1
21	BovineHD2100018663	63598280	2.94e-06	-	-
22	BovineHD2200010584	37258935	1.692e-06	12 992bp from *PRICKLE2*	Prickle planar cell polarity protein 2
22	BovineHD2200011718	40930564	1.953e-06	*FHIT*	Fragile histidine triad diadenosine triphosphatase
24	BovineHD2400011710	42265105	1.417e-06	*RAB31*	RAB31, member RAS oncogene family
25	BovineHD2500012018	25848107	5.852e-07	*GSG1L*	GSG1 like (germ cell-specific gene 1-like protein)
26	BovineHD2600010822	39451175	1.794e-06	-	-

© University of Pretoria

Chapter 5

A protocol for the identification of polled and scurs phenotypes in South African Bonsmara beef cattle

© University of Pretoria

5.1 Introduction

The South African Bonsmara breed is a composite breed developed through scientific breeding experiments and objectively recorded performance data from 1937 to 1963 at the Mara and Messina Research stations in the Limpopo province of South Africa (Bonsma, 1980). The development of this breed was commissioned by the government in 1935 with the objective of developing a breed adapted to the harsh environmental conditions of South Africa, with good performance of economically important traits (Bonsma, 1980). During the 1950's, the first Bonsmara bulls were made available to commercial cattle breeders and were quickly widespread across South Africa. Scientific measurement formed the basis of breed development and the requirement for compulsory performance testing and measuring production and reproduction traits are still implemented in the minimum breed standards set by the Bonsmara Breeder's Society (https://bonsmara.co.za/wp-content/uploads/2019/09/Bonsmara-Comprehensive-Guide.pdf).

The Bonsmara consists of 5/8 Afrikaner and 3/8 exotic *Bos taurus*, attained from the British Shorthorn and Hereford breeds. The Afrikaner is a horned Sanga breed and even though the Bonsmara was developed from the polled Hereford breed, the Bonsmara was not established as a polled breed (Bonsma, 1980). The polled trait's ancestry is either from the breeds used to develop the Bonsmara (Shorthorn/Hereford ancestors) or from more recent crosses by upgrading Red Poll and Red Angus cows to Bonsmara stud status (Addendum A). Since the development of the breed, it was required that calves be dehorned within six weeks after birth and breeding for polledness was not advocated (Bonsma, 1980). Although recording of the polled phenotype is compulsory, no guidelines for recording the horn status of Bonsmara animals are available. Breeding for the polled condition in Bonsmara became of interest only over the past 20 years with an increased demand for polled animals and market preferences, especially for exportation of embryos of polled Bonsmara animals. Additional benefits were the reduced labour costs when breeding polled animals rather than dehorning calves (Pers. Comm., C. Uys, November 2017, charluys@vodamail.co.za), as well as improved animal welfare.

The current research study indicated discrepancies and difficulties in recording of the polled phenotype (Grobler *et al.*, 2018; Chapter 3 of this dissertation), which is exacerbated by the prevalence of the scurs phenotype in the Bonsmara breed. It can be difficult to distinguish the scurs phenotype from horns, due to both the late development of scurs and dehorning practices. The extensive systems that are characteristic of the majority of South African beef production systems also makes accurate phenotypic recording of horn status difficult. These challenges indicated a need for the development of practical guidelines and specific standards for phenotyping Bonsmara animals for the polled and scurs phenotypes. This protocol aims to assist breeders to accurately identify polled and scurred animals, so that appropriate selection strategies can be implemented to increase the number of polled animals and to manage scurs.

© University of Pretoria

5.2 Phenotypic identification

Even though horn growth is evident within a few weeks after birth, the physical horn buds only start to develop during the first two months of life. The horn bud is not yet attached to the skull (Figure 5.1 A) and is free floating in the skin layer above the skull up to approximately two to three months of age (Newman & Partridge, 2007). Hence, the horn bud will be moveable during this time and contributes to the difficulty to distinguish between horns and scurs in calves up to two to three months of age. Between two and six months of age the horn bud gradually fuses with the skull; the horn starts to grow and becomes a bony extension of the skull (Irrgang, 2012). At approximately six months of age, the bony cone of the horn fuses with the caudal frontal sinus (Habel & Budras, 2003) (Figure 5.1 B). Between six and eight months of age, the bony horn cone are gradually pneumatised from the caudal frontal sinuses, resulting in the hollow interior of the horn to be connected directly with the frontal sinuses of the skull (Habel & Budras, 2003).

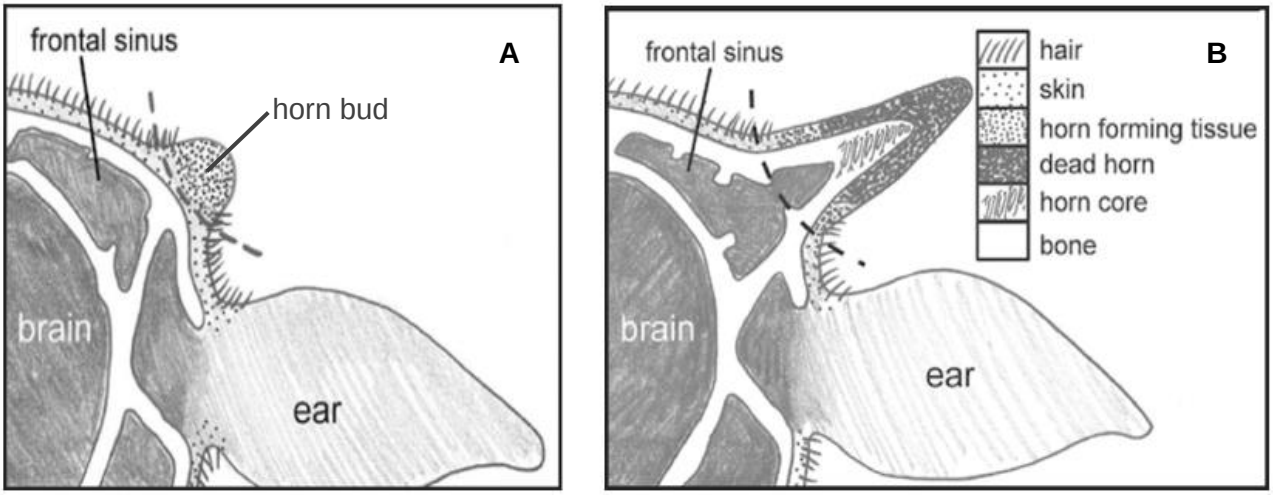


Figure 5.1 The anatomy of horn growth in calves of approximately two to three months of age (A) and between six and eight months of age (B) (Newman & Partridge, 2007)

The polled phenotype can be observed early in life and do not change over time. For the Bonsmara animals in this study, specific morphological characteristics associated with the polled phenotype have been identified in calves and can be used to identify the polled phenotype at birth. These are listed in Table 5.1 and visually presented in Figure 5.2. After approximately 12 months of age the rounded peaked poll (central prominence on the head) of polled animals will be more pronounced and clearly visible.

© University of Pretoria

Table 5.1 Morphological characteristics of Bonsmara calves at birth associated with the polled and horned phenotypes

Polled calf	**Horned calf**
Distinct cowlick (tuft) in the hair between the ears	No distinct cowlick (tuft) in the hair between the ears
Round, peaked poll*	Flat poll*
No indication of a horn bud	Observable horn bud

* Central prominence on the head

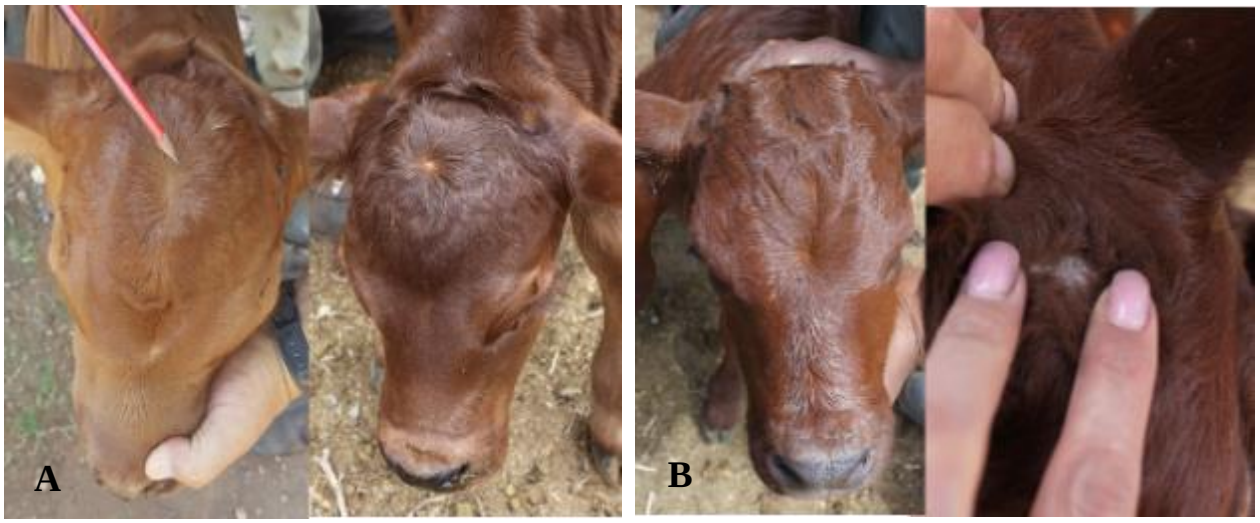


Figure 5.2 The distinct cowlick in the hair between the ears that can be observed at birth for polled Bonsmara calves (A) versus the horn bud that is visible for horned Bonsmara calves (B) (Photographs: R. Grobler)

Between approximately three and five months of age all polled calves should be inspected to confirm their polled status, i.e. no signs of horn growth. If there are any signs of horn growth, this would indicate the development of scurs. Scurs only appear after approximately four months of age or in some cases, animals that were phenotyped as polled at birth and weaning can develop scurs between 12 and 24 months of age. Scurs are usually not fused to the frontal bone of the skull (Medugorac *et al.*, 2012), i.e., scurs are loosely attached to the head and have the ability to move (Asai *et al.*, 2004).

Between three and five months of age it is important to note that in calves of a similar age, horn growth will be more distinct, and horns would be larger compared to scurs (Figure 5.3). Scurs can be distinguished from horns primarily based on the head shape of the calves. Calves with scurs will have a rounded poll (central prominence on the head) and the distinct cowlick in the hair between the ears will still be visible (Figure 5.3 B) whereas horned calves will have a flat poll (central prominence on the head) (Figure 5.3 A). Additionally, scurs will be attached to the skin and therefore moveable, while

© University of Pretoria

horns will be attached to the skull and not moveable. Calves with horns and scurs should be dehorned at this stage.

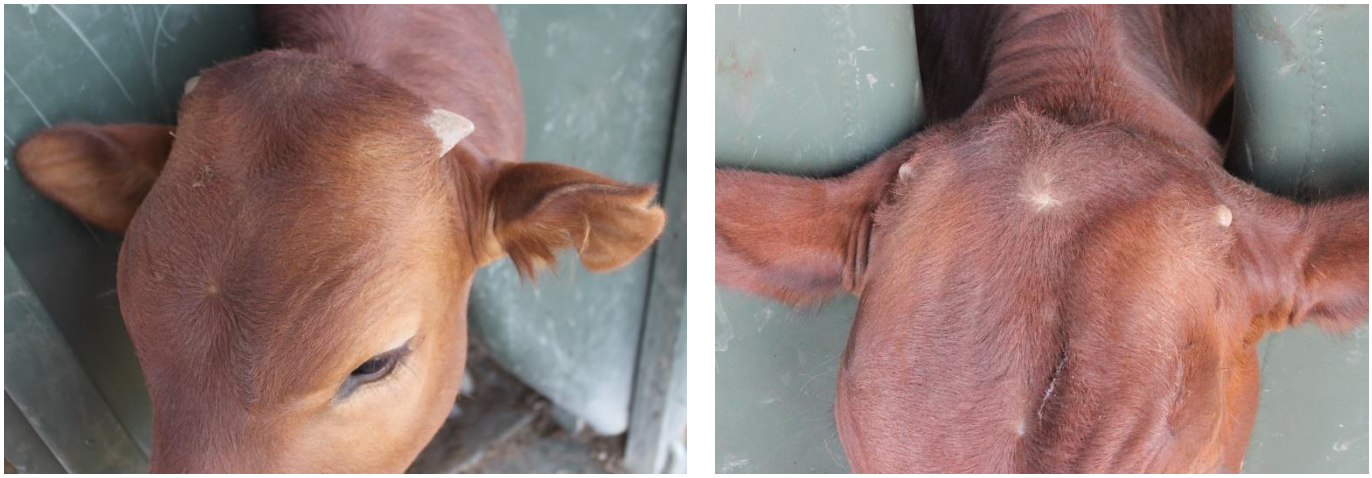


Figure 5.3 The horned (A) and scurs (B) phenotypes in Bonsmara calves of a similar age, i.e. at five months of age (Photographs: R. Grobler)

Polled calves between three and five months of age can have small scurs that are not visible without closer inspection and these calves might appear smooth polled, i.e., no signs of horn growth. Therefore, all polled calves should be checked for scurs, by inspecting the hair on the head where horns would have been. If small scurs are present, it will appear as small scab-like growths between the hairs (Figure 5.4) and will not be visible without parting the hairs (Figure 5.4). In this study it was observed that scurs generally developed earlier in male calves and that the growth of scurs are more pronounced in male calves, compared to female calves. Small scab-like scurs between the hairs on the head were more frequently observed in female calves.

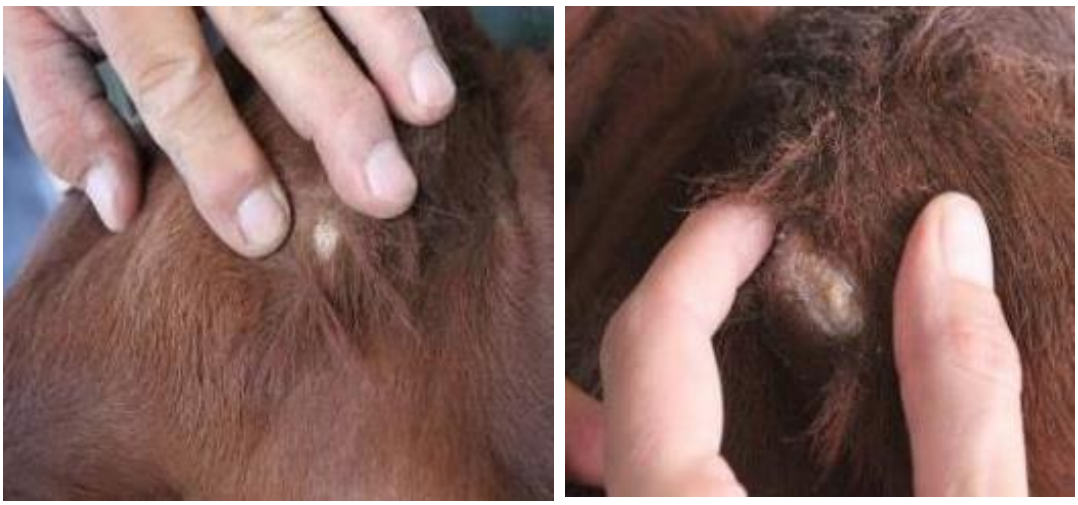


Figure 5.4 Small scab-like scurs observed in polled Bonsmara calves between the hairs on the head where horns would have been (Photographs: R. Grobler)

© University of Pretoria

In most animals, scurs typically develop after 12 months of age, thus many animals that are initially identified as polled will develop scurs at a later age, i.e., after 12 months of age. The scurs phenotype can be identified as follows for animals after 12 months of age: i) corneous appendages that grow in a similar place on the head as horns, ii) for animals of the same age, these appendages are smaller than horns and iii) are loosely attached to the head and are in most cases moveable. In the Bonsmara breed, scurs vary to a large degree among individuals, sexes and age groups in terms of different shapes of the scurs and sizes, varying from a small scab-like growth to large scurs in older animals (Addendum B). Initially scurs present as a round or oval shaped scab-like growth in between the hairs on the head where horns would have been (Figure 5.5 A), that is moveable on the head and is not attached to the skull. In some animals this scab-like structure remains and never forms larger scurs, but in most cases the scab-like structure will develop into larger scurs in older animals (Figure 5.5 B). Scurs usually grows as a round structure and longer scurs tend to grow inwards towards the head (Addendum B). Scurs can also be observed asymmetrically: one side of the head has scurs, while the other side are polled, or the scurs on the one side of the head is larger than the other side (Addendum B).

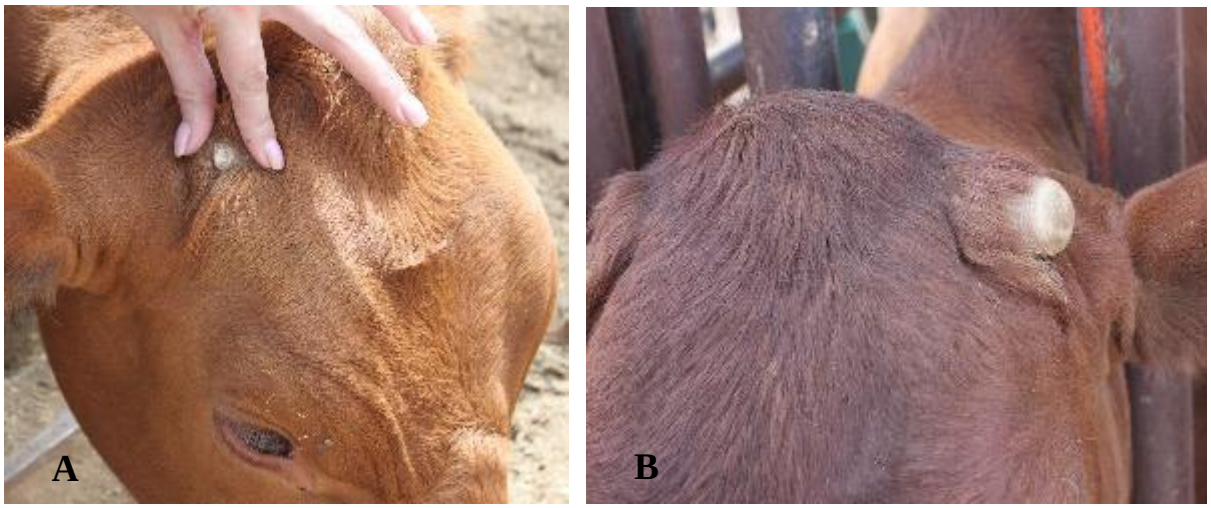


Figure 5.5 Scurs observed in Bonsmara animals after 12 months of age. Initially scurs present as a scab-like growth between the hairs on the head where horns would have been (A), but in most cases the scab-like structure will develop into larger scurs in older animals (B) (Photographs: R. Grobler)

Since horns will continue to grow during the animal's entire life and to conform with the minimum breed standards set by the Bonsmara Breeder's Society, it is compulsory that all animals be dehorned within a few months after birth. It is recommended that animals with scurs should also be dehorned for a smooth appearance and to conform to the Bonsmara breed standards.

© University of Pretoria

5.3 Genotypic identification

An important aspect of selection for polled animals is the identification of the *POLLED* gene for confirmation of polledness on a genetic level, and to distinguish between heterozygous and homozygous polled individuals. Genotypic identification of polledness can be done by screening animals for the Celtic variant of the *POLLED* locus, which has been confirmed in the Bonsmara breed (Grobler *et al.*, 2018). Animals can be screened for the Celtic variant by sending a hair sample to a relevant laboratory that screens for this variant, either by a PCR based diagnostic test or by SNP genotyping. The screening identifies the two alleles for the Celtic variant of the *POLLED* locus, i.e., the dominant polled allele (P) and the recessive horned allele (p). These diagnostic tests can, however, not be used to identify the scurs phenotype on a genetic level, since both polled and scurred animals test genetically heterozygous polled at the *POLLED* locus (Grobler *et al.*, 2018). The causative mutation for scurs has not yet been identified.

For accurate recording of genetic testing for the *POLLED* locus and to ensure consistency for which variant is tested for, it was decided to indicate the results on the SA Stud Book Logix's system by using the two alleles of the *POLLED* locus and to indicate that the animals were tested for the Celtic variant, a C will follow the polled status, i.e., PP (c) and Pp (c).

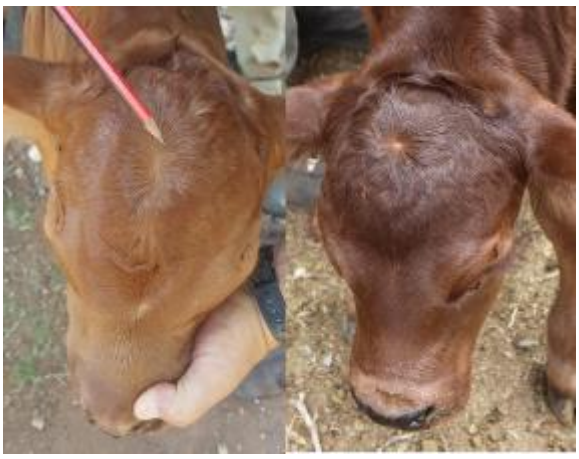
5.4 Protocol for Bonsmara farmers

The following protocol was compiled to assist Bonsmara breeders to identify the polled, horned and scurs phenotypes at various ages, and to indicate how to record these phenotypes.

Identification at birth

To identify the polled phenotype at birth:
- ➢ A distinct cowlick in the hair between the ears
- ➢ No indication of a horn bud
- ➢ A rounded peaked poll

→ Record calves as polled in the calf book; indicated as **P**

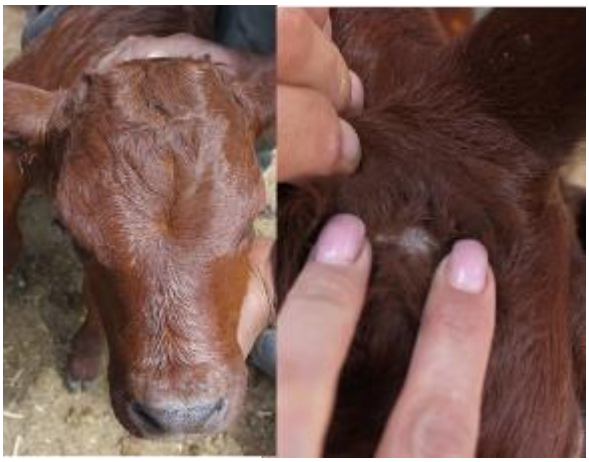


© University of Pretoria

To identify calves that will be horned:

- Absence of the cowlick in the hair between the ears
- The horn bud can be observed between the hairs on the head
- A flat poll

→ Record calves as horned in the calf book; indicated as **H**



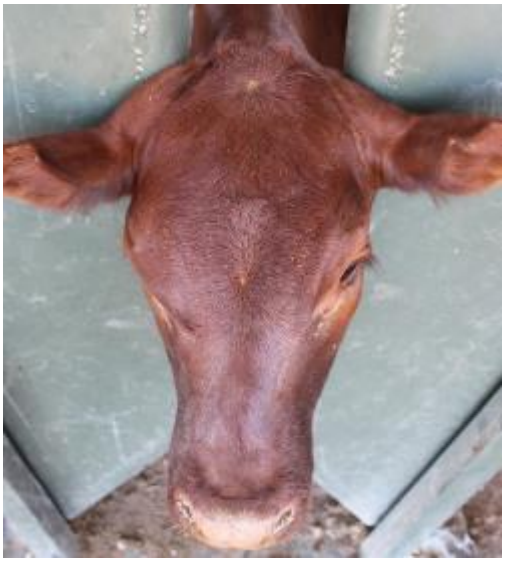
Identification between three and five months of age

- Phenotyping should now be repeated to confirm the phenotype of calves that was recorded at birth
- All polled calves should be checked to confirm that they are still polled, i.e., any signs of horn growth would indicate the development of scurs

To confirm the polled phenotype:

- Polled calves will show no sign of horn growth
- The cowlick in between the ears can still be observed

→ Confirm phenotype with a **P** in the calf book



To confirm the horned phenotype:

- Horns will be clearly visible
- Horns are not yet firmly attached to the skull, and might be moveable
- All horned calves that have not been dehorned yet, should be dehorned

→ Horned animals should be indicated with a **H** in the calf book

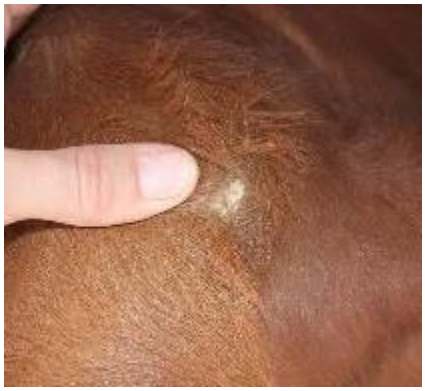
© University of Pretoria

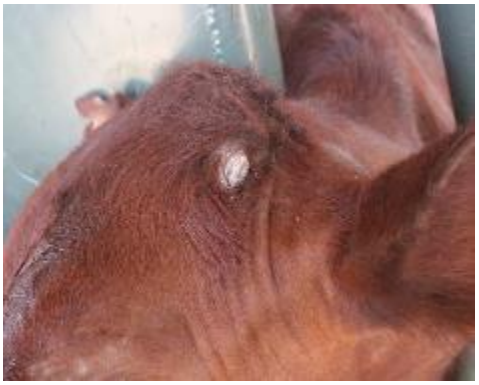
Polled animals that show signs of horn growth might indicate scurs, which can be identified as follows:

- A rounded peaked poll
- Scurs grow in a similar position on the head as horns
- The hair on the head, where horns would have been, should be checked for scurs
- A round or oval shaped scab-like growth between the hair
- Appears as if the scab-like growth is attached to the skin
- OR Growths similar to small horns
- These growths are smaller compared to horns at the same age
- And are loosely attached to the head, i.e., moveable

→ Polled animals that are identified with scurs at this stage, should be recorded as **PSc**

→ Animals with scurs should be dehorned for a smooth appearance

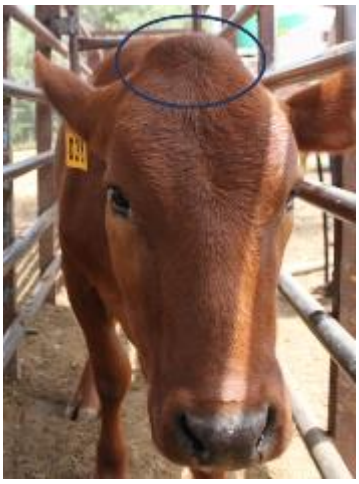




Identification after 12 months of age

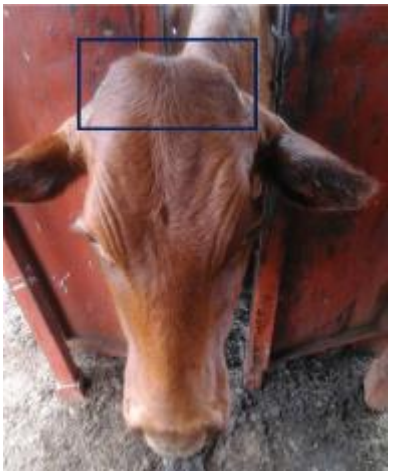
To confirm the polled phenotype:

- The polled phenotype should be more pronounced and clearly visible
- Polled animals present with a rounded more peaked poll



To confirm the horned phenotype:

- Horned animals will have a flat poll (i.e., horn crown)
- Horned animals would have been dehorned at ± 5 months of age; no horns will be visible



© University of Pretoria

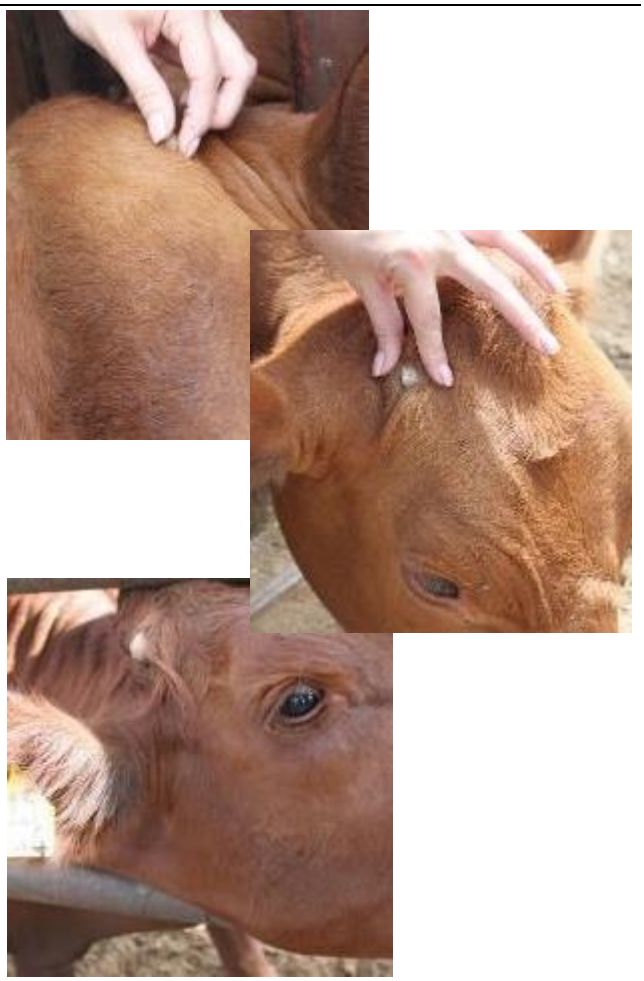
All polled animals should be re-evaluated, since animals that were recorded as polled at birth and at approximately five months of age can develop scurs after 12 months of age

For polled animals, any signs of horn growth might indicate scurs, which can be identified as follows:

- Follow the same procedure as for five months of age and take note of the following:
- Feel if the growth is firmly attached to the skull or if it is moveable
- This scab-like structure can develop into larger scurs
- Scurs are smaller compared to horns at the same age
- The larger scurs tend to grow inwards towards the head

→ Animals that are identified as scurred, should be recorded as **PSc**

→ These animals should be dehorned for a smooth appearance



Genotypic identification

Celtic variant of the *POLLED* gene

- To confirm polledness on a genetic level
- To distinguish between heterozygous and homozygous polled individuals

Collect a hair sample for each animal as follows:

- Collect 25 - 40 hairs (with intact follicles) from the tail of the animal
- Place collected hair into a separate envelope for each animal
- Mark each envelope clearly with the following:
  - Animal identification number
  - Sex of the animal
  - Phenotype of the horn status of the animal
  - The breeder's details
- Send samples to a laboratory that screens for the Celtic variant of the *POLLED* gene
- Results are indicated as PP (c), Pp (c) or pp:
  - PP (c) - Homozygous polled
  - Pp (c) - Heterozygous polled
  - pp - Horned
- Results of the *POLLED* tests should be sent to SA Stud Book to upload the data on the national database

© University of Pretoria

5.5 Benefits of phenotypic recording

Identifying the horn status of animals at an early age will assist in the management of animals, e.g., accurate phenotypic recording before dehorning, especially for breeders who select for the polled trait in their herds. Accurate phenotypic identification of the polled and scurs phenotypes in the Bonsmara will contribute towards more effective selection and faster fixation of the polled allele in Bonsmara herds. If the scurs phenotype can be accurately identified, it will decrease the indiscriminate culling of animals that are incorrectly phenotyped as horned. Due to the epistatic interaction between the *POLLED* and *SCURS* loci, scurred animals are heterozygous polled on a genetic level (Grobler *et al.*, 2018) and can still be incorporated into polled breeding programs.

The prevalence of the scurs phenotype and the number of polled animals in breeding programs can be managed by increasing the number of homozygous polled animals within the herd, by setting up a breeding program with polled sires and dams. When mating a homozygous polled animal with a horned animal, all offspring will be heterozygous polled (Table 5.2), but there is a chance that some of the offspring can develop scurs. If polled bulls are mated with polled cows, the chances of getting polled offspring increases and less horned calves will be born (Table 5.2). The proportion of polled offspring, and the chance for homozygous polled offspring, will increase if homozygous polled animals are mated with either heterozygous or homozygous polled animals (Table 5.2).

Table 5.2 The expected genotype and phenotype outcomes for different mating situations of polled and horned animals

Mating	Subsequent genotype	Resulting phenotype
PP x pp	100 % Pp	Polled
Pp x pp	50 % Pp	Polled
	50 % pp	Horned
Pp x Pp	25 % PP	Polled
	50 % Pp	Polled
	25 % pp	Horned
PP X Pp	50 % PP	Polled
	50 % Pp	Polled

Early identification of the polled status of animals will assist in the genetic selection of the polled trait and will result in faster introgression of the *POLLED* gene in the Bonsmara population. Identifying the genetic polled status of animals can have an economic benefit to breeders, since bulls can be marketed as certified polled, and this information can be incorporated into catalogues for auctions (Figure 5.6). Since the number of homozygous polled bulls are limited in the Bonsmara breed (Pers. Comm., C. Uys, November 2019, charluys@vodamail.co.za), certified homozygous polled bulls usually reach higher prices at auctions. More polled bulls in the market will also have the added advantages of

© University of Pretoria

decreased labour cost and increased welfare since dehorning animals will become redundant over time. Breeding polled Bonsmara animals is a welfare friendly long-term solution to dehorning cattle.

BULLS

1	MAROCHEL BONSMARAS	MCU 170024 Pp(c) SP	MCU 130126 PP(c) ☑ / MCU 140187 ☑	GT / DNA 20.04.17
2	MAROCHEL BONSMARAS	MCU 170203 Pp(c) SP	MCU 150204 Pp(c) ☑ / MCU 140104 ☑	GT / DNA 02.11.17
3	MAROCHEL BONSMARAS	MCU 170032 PP(c) SP	MCU 130126 PP(c) ☑ / MCU 140103 Pp(c) ☑	GT / DNA 27.04.17
4	MAROCHEL BONSMARAS	MCU 170202 Pp(c) SP	MCU 130151 PP(c) ☑ / MCU 120065 ☑	GT / DNA 01.11.17
5	MAROCHEL BONSMARAS	MCU 170037 PP(c) SP	MCU 130126 PP(c) ☑ / MCU 140069 Pp(c) ☑	GT / DNA 05.05.17
6	MAROCHEL BONSMARAS	MCU 170154 Pp(c) SP	MCU 140092 Pp(c) ☑ / MCU 090029 ☑	GT / DNA 13.10.17

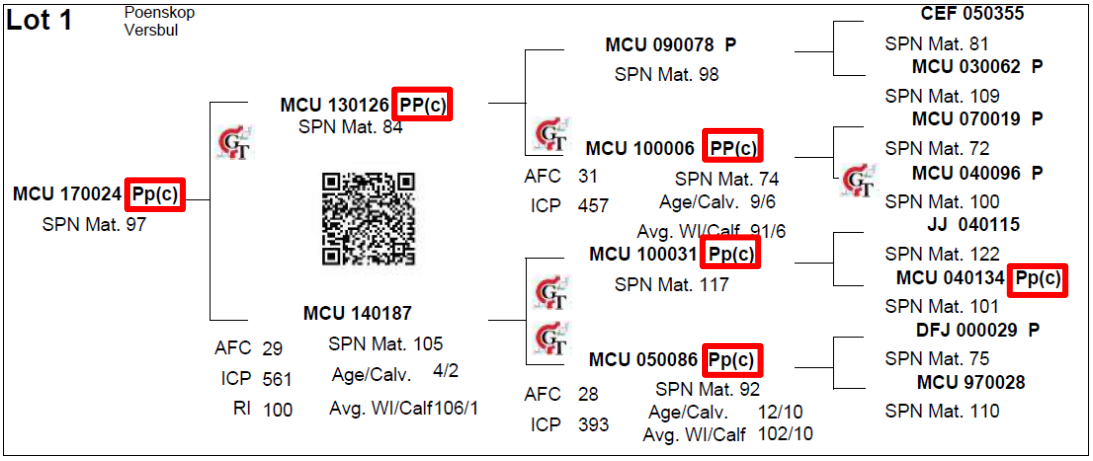


Figure 5.6 Sections from a catalogue for a Bonsmara production auction, indicating the animals that were genetically tested for the Celtic variant of the *POLLED* locus (tested animals indicated with red blocks)

5.6 Conclusion

This protocol recommends that phenotyping is done as early as possible, i.e., at birth or soon thereafter, and to re-evaluate animals between three and five months of age to confirm the initial phenotype that was recorded. Genetic testing for polledness is also recommended to distinguish between heterozygous and homozygous polled animals, and especially for animals where a clear distinction cannot be made between scurs and horns. Early and accurate identification of the polled and scurs phenotypes will assist with the management of these phenotypes. The genotypic information of the horn

© University of Pretoria

status of animals can be effectively incorporated into a polled breeding program to increase the number of polled animals and to manage the prevalence of the scurs phenotype.

Acknowledgements

I would like to acknowledge Mr. Charl Uys for his guidance towards the phenotypic identification of the animals and for sharing his knowledge. The Bonsmara Breeder's society is herewith acknowledged for consent to conduct the research for this study.

References

Asai, M., Berryere, T.G. & Schmutz, S.M., 2004. The scurs locus in cattle maps to bovine chromosome 19. Animal genetics. 35, 34-39.

Bonsma, J.C., 1980. Cross-breeding, breed creation and the genesis of the Bonsmara. In: Livestock Production - a Global Approach. Tafelberg, Cape Town, South Africa. pp90–110.

Grobler, R., Visser, C., Capitan, A. & van Marle-Köster, E., 2018. Validation of the *POLLED* Celtic variant in South African Bonsmara and Drakensberger beef cattle breeds. Livestock Science. 217, 136-139.

Habel, R. & Budras, K.H., 2003. Skull with paranasal sinuses and horns. In: Bovine Anatomy. Budras, K. H. & Habel, R. (Eds.), Schlütersche, Hannover, Germany, pp.34–35.

Irrgang, N., 2012. Horns in cattle - implications of keeping horned cattle or not. Doctoral dissertation, University of Kassel, Germany.

Medugorac, I., Seichter, D., Graf, A., Russ, I., Blum, H., Göpel, K.H., Rothammer, S., Förster, M. & Krebs, S., 2012. Bovine polledness – an autosomal dominant trait with allelic heterogeneity. PLoS One, 7, e39477.

Newman, R. & Partridge, I., 2007. A guide to best practice husbandry in beef cattle. Branding, castration and dehorning. In: 'Queensland Department of Primary Industries and Fisheries. Meat and Livestock Australia Limited'. (Ed. Meat and Livestock Australia Limited)(Queensland).

© University of Pretoria

Chapter 6
Critical review and conclusion

6.1 Critical discussion and recommendations

The routine practice of dehorning has increasingly raised welfare concerns, as it is well documented that dehorning is associated with stress and acute pain (Graf & Senn, 1999; Stafford & Mellor, 2011). Public scrutiny and welfare concerns has especially mounted as very few farmers use any form of medication to alleviate pain (Cozzi *et al.*, 2015; Kling-Eveillard *et al.*, 2015). From a farmer's point of view, dehorning also negatively affects weight gains, especially during the first two to six weeks after dehorning (Winks *et al.*, 1977; Goonewardene *et al.*, 1999), which can have a considerable economic impact (Goonewardene *et al.*, 1999; Prayaga, 2007). All these factors contribute towards the need for sustainable alternatives to dehorning of cattle. In this regard, breeding genetically polled animals would be a welfare friendly alternative, as well as a long-term solution to dehorning.

At the start of this project, there was no diagnostic testing for polledness available in South Africa. The few Bonsmara breeders that were interested in breeding polled animals primarily relied on phenotypic selection. With the increased availability of marker assisted selection, some breeders utilized a diagnostic test that was developed in Australia to identify polled genotypes. This DNA test, initially released in 2010, was refined in 2014 to a haplotype diagnostic test to assign polled and horned genotypes in Australian beef cattle breeds (Piper *et al.*, 2014). By 2018 a total of 182 Bonsmara animals were tested with the Australian haplotype test, but a poor genotype assignment rate of 62.64% was observed (Connors *et al.*, 2018). More recently, the Celtic (P_C) and Friesian (P_F) polled variants became routinely available on various bovine SNP arrays, however, in South Africa routine genotyping (e.g., for genomic selection) is not standard practice. Even though SNP genotyping is now available, mainly through animal recording and genetic improvement companies, very few farmers are able to genotype their entire herd due to the cost implication. Therefore, there is a need for simple and cost effective diagnostic testing, especially for farmers with large herds who want to incorporate the results of diagnostic testing for polledness into their breeding programs.

In this project, the Celtic variant of the *POLLED* locus was validated as the causative mutation for polledness in the Bonsmara breed. All animals that were phenotyped as polled or scurred were found to carry at least one copy of the Celtic allele (P_C) whereas all horned animals were homozygous wildtype (pp). For the Bonsmara animals in this research (Chapter 4), very few homozygous polled animals (frequency of P_CP_C = 0.170) were identified, and the majority of animals were heterozygous polled (frequency of P_Cp = 0.640). These results confirm that selection for polledness were only emphasized during the last two decades. Even though the stud breeders included in this study specifically select for polled Bonsmara cattle, they have limited homozygous polled sires available for inclusion in their breeding programs. Stud breeders supply genetic material to commercial farmers, which in turn produce

© University of Pretoria

weaner calves for commercial feedlot operations. The genetic merit of stud breeding animals should remain high to ensure genetic improvement for economically important traits, i.e., growth and reproductive traits. Due to the limited number of available polled Bonsmara bulls, the majority of the Bonsmara population remains predominantly horned.

This research clearly demonstrated a high prevalence of scurs in the Bonsmara herds included in this project. According to the epistatic effect between the *POLLED* and *SCURS* loci, and the sex-influenced expression pattern of scurs, animals that are phenotypically scurred should present with a heterozygous polled genotype (Long & Gregory, 1978). This implies that heterozygous polled animals can either express a polled or scurred phenotype. In this study a high proportion (0.427; Chapter 4) of the animals that had a heterozygous polled genotype were phenotypically scurred.

Contrasting to previous findings, in this study not all the animals phenotyped as scurred had a heterozygous polled genotype. Even though the majority of scurred samples tested heterozygous polled for the Celtic variant, a proportion of animals tested genetically horned (pp). This discrepancy was more noticeable in females (0.102; Chapter 4) compared to only a few cases in males (0.053; Chapter 4). Firstly, this discrepancy might be attributed to incorrect phenotyping and difficulty to distinguish scurs from horns in some animals. However, based on the phenotypic identification of scurs, some of these animals are undeniably scurred (i.e., small loosely attached appendages) (Addendum C). It could be postulated that a novel mutation influencing scurs, that is not linked to the *POLLED* locus, might be present in the Bonsmara breed. It is recommended that these animals should be further investigated by sequencing analysis and that the findings and hypothesis should be validated in a wider sample group.

The challenge regarding accurate phenotypic recording in previous (Capitan *et al.*, 2009; Randhawa *et al.*, 2020), and the current studies, emphasises the need for a diagnostic test for the *SCURS* locus, especially considering the inconsistencies observed with regards to the polled genotype of scurred animals. Currently, screening for the Celtic variant of the *POLLED* locus has benefits for the identification of polled animals and to confirm that scurred animals are genetically polled. However, this screening does not resolve the identification of scurs on a genetic level.

This research identified four protein coding candidate genes across three chromosomes associated with scurs in Bonsmara cattle: *PHGDH* and *LRIG2* on BTA3, *FBN1* on BTA10 and *GUCY1B1* on BTA17. These results indicate genetic heterogeneity and suggest that the development of scurs is influenced by more than one gene in the Bonsmara beef cattle breed. Putative candidate genes associated with the scurs phenotype in Bonsmara cattle have not been identified by previous research (Asai *et al.*, 2004; Tetens *et al.*, 2015; Ketel, 2020). The candidate genes (*FBN1*, *GUCY1B1*, *LRIG2*, *PHGDH*) identified in this study are involved in a variety of molecular functions and biological processes. It is postulated that, besides involvement in the molecular regulation in the development of scurs, these genes might play a role towards the attachment of scurs to the skull. Unlike horns which consist of keratinized epithelium, scurs primarily consist of cartilaginous tissue (Brenneman *et al.*, 1996). Furthermore, scurs are typically not fused to the skull but is rather attached to the skull by soft tissue. Specifically, *FBN1*

© University of Pretoria

encodes for the extracellular matrix protein fibrillin-1, which provides structural support in elastic and non-elastic connective tissue.

It is, however, not certain how these genes interact to express the scurs phenotype and gene expression studies are recommended. Since the exact genetic mechanism and molecular regulation of horns and scurs are still unknown, functional studies in horned, polled and scurred cattle are required to resolve the exact pathways involved. It is furthermore recommended that these candidate genes, and the surrounding regions of interest, be further investigated by sequencing analysis.

The statistical power to detect significant associations and the number of significant SNPs detected by a GWAS study, is largely dependent on the sample size of the study population, as well as the density of the SNP panel used (Visscher et al., 2017). Bonsmara animals in this study were genotyped with the Geneseek Genomic Profiler 150K SNP array, consisting of 139 376 SNPs, instead of the Bovine 50K bead chip (version 3 contains 53 714 SNPs) that is generally used. The sample size used in the GWAS (220 animals) was small, but to increase the power of detecting associations, a crucial step in the study design was to specifically define the phenotypes of the case (scurs) and control (polled) samples that were included in the GWAS (Zondervan & Cardon, 2007). This study design was also implemented to account for potential population substructure, but this approach might have limitations in accounting for herd effect. It is therefore recommended that future GWAS strategies be optimized in terms of accounting for existing herd effects and between-animal relationships, and this could be achieved through utilizing alternative software programs (e.g., GCTA or WOMBAT) that take these factors into consideration. Further recommendations would include to validate the GWAS findings of this study in an independent group of Bonsmara animals and that future studies should increase the sample size of the dataset.

To aid in the validation of the initial mapping results of the *SCURS* locus in this study, a substantial number of phenotypes will be required for GWAS. The primary challenge lies in accurately phenotyping and recording the scurs phenotype within breeds. The findings of this research, specifically the discrepancies between the on farm phenotype and Celtic genotype results observed in Chapter 3 and Chapter 4, highlights poor phenotypic recording amongst breeders, with the major problem being to distinguish between horned and scurred phenotypes. Phenotyping protocols and guidelines that were developed breed specifically will aid in the collection of accurate phenotypes for horn status.

The study was concluded by compiling a protocol for phenotyping Bonsmara animals for polled and scurs phenotypes at various ages. This protocol aimed to assist breeders to accurately identify polled and scurred animals, so that appropriate selection strategies can be implemented to increase the number of polled Bonsmara animals and to manage scurs. This protocol should coincide with farmers' management practices and ensure that polled, horned and scurs phenotypes are recorded up to at least 24 months of age, with emphasis on the identification of scurs.

There is a need to increase the number of homozygous polled animals, and especially homozygous polled bulls, in the Bonsmara breed. Accurate phenotypic identification of the polled and scurs

© University of Pretoria

phenotypes in the Bonsmara will contribute towards more effective selection and faster fixation of the polled allele in Bonsmara herds. Early and accurate identification of the scurs phenotype will assist with the management of this phenotype and eliminate indiscriminate dehorning or culling of animals that are incorrectly phenotyped as horned and could also assist with improved selection of the polled trait. It is recommended that selection strategies for polledness should utilize polled animals with known genotypic status (PP versus Pp) in a well-developed breeding program.

6.2 Future research

It is recommended that future research studies investigating the polled and scurs phenotypes, should include other South African beef cattle breeds. During this research project, other breeds (e.g., Drakensberger, Hereford and Tuli) showed interest in participating in the Polled project at the University of Pretoria but insufficient samples were available and there was also a lack of accurate phenotypic records to investigate these breeds. To overcome this challenge, the current phenotyping protocol developed for the Bonsmara breed in this study can be used to develop breed specific protocols and guidelines to facilitate accurate recording of horn status phenotypes.

The results of this study indicate genetic heterogeneity for the scurs phenotype and suggest the involvement of more than one gene in the expression of the scurs phenotype in the Bonsmara breed. It is unclear how the genes identified in this study interact to express the scurs phenotype. It is recommended that future studies sequence the candidate genes and regions of interests identified in this study to investigate specific mutational differences between scurred and non scurred animals. Furthermore, gene regulation could play an important role in horn development, and it may be useful to study the regulatory sequences of the loci associated with the polled and scurs phenotypes (Liang *et al.*, 2016). Future studies should focus on discovering genomic elements influencing the expression of polledness and scurs, as well as investigating the potential molecular pathways governing these phenotypes. It is recommended that future research include functional genomic and multi-omic approaches to investigate such genomic elements. These approaches may contribute towards an improved understanding of horn ontogenesis. Even though these advanced modern approaches hold potential, it should be noted that the lack of availability of large data sets with accurate phenotypes is a limiting factor for application.

6.3 Conclusion

There is a need to increase the number of homozygous polled animals in the Bonsmara breed, especially animals with superior genetics that can be included in well designed breeding programs. Additionally, by increasing the number of polled animals in the Bonsmara breed, welfare of beef cattle production systems in the South African industry can be improved by decreasing the number of horned animals since dehorning might become redundant over time, especially in the absence of more humane methods of dehorning. The commercial application of the PCR based Celtic screening will assist in the

© University of Pretoria

identification of genetically polled animals, especially early in life, which would result in faster introgression of the *POLLED* gene. This would also lead to faster genetic progress and more efficient selection for polledness. However, this Celtic screening does not resolve the identification of scurs on a genetic level. Sequencing regions of interest and fine mapping of candidate genes associated with the scurs phenotype may provide valuable information for identifying causal mutations which can be used in future SNP based selection approaches.

References

Asai, M., Berryere, T.G. & Schmutz, S.M., 2004. The scurs locus in cattle maps to bovine chromosome 19. Animal genetics. 35, 34-39.

Brenneman, R.A., Davis, S.K., Sanders, J.O., Burns, B.M., Wheeler, T.C., Turner, J.W. & Taylor, J.F., 1996. The polled locus maps to BTA1 in a Bos indicus× Bos taurus cross. Journal of Heredity. 87, 156-161.

Capitan, A., Grohs, C., Gautier, M. & Eggen, A., 2009. The scurs inheritance: new insights from the French Charolais breed. BMC Genetics. 10,1-11.

Connors, N.K., Tier, B. & Johnston, D.J., 2018. Current status of Australia's diagnostic poll haplotype test. In World Congress on Genetics Applied to Livestock Production (p. 344).

Cozzi, G., Gottardo, F., Brscic, M., Contiero, B., Irrgang, N., Knierim, U., Pentelescu, O., Windig, J.J., Mirabito, L., Eveillard, F.K. & Dockès, A.C., 2015. Dehorning of cattle in the EU Member States: A quantitative survey of the current practices. Livestock Science. 179, 4-11.

Graf, B. & Senn, M., 1999. Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. Applied Animal Behaviour Science. 62, 153-171.

Goonewardene, L.A., Pang, H., Berg, R.T. & Price, M.A., 1999. A comparison of reproductive and growth traits of horned and polled cattle in three synthetic beef lines. Canadian Journal of Animal Science. 79, 123-127.

Ketel, C.R., 2020. Investigating Scur Candidate Genes in *Bos taurus* cattle. MSc dissertation, Department of Animal and Poultry Science, University of Saskatchewan.

Kling-Eveillard, F., Knierim, U., Irrgang, N., Gottardo, F., Ricci, R. & Dockès, A.C., 2015. Attitudes of farmers towards cattle dehorning. Livestock Science. 179, 12-21.

Liang, C., Wang, L., Wu, X., Wang, K., Ding, X., Wang, M., Chu, M., Xie, X., Qiu, Q., & Yan, P., 2016. Genome-wide Association Study Identifies Loci for the Polled Phenotype in Yak. PLoS One 11, e0158642.

Long, C.R. & Gregory, K.E., 1978. Inheritance of the horned, scurred, and polled condition in cattle. Journal of Heredity. 69, 395-400.

Piper, E.K., Tier, B. & Henshall, J.M., 2014. A haplotype diagnostic for polled in Australian beef cattle. In Proceedings, 10th World Congress of Genetics Applied to Livestock Production (Vol. 10, pp. 1-3).

© University of Pretoria

Prayaga, K. C., 2007. Genetic options to replace dehorning in beef cattle – a review. Crop and Pasture Science. 58, 1-8.

Randhawa, I.A., Burns, B.M., McGowan, M.R., Porto-Neto, L.R., Hayes, B.J., Ferretti, R., Schutt, K.M. & Lyons, R.E., 2020. Optimized genetic testing for polledness in multiple breeds of cattle. G3: Genes, Genomes, Genetics. 10, 539-544.

Stafford, K.J. & Mellor, D.J., 2011. Addressing the pain associated with disbudding and dehorning in cattle. Applied Animal Behaviour Science. 135, 226-231.

Tetens, J., Wiedemar, N., Menoud, A., Thaller, G. & Drögemüller, C., 2015. Association mapping of the scurs locus in polled Simmental cattle – evidence for genetic heterogeneity. Animal Genetics. 46, 224-225.

Visscher, P.M., Wray, N.R., Zhang, Q., Sklar, P., McCarthy, M.I., Brown, M.A. & Yang, J., 2017. 10 years of GWAS discovery: biology, function, and translation. The American Journal of Human Genetics. 101, 5-22.

Winks, L., Holmes, A.E. & O'Rourke, P.K., 1977. Effect of dehorning and tipping on liveweight gain of mature Brahman crossbred steers. Australian Journal of Experimental Agriculture and Animal Husbandry. 17, 16–19.

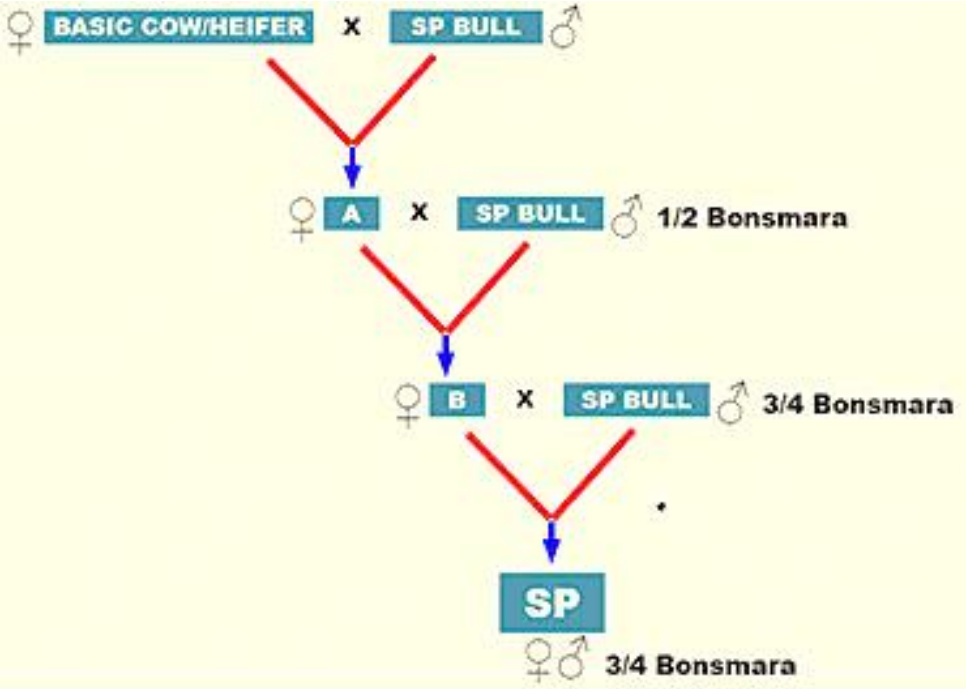
Zondervan, K.T. & Cardon, L.R., 2007. Designing candidate gene and genome-wide case–control association studies. Nature Protocols. 2, 2492-2501.

© University of Pretoria

Addendum A

The Bonsmara upgrading system

In the diagram below, SP refers to Stud Book proper and indicates an animal which is fully registered as a Bonsmara stud animal



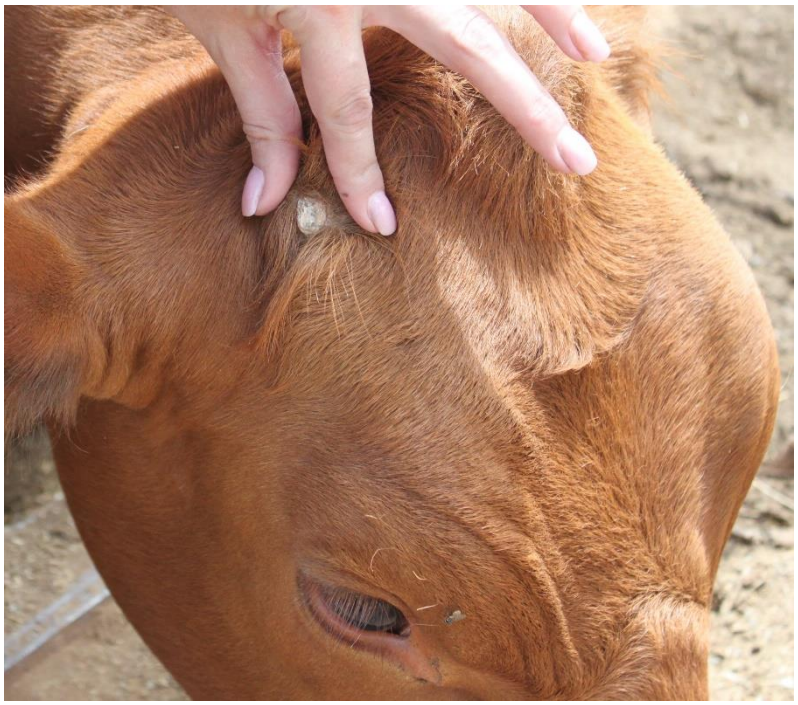
© University of Pretoria

Addendum B

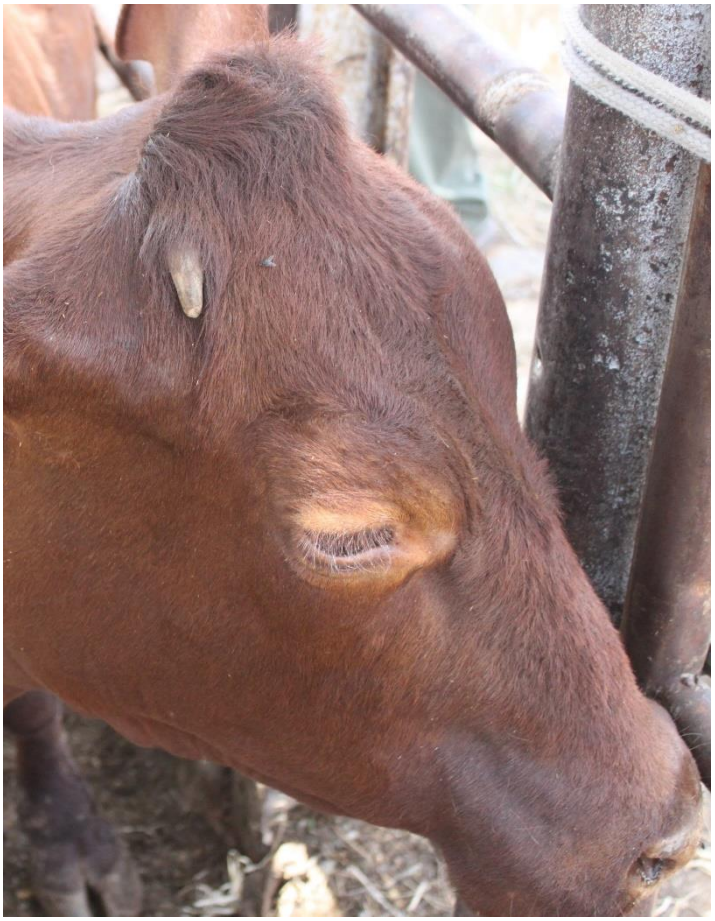
The scurs phenotype

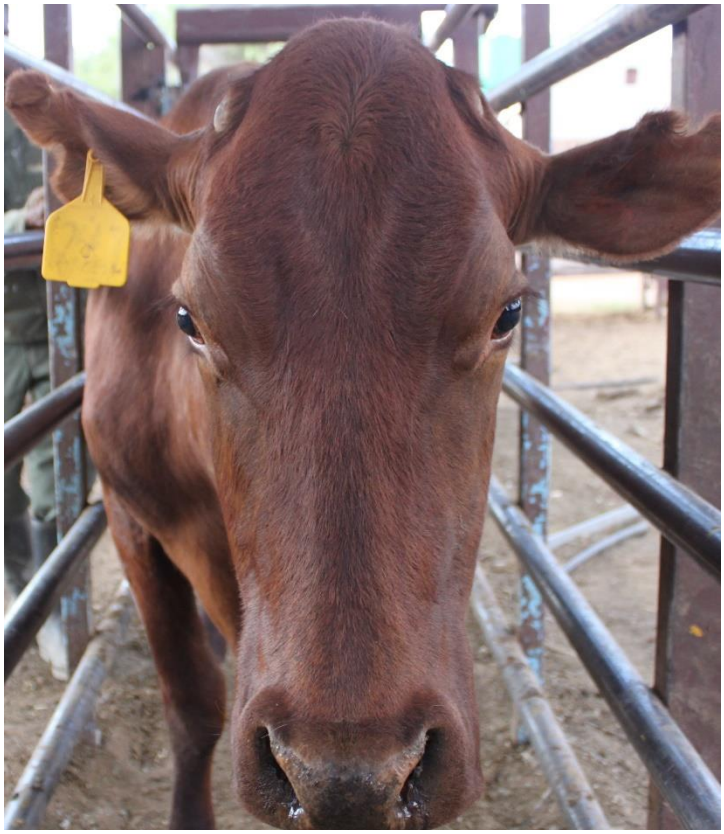
In the Bonsmara breed, scurs vary to a large degree among individuals, sexes and age of individual in terms of different shapes of the scurs and sizes, varying from a small scab-like growth to large scurs in older animals (Photographs: R. Grobler and S. Lashmar)

Females

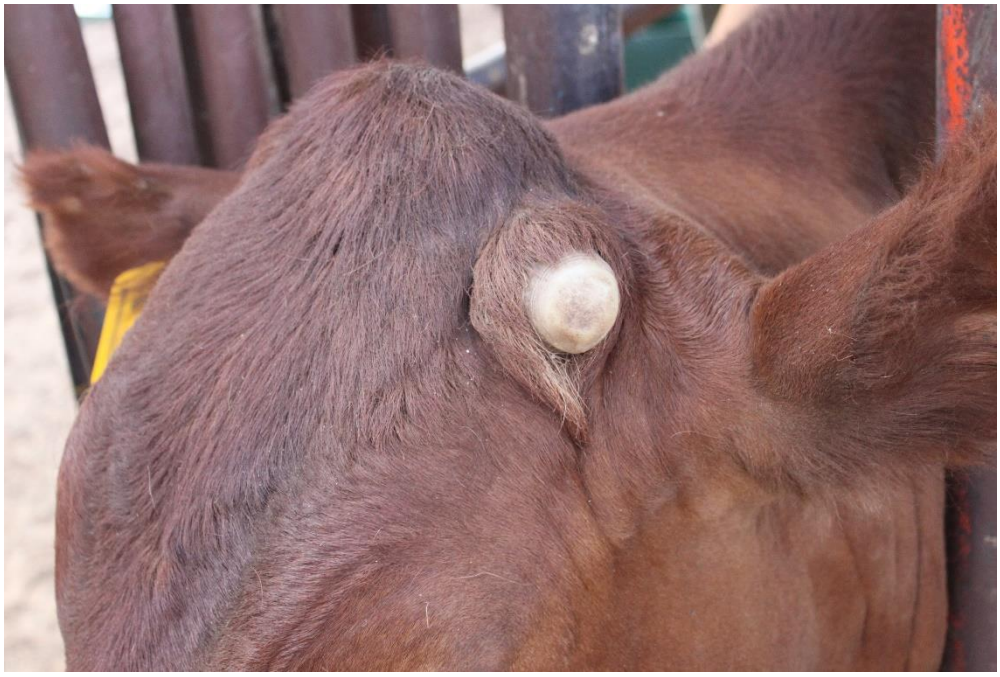


© University of Pretoria

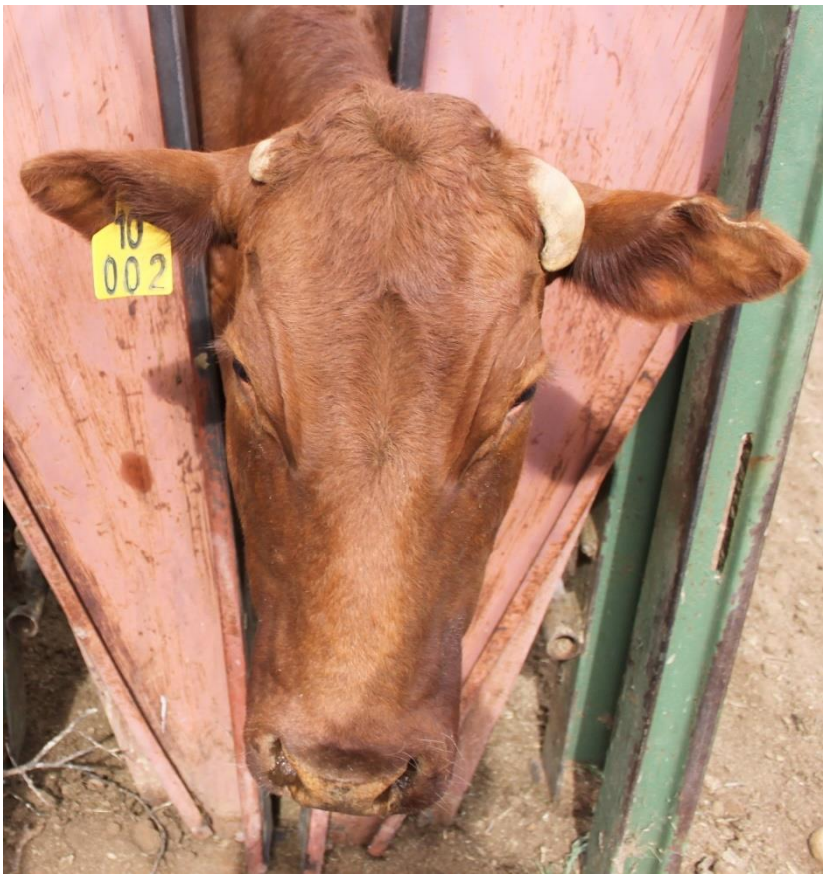




© University of Pretoria

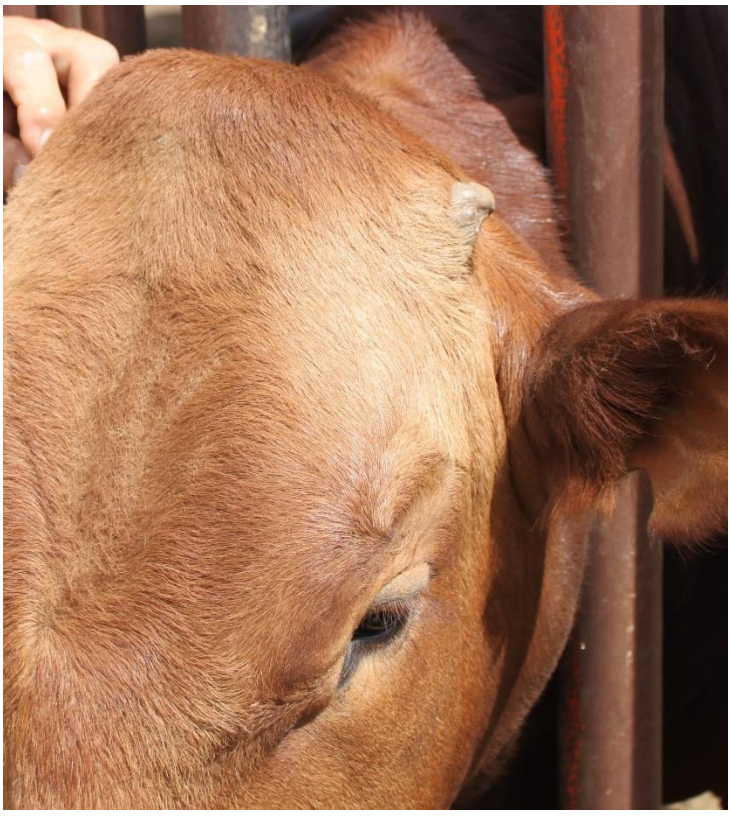


© University of Pretoria

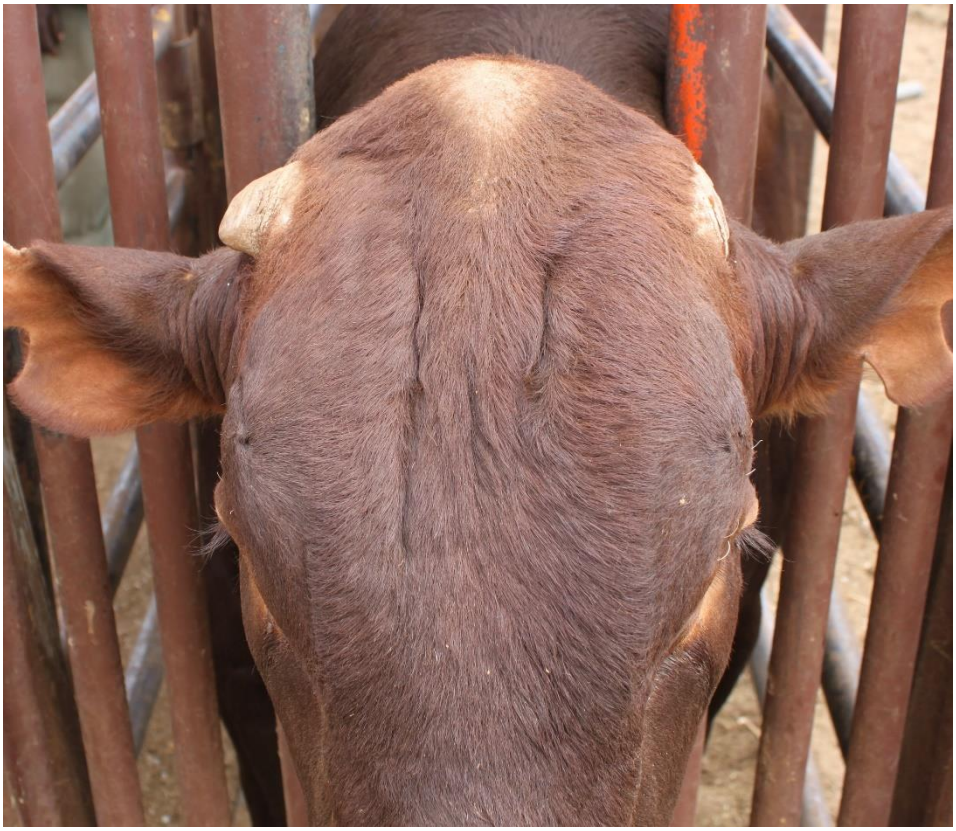


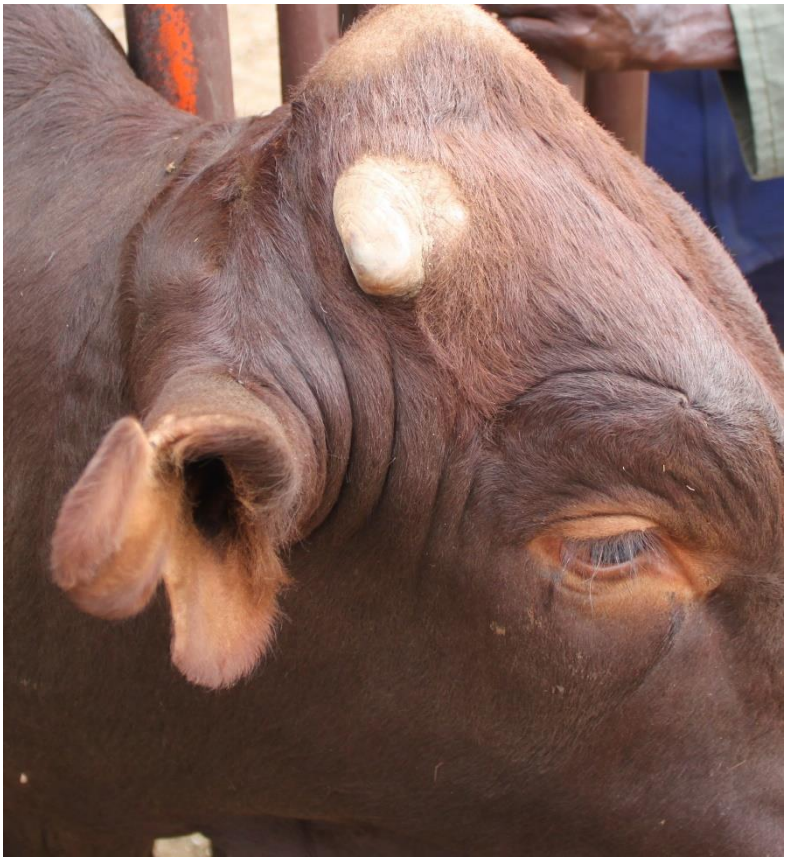
© University of Pretoria

Males

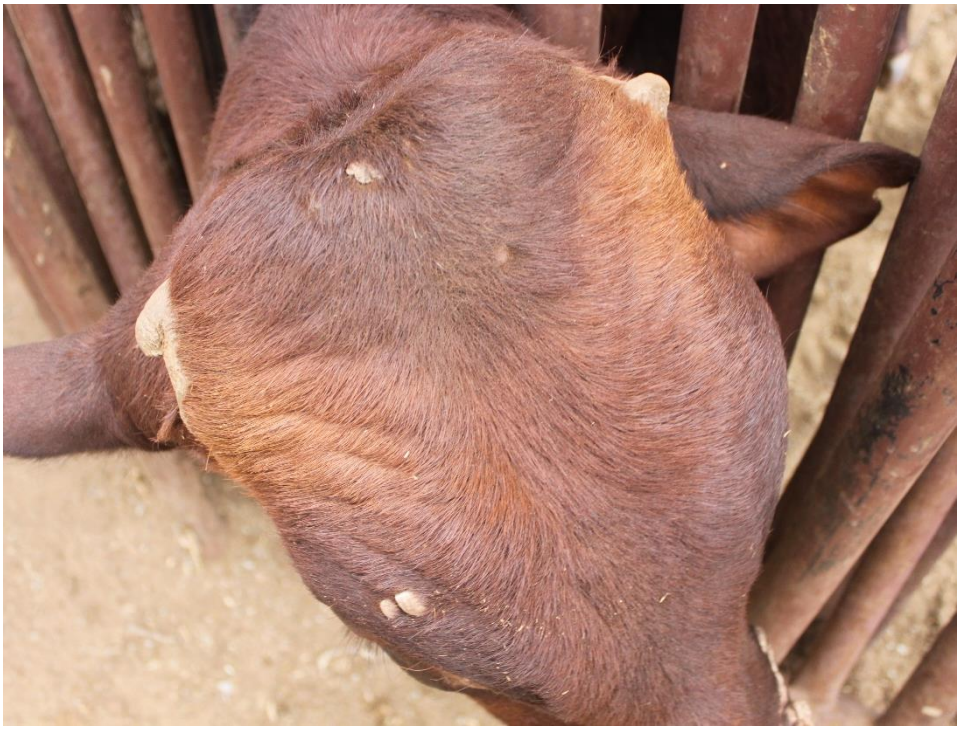


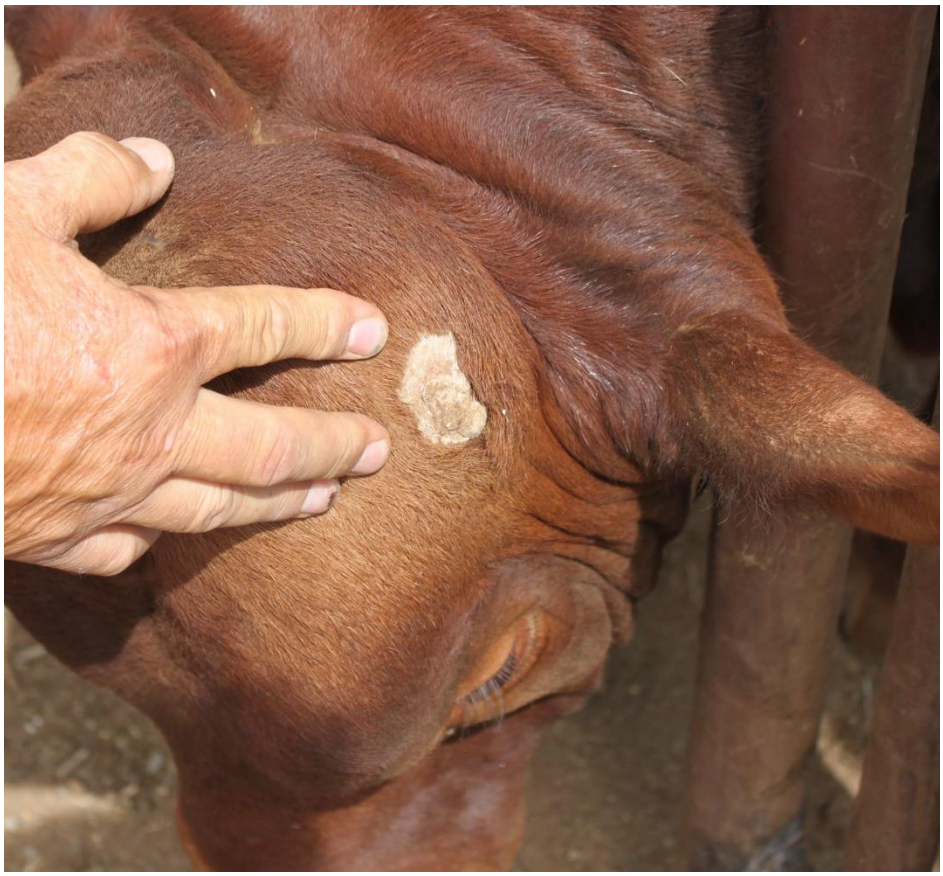
© University of Pretoria





© University of Pretoria

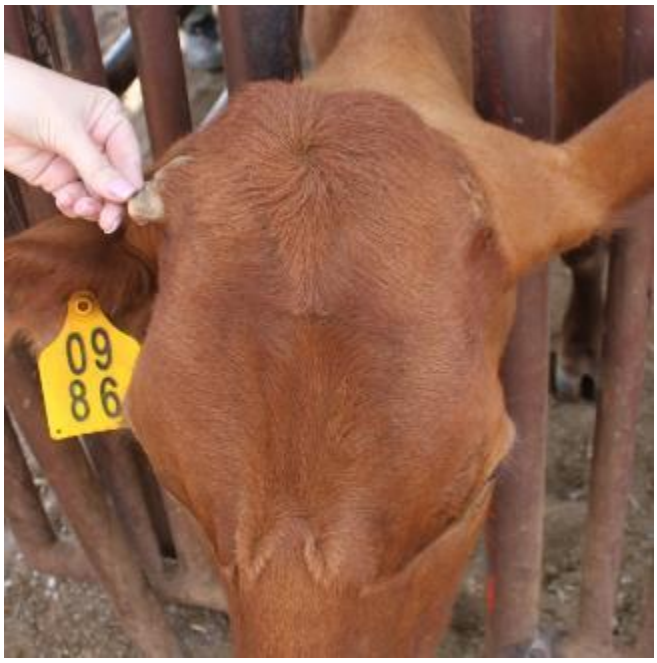




© University of Pretoria

Addendum C

Phenotypically scurred animals genotyped as horned (based on the Celtic variant of the *POLLED* locus)



© University of Pretoria