



**UNIVERSITI PUTRA MALAYSIA**

***PHENOTYPIC AND GENOMIC CHARACTERIZATION OF KEDAH-  
KELANTAN BULLS AND COMPARATIVE ASSESSMENT OF SEMEN  
CHARACTERISTICS***

**ISLAM MOHAMMED SIRAJUL**

**FPV 2022 10**



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By

**ISLAM MOHAMMED SIRAJUL**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Doctor of Philosophy**

**May 2022**

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## DEDICATION

To beloved my late mother, Halima Khatun, who was my best friend and inspiration in bringing me to this position, but who passed away on March 11, 2021, while I was studying at Universiti Putra Malaysia (UPM), Malaysia. Oh, Almighty Allah SWT, please accept my mother as a permanent resident of the Everlasting Paradise. Ameen.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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**May 2022**

**Chairman : Associate Professor Nurhusien Yimer Degu, PhD**  
**Faculty : Veterinary Medicine**

Kedah-Kelantan (KK) cattle are a well-adapted indigenous cattle breed in Malaysia. Despite having a slower growth rate than crossbred cattle, synthetic breeds, and imported beef breeds, KK cattle represents the primary source of more than 63 percent of domestic beef production in Malaysia. Nevertheless, purebred KK cattle are suffering from major germplasm dilution issues because of the current widespread and haphazard crossbreeding practices. The phenotypic and genomic characterization have not yet been recorded in order to prevent genetic dilution and further genetic improvement of this potential KK breed. The second source of domestic beef production in the country is various crossbred cattle. One of the most important crossbred cattle is the KK crossed with Brangus bulls. Although there are few studies on the growth, scrotal or testicular biometry, and semen characteristics of KK bulls, a comparative study with KK x Brangus bulls at a young age has not been addressed yet. Therefore, this study was conducted to determine the phenotypic and genomic characterization of Malaysian KK bulls and their semen characteristics in comparison to KK x Brangus bulls. A total of 184 purebred KK cattle were visually assessed for phenotypic features across three distinct agro-ecological zones. Fifty-one purebred KK cattle, regardless of sex, were measured for morphometric traits. The genomic DNA samples of two purebred KK bulls were sequenced using the Illumina NovaSeq 6000 platform after library construction. The sequence alignment, read mapping, assembly, and variant calling steps were all completed using the standard bioinformatics pipeline. For the whole genome resequencing of this investigation, the bovine reference genome located from [https://www.ncbi.nlm.nih.gov/assembly/GCF\\_000247795.1](https://www.ncbi.nlm.nih.gov/assembly/GCF_000247795.1) was utilized. Various software were used to determine existence of genomic variants in KK bull's genome. Monthly body weight (BW) and scrotal testicular biometry were measured from 10 experimental bulls consisting of 6 purebred KK and 4 KK x Brangus bulls. Sperm chromatin condensation (SCC) and sperm DNA

integrity of 48 frozen semen samples were measured by using the aniline blue (AB) and acridine orange (AO) test, respectively. One hundred twenty blood samples were assessed for testosterone (T), Gonadotrophin releasing hormone (GnRH), and insulin-like growth factor 1 (IGF1) of the experimental bulls. Puberty was defined when a bull produces an ejaculate containing a minimum number of  $50 \times 10^6$  sperm cells with a minimum of 10% motility. Data were analyzed using SPSS computer software program. Brown was the predominant coat color in KK cattle with small body size. The overall means of wither height, heart girth, and body length in KK were  $104.30 \pm 7.13$ ,  $127.41 \pm 13.16$ , and  $98.30 \pm 12.27$  cm, respectively. The genomic part of the study on the two KK bulls revealed an average number of single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), structural variations (SVs), and copy number variation (CNVs) of 8,823,792 (85%); 1,573,661 (15%); 30,547 (0%), and 24,254 (0%), respectively. Moreover, out of 57,855 SNPs found in the gene coding regions of KK bulls, 35,093 (61%), 22,608 (39%), 212 (0%), and 42 (0%) for synonymous, non-synonymous, stop gain and stop loss, respectively. The functional classification of InDels such as deletion and insertion, were 848,972 (54%) and 722,563 (46%), respectively. On the other hand, the comparison between the KK and KK x Brangus bulls uncovered significantly greater ( $p < 0.05$ ) body weight in KK x Brangus bulls ( $230.50 \pm 9.32$  kg) than KK bulls ( $204.50 \pm 13.22$  kg) at 24 months of age. Moreover, this study also showed that conventional semen quality, SCC, and sperm DNA integrity were significantly ( $p < 0.05$ ) greater in KK bulls than in their crossbred bulls. At the age of 18 months, the T levels in KK and KK x Brangus bulls were  $1.118 \pm 0.028$  and  $0.836 \pm 0.040$  ng/ml, respectively, and differed significantly ( $p < 0.01$ ). In addition, the average age at puberty was significantly ( $p < 0.01$ ) earlier in KK bulls ( $18.0 \pm 0.37$  months) than KK x Brangus bulls ( $23.5 \pm 0.33$  months). In conclusion, the phenotypic and morphometric measurements in the current study showed that Malaysian KK cattle generally possess brown coat types with a smaller body size. The identification of an almost similar number of different genomic variants compared with the other Zebu cattle breeds clearly shows the Zebu origin of the Malaysian KK cattle. Secondly, the heterozygous ratio of SNPs and InDels obtained in the genome certainly indicates the presence of genetic variation in the KK cattle. The results of this study revealed that KK bulls were superior in terms of reproductive indices such as SC, testicular parameters, earlier age at puberty, conventional semen quality and SCC, and sperm DNA integrity as compared to KK x Brangus bulls. However, KK x Brangus bulls were better in terms of body weight and average daily weight gain than KK bulls. The phenotypic and genomic characterization are brand-new knowledge that will serve as the basis for future genetic research on the Malaysian KK breed. The knowledge generated from this study would serve as a tool for the genetic improvement of this potential breed to promote and expand the Malaysian beef industry in the future.

**Keywords:** Kedah-Kelantan cattle, Kedah-Kelantan x Brangus bulls, phenotypic, morphometric, genomic, whole-genome sequencing, growth performance, reproductive indices, conventional semen analysis, puberty, sperm DNA integrity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN FENOTIP DAN GENOMIK LEMBU PEJANTAN KEDAH-  
KELANTAN DAN PENILAIAN PERBANDINGAN CIRI-CIRI AIR MANI**

Oleh

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Lembu Kedah-Kelantan (KK) merupakan baka lembu asli yang disesuaikan dengan baik di Malaysia. Walaupun mempunyai kadar pertumbuhan yang lebih perlahan berbanding lembu kacukan, baka sintetik dan baka lembu pedaging import, lembu KK adalah pengeluar utama lebih daripada 63 peratus daging lembu domestik di Malaysia. Lembu KK tulen, sebaliknya, mengalami masalah pencairan plasma nutfah utama kerana amalan kacukan yang berleluasa dan sembarangan. Pencirian fenotip dan genomik masih belum direkodkan untuk mengelakkan pencairan genetik dan perkembangan genetik selanjutnya bagi baka KK yang berpotensi ini. Sumber kedua pengeluaran daging lembu domestik di negara ini ialah pelbagai lembu kacukan. Salah satu lembu kacukan yang paling ketara di negara ini ialah KK kacukan dengan lembu Brangus. Sungguhpun terdapat beberapa kajian ke atas tumbesaran, biometri testis skrotum, dan ciri-ciri air mani lembu jantan KK, kajian perbandingan dengan lembu jantan KK x Brangus dari peringkat umur muda belum dikaji lagi. Oleh itu, kajian ini dijalankan untuk menentukan pencirian fenotip dan genomik lembu jantan KK Malaysia dan ciri air maninya berbanding lembu jantan KK x Brangus. Sebanyak 184 lembu KK baka tulen telah dinilai secara visual untuk ciri fenotip merentas tiga zon agro-ekologi yang berbeza. Lima puluh satu lembu KK baka tulen, tanpa mengira jantina, diukur untuk ciri morfometrik. Genom dua ekor lembu jantan KK baka tulen telah disusun menggunakan platform Illumina NovaSeq 6000 selepas pembinaan perpustakaan. Penjajaran jujukan, pemetaan baca, pemasangan dan langkah panggilan varian semuanya telah dilengkapkan menggunakan salur paip bioinformatik standard. Untuk keseluruhan penjujukan genom penyiasatan ini, genom rujukan lembu yang digunakan terletak dari [https://www.ncbi.nlm.nih.gov/assembly/GCF\\_000247795.1](https://www.ncbi.nlm.nih.gov/assembly/GCF_000247795.1). Pelbagai perisian digunakan untuk menentukan varian genom wujud dalam genom lembu jantan KK. Berat badan bulanan (BW) dan biometri testis skrotum diukur daripada 10 ekor lembu ujikaji yang terdiri daripada 6 ekor lembu KK dan 4 ekor lembu KK x Brangus. Pemeluwapan kromatin sperma (SCC) dan integriti DNA sperma bagi 48 sampel air mani beku diukur dengan menggunakan ujian



aniline blue (AB) dan oren acridine (AO). Seratus dua puluh sampel darah lembu ujikaji dinilai untuk testosteron (T), hormon pelepas gonadotropin (GnRH), dan faktor pertumbuhan seperti insulin 1 (IGF1). Akil baligh ditakrifkan sebagai apabila seekor lembu jantan mengeluarkan ejakulasi yang mengandungi sekurang-kurangnya  $50 \times 10^6$  sel sperma dengan motiliti minimum 10%. Data dianalisis menggunakan program perisian komputer SPSS. Warna coklat merupakan warna bulu yang paling dominan dalam lembu KK dengan saiz badan yang kecil. Cara keseluruhan ketinggian layu, lilitan jantung dan panjang badan dalam KK ialah  $104.30 \pm 7.13$ cm,  $127.41 \pm 13.16$ cm, dan  $98.30 \pm 12.27$ cm, masing-masing. Purata bilangan polimorfisme nukleotida tunggal (SNP), sisipan dan pemadaman (InDels), variasi struktur (SV), dan variasi nombor salinan (CNV) dalam genom lembu jantan KK ialah 8,823,792 (85%); 1,573,661 (15%); 30,547 (0%), dan 24,254 (0%), masing-masing. Selain itu, daripada 57,855 SNP yang ditemui di kawasan pengekodan gen lembu jantan KK, 35,093 (61%), 22,608 (39%), 212 (0%) dan 42 (0%) untuk sinonim, tidak sinonim, stop gain dan stop kerugian, masing-masing. Klasifikasi berfungsi InDels seperti pemadaman dan sisipan masing-masing ialah 848,972 (54%) dan 722,563 (46%). Keputusan menunjukkan berat badan yang lebih besar ( $p < 0.05$ ) dalam lembu jantan KK x Brangus ( $230.50 \pm 9.32$ kg) berbanding lembu jantan KK baka tulen ( $204.50 \pm 13.22$  kg) pada umur 24 bulan. Selain itu, kajian ini juga menunjukkan bahawa kualiti air mani konvensional, SCC, dan integriti DNA sperma adalah ketara ( $p < 0.05$ ) pada lembu jantan KK berbanding lembu kacukan mereka. Pada usia 18 bulan, paras testosteron dalam lembu jantan KK dan KK Brangus ialah  $1.118 \pm 0.028$  ng/ml dan  $0.836 \pm 0.040$  ng/ml, dan berbeza dengan ketara ( $p < 0.01$ ). Di samping itu, umur purata semasa akil baligh adalah ketara ( $p < 0.01$ ) lebih awal dalam lembu jantan KK ( $18.0 \pm 0.37$  bulan) berbanding lembu jantan KK x Brangus ( $23.5 \pm 0.33$  bulan). Kesimpulannya, ukuran fenotip dan morfometrik dalam kajian semasa menunjukkan bahawa lembu KK Malaysia umumnya mempunyai jenis bulu coklat dengan saiz badan yang lebih kecil. Terutamanya, bilangan varian genomik berbeza yang dikenal pasti hampir sama berbanding dengan baka lembu Zebu yang lain jelas menunjukkan asal usul Zebu lembu KK Malaysia. Kedua, nisbah heterozigot SNP dan InDels yang diperolehi dalam genom pasti menunjukkan kehadiran variasi genetik dalam lembu KK. Keputusan kajian ini mendedahkan bahawa lembu jantan KK lebih unggul dari segi indeks pembiakan seperti SC, parameter testis, umur awal akil baligh, kualiti air mani konvensional dan SCC, dan integriti DNA sperma berbanding lembu jantan KK x Brangus. Walau bagaimanapun, lembu jantan KK x Brangus adalah lebih baik dari segi berat badan dan purata kenaikan berat badan harian daripada lembu jantan KK. Pencirian fenotip dan genomik adalah pengetahuan baharu yang akan menjadi asas untuk penyelidikan genetik masa hadapan tentang baka KK Malaysia. Pengetahuan yang dijana daripada kajian ini akan menjadi alat untuk penambahbaikan genetik baka berpotensi ini untuk mempromosikan dan mengembangkan industri daging lembu Malaysia pada masa hadapan.

**Kata kunci:** Lembu lembu Kedah-Kelantan, lembu Kedah-Kelantan x Brangus, fenotip, morfometrik, genomik, penjujukan genom keseluruhan, prestasi pertumbuhan, indeks pembiakan, analisis air mani konvensional, akil baligh, integriti DNA sperma.



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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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## LIST OF ABBREVIATIONS

µl	Microliter
A	Adenine
AB	Aniline blue
ABT	Aniline blue test
ADG	Average daily gain
AI	Artificial insemination
AnGR	Animal genetic resources
AOT	Acridine orange test
ART	Assisted reproductive technology
AV	Artificial vagina
BCS	Body condition score
BL	Body length
BLAST	Basic Local Alignment Search Tool
Bp	Base pairs
BSE	Bull breeding soundness evaluation
BW	Body weight
BWA	Burrows-Wheeler Aligner
C	Cytosine
cDNA	Complementary DNA
CNV	Copy number variation
CSA	Conventional semen analysis
CV	Coefficient of variation
DbSNP	Database of single nucleotide polymorphisms
DEL	Deletion
DNA	Deoxyribonucleic acid
DVS	Department of veterinary services
DW	Distilled water
EE	Electro-ejaculator

EL	Ear length
ELISA	Enzyme-linked immune sorbent assay
EW	Ear width
FAO	Food and agricultural organization
FG	Flank girth
FL	Face length
FSH	Follicular stimulating hormone
FW	Face width
g	gram
G	Guanine
Gbp	Giga base pairs
gDNA	Genomic DNA
GH	Growth hormone
GnRH	Gonadotrophin releasing hormone
HA	Head abnormality of sperm
HG	Heart girth
HL	Head length
HRP	Horseradish peroxidase
ICE	Capillary electrophoresis
IGF1	Insulin like growth factor 1
InDel	Insertion and deletion
INS	Insersion
INV	Inversion
4IR	The Fourth Industrial revolution
ITC	Inter-chromosomal translocations
ITX	Intra-chromosomal translocations
Kbps	kilo base pairs
Kg	kilogram
KK	Kedah-Kelantan

LH	Luteinizing hormone
LN	Liquid nitrogen
MA	Mid piece abnormality of sperm
MARDI	Malaysian Agricultural Research and Development Institute
Mbps	Mega base pairs
MCO	Movement control order
ml	Mili litter
mRNA	Messenger RNA
MW	Molecular weight
NAP2	National agro-food policy 2
NCBI	National Centre for Biotechnology Information
ng	nano gram
NaCl	Sodium chloride
Ng/ml	Nano grams per millilitre
NGS	Next generation sequencing
NS	Normal sperm
OD	Optical density
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PE	Paired end sequencing
PKC	Palm kernel cake
PM	Progressive motility
PTW	Paired testicular weight
PVA	Polyvinyl alcohol
QC	Quality control
QTL	Quantitative trait loci
RefSeq	Reference Sequence Database
SBS	Sequencing by synthesis
SC	Scrotal circumference

SCC	Sperm chromatin condensation
SD	Standard deviation
SDG	Sustainable development goals
SE	Single end sequencing
SEM	Standard error of mean
SMRT	Single-molecule real-time
SNP	Single nucleotide polymorphism
SRA	Sequence read archive
SV	Semen volume
SV	Structural variation
T	Testosterone
T	Thymine
TA	Tail abnormality of sperm
TB	Tail base
TL	Testicular length
TPU	Taman Pertanian Universiti
tRNA	Transfer RNA
Ts	Transition
Tv	Transversion
TW	Testicular width
U	Uracil
UPM	Universiti Putra Malaysia
USA	United States of America
V/V	Volume per volume
WGS	Whole genome sequencing

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of the Study

The demand for beef as an animal protein source is gradually increasing in Malaysia. Livestock production shows a substantial role in ensuring valued animal protein for the 32.7 million population of Malaysia (Department of Statistics, 2021). Over the previous two decades, Malaysia's beef cattle industry has experienced steady expansion (Abdulla et al., 2016). According to per capita consumption of animal products by commodity in Malaysia, annual beef consumption is projected to be 6.1kg/capita (Department of Veterinary Services (DVS), 2020). Malaysia imported more than 78% beef from different countries. As a result, various importers imported non-certified beef as halal beef that was published in different daily newspapers under the heading "neither beef nor halal" (Basyir, 2020; Solhi, 2020). Consequently, consumers have avoided imported beef and related products considering the lack of trustworthiness and quality of beef. On the contrary, demand for domestic beef has been increasing dramatically to meet the growing demand and reduce the importation of beef. Domestic beef production can only cover roughly 22% of demand using purebred Kedah-Kelantan (KK) and crossbred cattle (DVS, 2020). In terms of self-sufficiency, KK cattle account for more than 63 percent of domestic beef output, while KK crossbred cattle accounting for the remaining. KK cattle (*Bos indicus*) are well-adapted indigenous cattle of beef production under subsistence farming system in Malaysia. Survivability, adaptability, sustainability, fertility, and reproducibility are all significant characteristics of Malaysian KK cattle (Raymond and Abu-Hassan, 2012; Ariff et al., 2015; Mastura et al., 2019). KK cattle are more profitable than other existing cattle breeds because they require low maintenance costs with locally available feedstuffs on grazing lands (Loh, 2004). In addition, farmers get high price of KK cattle due to its tastiness and lean meat despite lower growth performance. However, the Government of Malaysia imported more than seventeen beef cattle breeds from different countries to increase the beef production. Although, the exotic breeds did not contribute significantly in the beef sector with the efforts of more than 55 years because of high mortality and poor reproductive performance (Johari and Jasmi, 2009). Simultaneously, Universiti Putra Malaysia initiated to cross KK cows with imported Brahman and Hereford bulls with the view of getting a higher meat yield than purebred KK cattle for promoting and expanding the beef industry (Ariff et al., 1993). Consequently, crossbreeding programmes have continued to this day to achieve genetic improvement of KK cattle in order to strengthen Malaysia's beef industry through governmental and private sectors initiatives (Ariff et al., 1993; Johari et al., 1994; Raymond and Abu-Hassan, 2012; Jamaludin et al., 2014; Ariff et al., 2015). However, KK crossbred cattle did not show full potential for sustainable and profitable beef cattle production owing to poor reproductive performance (Johari and Jasmi, 2009; Fatima, 2015; Abdulla et al., 2016). Moreover, extensive crossbreeding activities lead to the genetic

dilution of KK purebred cattle. In addition, unselected breeding bulls and disorganized breeding systems have led a decline in the purity of the purebred KK breed. For long-term conservation of KK cattle, it is critical to maintain the purity and genetic quality of KK cattle by preserving them from any threat of germplasm dilution. The phenotype of an individual animal contributes to determining that individual's breed traits. Morphological measurements have a significant role in individual body conformation. Prioritizing the conservation of a breed's genetic diversity and potentiality is essential (Nyamushamba et al., 2017; Chen et al., 2018a). As a result, genomic study on KK cattle using whole genome sequencing (WGS) is necessary. The phenotypic and genotypic traits of the KK cattle, which are important for further improvement, have yet to be described, despite their outstanding reproductive ability and remarkable adaptation capacity.

Breeding bulls are always regarded as far valuable than cows (Okere et al., 2014) because sperm from a single fertile bull may be used on thousands of cows (Devkota et al., 2008). Breeding bulls need to be assessed at young stage to ensure optimum semen quality (Penitente-Filho et al., 2018). Scrotal biometry is important for assessing the potential breeder bulls (Brito, 2014; Barth, 2018). Moreover, libido status along with testosterone level can influence the ejaculatory efficiency and the quality of semen in an ejaculation (Pound et al., 2002; Levis and Reicks, 2005). Semen analysis in the conventional method is frequently used for the assessment of bull breeding soundness. However, there is still no standard predictive inception for the success of conventional semen parameters despite of having a direct relationship of semen quality along with reproductive efficiency of bulls. This is because conventional semen analysis does not evaluate all features of testicular functions and sperm quality (Aoki et al., 2005; Carrell et al., 2007; Dada, 2017; Agarwal et al., 2021). Therefore, conventional semen analysis has been proven to be a poor indicator of reproductive potential of the breeding bulls. In this situation, sperm chromatin condensation and sperm DNA integrity may be the better diagnostic and prognostic biomarkers of sperm reproductive potential to overcome the drawbacks associated with conventional semen analysis (Shamsi et al., 2011).

## **1.2 Problem Statement**

In Malaysia, the KK cattle produce almost twice as much beef (63%) than crossbred cattle. However, because to the current haphazard and widespread crossbreeding operations, purebred KK cattle were having serious issues with germplasm dilution (Johari and Jasmi, 2009; Fatimah, 2015; Abdulla et al., 2016). The phenotypic and genomic characterization effort can effectively prevent against the genetic dilution of indigenous breeds like KK cattle, according to Pohler et al. (2019). Additionally, using a selective breeding bull in association with a selective breeding approach can significantly reduce the risk of germplasm degradation. Because bulls are always much more crucial for effective breeding, reproduction, and genetic improvement than cows. In order to select the ideal breeding bull, purebred younger KK bulls must be compared



with counterparts. To avoid genetic dilution and continue the genetic development of this prospective KK breed, phenotypic and genomic characteristics have not yet been documented. The growth, scrotal testicular biometry, and semen characteristics of KK bulls have not also been studied in order to compare KK with KK x Brangus bulls. Therefore, it is immediately important to take the appropriate measures for thorough, systematic study in order to address the challenges and issues outlined before. The findings of the phenotypic and genomic characterization of KK bulls are suggested to serve as the first basis for reference work, which would be extremely valuable for the further genetic improvement of this breed.

### 1.3 General Objective

To determine the phenotypic and genomic characteristics of Kedah-Kelantan bulls and reproductive indices compared to KK x Brangus bulls.

The Specific Objectives of this Study are:

1. To evaluate phenotypic and morphometric characteristics of KK cattle
2. To determine the genomic variants that exist in KK bulls using whole genome sequencing
3. To compare growth and semen characteristics between KK and KK x Brangus nulls
4. To assess hormonal profile and reproductive indices in KK and KK x Brangus bulls at puberty

Hypothesis of this Study:

#### Objective 1 and 2:

Ho :  $\mu_1 = \mu_2$  ; The morphometric measurements and genomic variants are not closely related to contemporary indigenous breeds

Ha :  $\mu_1 \neq \mu_2$  ; The morphometric measurements and genomic variants are closely related to contemporary indigenous breeds

#### Objective 3:

Ho :  $\mu_1 = \mu_2$  ; There is no relationship of growth and semen characteristics in KK and KK x Brangus bull's semen

Ha :  $\mu_1 \neq \mu_2$  ; There is a relationship of growth and semen characteristics in KK and KK x Brangus bull's semen

**Objective 4:**

Ho :  $\mu_1 = \mu_2$  ; There is no significant difference in hormonal level and reproductive indices in KK bulls and KK x Brangus bulls at puberty

Ha :  $\mu_1 \neq \mu_2$  ; There is a significant difference in hormonal level and reproductive indices in KK bulls and KK x Brangus bulls at puberty



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