UNIVERSITY OF COPENHAGEN

Veterinary MSc Thesis

Age assessment of the bovine fetus

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Submitted August 1st 2017

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Title:	Age assessment of the bovine fetus
Topic description:	Examination of age associated macroscopic morphological features of the bovine fetus for on-site age assessment at times of slaughter or abortion.
Supervisors:	Søren Saxmose Nielsen, Jørgen Steen Agerholm
Submitted on:	August 1st 2017
Number of ECTS points:	30
Number of characters:	117.801

Cover illustration: *Courtesy of Godelind Wolf-Jäckel*

Abstract

Methods for more accurate age assessment regarding the bovine fetus are required to determine gestational stage. This due to recent evidence supporting the theory that fetuses in the 3rd trimester of gestation are potentially capable of experiencing pain, particularly in situations of slaughter. Literature on age assessment lacks information to accurately assess and does not reflect the current genetic composition of dairy cows, being that it is of older date.

To age assess, fetuses (n=349) were collected at the viscera line of Danish Crown Beef, Aalborg and at educational caesarean sections at The University of Copenhagen (n=2) during March and April, 2017. All fetuses were uniformly assessed for the macroscopic morphological external features; presence of eyelids on embryos, gender and testicular descencus, presence of genital tubercle, tongue papillae, tactile hair on muzzle, eyebrow and eyelash, the opening of eyes, pigmentation and hair growth, presence and eruption of deciduous incisors 1-3 and canine teeth. Fetometric data on head width, head length, fetal weight and crown-rump length were recorded. Presence of 6 erupted incisors was assessed as an age determining factor in relation to the 252 days age limit for the slaughtering of pregnant cows. Lateral height of incisor 1 was assessed for the correlation to age.

Benchmarks with 95% confidence intervals for the developmental stages were established. As an example, the 2nd trimester holds visible tactile hair on muzzle and around eyes and complete pigmentation. Fur starts appearing early in the 3rd trimester starting in the ears, and is completed around day 260 of gestation. Incisor 1 is visible through the gingival membrane between day 198 and 233 of gestation and erupts between day 231 and 273 of gestation. Fetometric data revealed strong correlation between gestational age in days with head width (R^2 0.95), head length(R^2 0.96), weight(R^2 0.88) and crown-rump length(R^2 0.96). This study revealed the probability of a fetus being older than 252 gestational days and having ‘at least 6 erupted incisors’ to be 78%. No statistically significant difference was found between the groups having ’at least 6 erupted incisors’ and having ‘1-5 erupted incisors’ with regards to gestational age in days. In addition, a statistically significant correlation between lateral height of right incisor 1 and gestational age in days was found (R^2 0.67).

Keywords: Bovine fetus; Fetal development; Age assessment; External fetal features; Fetometry

Resume

Behovet for mere præcise måder hvorpå man kan aldersbestemme kvægfostre er opstået grundet et øget fokus på fostres potentielle evne til at føle smerte i den sidste tredjedel af drægtigheden, især i forbindelse med slagtning af moderdyret. Litteratur på området er af ældre dato og repræsenterer ikke den nutidige danske malkekos genetiske sammensætning, ydermere mangles information om præcise metoder til aldersbestemmelse.

Til at aldersbestemme, indsamledes fostre (n = 349) på Danish Crown Beef, Aalborg på slagtegangen og ved undervisningskejsersnit (n = 2) på Københavns Universitet i marts og april 2017. Alle fostre undersøgtes ens med henblik på de morfologiske makroskopiske udvendige karakteristika; tilstedeværelse af øjenlåg, køn og tilstedeværelsen af nedstegne testikler, tilstedeværelse af en genital tuberkel, smagsløg, taktil hårvækst på mule, øjenomgivelser og øjenvipper, åbning af øjne, pigmentering, hårvækst i form af pels, tilstedeværelse og gennembrud af temporære incisiver 1-3 samt dens caninus bilateralt. Målinger i form af hovedbredde, hovedlængde, vægt og ’crown-rump’ længde blev målt. Tilstedeværelsen af 6 gennembrudte temporære incisiver blev undersøgt i relation til aldersbestemmelse omkring slagtefristen for drægtige køer, dag 252 i drægtigheden. Lateral højde af incisiv 1 blev vurderet og undersøgt for mulig sammenhæng med alder. Aldersintervaller for udviklingsstadier blev fundet for de morfologiske karakteristika med 95% konfidensintervaller. Anden trimester indeholder blandt andet fremvækst af taktil hårvækst omkring mule og øjne samt færdigudviklet pigmentering. I tredje trimester starter udviklingen af pels, først indeni ørene og færdigudvikles omkring dag 260 i drægtigheden. Incisiv 1 bliver synlig gennem den gingivale slimhinde omkring dag 198 – 233 i drægtigheden og gennembryder slimhinden mellem dag 231 – 273. Ved gennemgang af målinger fandtes stærke korrelationer mellem alder i dage og hovedbredde (R^2 0,95), hovedlængde (R^2 0,96), vægt (R^2 0,88) og crown-rump længde (R^2 0,96).

Studiet viste en sandsynlighed for at være ældre end 252 dage og have 6 gennembrudte incisiver til værende 78%. Ingen statistisk forskel blev fundet mellem grupperne ’mindst 6 gennembrudte’ og ’1-5 gennembrudte’ med hensyn til alder. Der fandtes en middelstærk statistisk korrelation mellem lateral tandhøjde på incisiv 1 og alder i dage (R^2 0,67).

Nøgleord: Bovint foster; Fosterudvikling; Aldersvurdering; Morfologiske karakteristika

Preface

This MSc thesis constitutes 30 ECTS points and is a part of the MSc programme in Veterinary Medicine at the University of Copenhagen. It reflects my interest in bovine obstetrics and in particular my desire to contribute to age assessment of the bovine fetus with a view to a possible re-evaluation of the ethics concerning the slaughter of pregnant dairy cattle in the final stages of gestation.

The intention of this thesis was to look into age assessment of the bovine fetus and seek morphological features presumably correlated to age. This to ease future on-site age determination of bovine fetuses in dairy cattle at slaughter and abortion through simple means of measuring.

I would like to thank my supervisors Søren Saxmose Nielsen, Professor in Disease and Diagnostic Modelling, Section of Animal Welfare and Disease Control and Jørgen Steen Agerholm, Professor in Veterinary Reproduction and Obstetrics for advice and support in an intense process.

Furthermore, a number of people were involved in the ongoing pocess and I would like to express my deepest gratitude to the following:

Danish Crown Beef, Aalborg technicians and veterinarians for cooperation and assistance

Viking Genetics, Karsten Bording and inseminators of Viking Genetics for sparring, protective gear and an on-site office with plenty supply of coffee

Michael Wraae Folting for patience and editing

Kirstin Godsk, accommodation, entertainment, and feeding during sample collection

Godelind Wolf-Jäckel, for professional advice, knowledge, pictures and excellent proofreading

Family and friends for support and patience

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Abbreviation key

CHR:	Det Centrale Husdyrbrugsregister, the Danish central register for domestic farm animals
CKR:	a unique number to identify a each Danish bovine animal; consists of the CHR number followed by a serial number
CRL:	Crown-Rump-Length
HW:	Head width
HL:	Head length
Non-diff:	refers to non-differentiated gender in gender assessment
D Red:	Danish Red, dairy breed
DI1-3:	deciduous incisor teeth
DC:	deciduous canine tooth
IQR:	interquartile range, range between first quartile and third quartile
ANOVA:	analysis of variance

Definitions

Gestational age in days: defined as days from insemination date to recording date

Gestational trimesters: 3 time-periods of approximately 93 days each, given a theoretical gestational length of 280 days

- 1st trimester = 0 - 93 days
- 2nd trimester = 94 – 189 days
- 3rd trimester = 190 – 280 days

252nd day: marks the beginning of the last 10th of gestation and the period in which it is illegal to slaughter pregnant animals in Denmark

CRL: crown-rump-length defined from crown of the forehead to Tuber ischiadicum

Abnormal gestational length: > 350 days of gestation

Introduction

In 2015 the entire Danish cattle population consisted of 1.553.671 individuals, hereof 495.300 were yield recorded dairy cows of four different dairy-related breeds. The largest contributor regarding breed, to Danish dairy production was by far Danish Holstein, representing 70% of the population followed by Danish Jersey representing 13%. Danish Red contributed only with around 6.5% and the remaining were Cross-breeds and other dairy related breeds in one category without further breed specification. The same year, 512.500 cattle were slaughtered. Of these, 176.100 were dairy cows of the formerly mentioned breeds, only surpassed by young bulls/bulls totalling 215.200 animals, making dairy cattle the second largest contributor to Danish beef production in number of animals slaughtered. However, in tonnes of meat produced, dairy cattle contributed in 2015 with 51.200 tonnes and young bulls/bulls with 51.500 tonnes, making them almost equally contributory (1).

For the farmer, there are financial aspects involved in slaughter of dairy cows. The short term financial benefit being the dairy cow still representing value in the form of beef after ended milk production. Other aspects are those of long term negative financial importance being e.g. poor performance levels, age and failure to reproduce (2), or health related issues. Providing the animal is suitable for slaughter, it is of interest to the farmer to receive the revenue associated with slaughter.

A Danish study concluded that approximately 30% of dairy cows at time of slaughter are pregnant, hereof 22% of the study population, were in the 3rd trimester (3). A German study states that approximately 50% of the culled pregnant cows (prevalence of pregnancy at slaughter 5-10%), are in the 2nd and 3rd trimesters (4). An English study stated a pregnancy prevalence of 23.5%, hereof 26.9% being in the 3rd trimester of gestation (5). According to the European Food Safety Authority (EFSA) on average 16% of all dairy cows in Europe, are pregnant at time of slaughter, and 3% in the final 3rd of gestation (6). Even though prevalence varies among the before mentioned studies, slaughter of dairy cows in the last trimester of gestation is observed in all studies. According to EFSA, reasons for slaughtering pregnant animals unknowingly can be lack of pregnancy testing, lack of supervised breeding or poor recording. Knowingly slaughtering pregnant animals can be due to management issues i.e. calmer animals when pregnant, health and welfare considerations i.e. mastitis, or out of financial necessity (6).

Reasons as the afore mentioned, can arise during a lactation period of 12-18 months, in which the cow may also be pregnant. Given that it is legal to transport and slaughter pregnant animals in Denmark until the 252nd day of gestation (7), pregnancy in itself is not reason to refrain from slaughtering the cow. This limit has been set due to the fact that transportation and slaughter are considered to be of significant inconvenience, to inflict pain and unnecessary suffering to the dam, and might induce premature calving (8). The limit is set on arbitrary grounds, but also due to the facts that the fetus is not likely to survive if born during the first 90% of gestation and the dam is not considered physically inconvenienced during this time period (9). In case of emergency slaughter, this rule can be overridden but euthanasia guidelines, to minimize unnecessary suffering for both dam and fetus, are unclear (10; 7). In the proposal setting the foundation for the current statute regarding pregnant animals (7), details on handling of a viable fetus, included the fetus being excised by trained personnel from a stunned dam, and thereafter, the dam being bled out and slaughtered. The entire procedure as well as the fetus' viability was to be assessed by a veterinarian (9). In the final statute, this information was not included, and the question of how viable fetuses in late pregnancy must be handled in situations of slaughter, remains unaddressed (7). When the dam is bled out at slaughter, the fetus is left to die from hypoxia as a result of cessation of blood supply. According to EFSA, 2017 (6) '*it is very likely to extremely likely (i.e. with 90–100% likelihood) that fetuses show measurable responses to hypercapnic hypoxia as induced by maternal circulatory collapse/sticking of the dam.'*

A study on human fetuses (11) considered that fetuses in the later stages of gestation, are potentially capable of feeling pain. Their results showed a consistent possibility of fetal pain perception late in pregnancy, however definitive evidence is still lacking. EFSA (6) has, based on literature review by experts, concluded that the probability of the bovine fetus experiencing pain in the final stages of gestation is 1-33%. This is considered to be the most likely scenario, however the possibility late term fetuses experience pain, is not discarded in EFSA's report.

In Germany, a legislative proposal was set forth in April 2017, suggesting a ban on slaughter of pregnant cows in the 3rd trimester with exception of emergency slaughter and slaughter during disease control programmes (12). These exceptions however, still present an incongruity with regards to animal welfare and according to the German Veterinary Association (DVG), they do not take fetal death by hypoxia into consideration. Euthanasia of the fetus can only be achieved by chemical euthanasia of the dam.

If this piece of legislation is implemented, the relevance of accurate age determination will increase, especially if other European countries choose to follow Germany's example and ban slaughter of cows in the 3rd trimester. It will be necessary to have accurate tools to separate the 2nd and 3rd trimester at an acceptable accuracy when pregnant animals are presented for slaughter on the viscera line.

According to the European Commission, Scientific Committee on Veterinary Measures relating to Public Health (13), the levels of chemical residues of the hormone oestradiol is five times higher in muscle tissue of pregnant cows than in the non-pregnant cows. It is also stated that pregnant animals are slaughtered rarely and that elevated hormone levels in meat for consumption is of very little importance. Furthermore, it is known that oestrogen levels in peripheral blood increase towards the end of gestation, especially after the 250th day of gestation (14). If hormonal residue in meat for consumption becomes accepted as a possible influence with regards to public health, having appropriate age assessment tools will be necessary to correctly classify meat from pregnant cows as according to levels of hormonal residue.

Given that pregnancy and parturition are conditions for subsequent lactation, it is in the dairy farmers interest to maintain a high reproductive rate in the herd, in order to achieve continuous milk production. Gestational length is 281±4.9 days[1] on average for Danish Holstein cows and varies slightly amongst the different breeds with Danish Jersey at 283±4.8 and Danish Red at 281±5.7 (15).

Gestation can be divided into three phases, the ovum stage day 0-13 days, embryonic stage 14-45 days and the fetal period from day 46 to parturition (16). Another classification refers to day 0-42 as the embryonic stage and day 43-260 as the fetal period and considers the fetus viable if born after day 260 (15). Human medicine divides the 9 months gestation into trimesters. Since gestational length in cattle on average corresponds to that of a human, 271±21.3 days (17), the concept of trimesters can be applied for bovine gestation as well and has been applied widely in bovine pregnancy related literature.

In cattle, the approximate age of the fetus can be assessed by transrectal palpation on the living dam, taking fetal size, size of placentomes and circumference of the uterine artery, into account (18). Pregnancy can earliest be detected at around day 35 of gestation by transrectal palpation performed by skilled personnel. At this stage, the amniotic vesicle can be palpated (19). Ultrasonography is another option, that requires skilled personnel and specialized equipment. With assistance of ultrasonography, pregnancy can be detected around 7-14 days earlier than by transrectal palpation, making reproduction more efficient (20).

Ultrasonography has also been applied in fetometry studies where age-assessment was the main objective. A study by Kähn,1989 (21) using 19 heifers with single fetuses, assessed different fetometric parameters to provide better age assessment tools for veterinarians when working with pregnant cattle. Linear regression models were made for various parameters e.g. crown-rump-length(CRL) (R^2=0.99) and size of external braincase (R^2= 0.97). There were however restrictions due to large fetuses being impenetrable to ultrasound waves towards the end of gestation, therefore, observations for CRL were only carried out on the fetuses with CRL up to 12 cm. Thereby the correlation model was limited to the first trimester of gestation. Since the fetal head was easier accessible also later in gestation, it was measured until the final two months of gestation. Fetometry has been, and still is, widely applied in both ultrasonography and in fetal studies in general. The measurements CRL and weight are often applied, but throughout literature, the predictive intervals offer little certainty. An often-cited study by Roberts, 1986 (18), show CRL intervals at the 240th day of gestation range between 65 and 85 cm, and weight intervals between 15-25 kg. Theses intervals are based on summaries from 14 different studies, however, creation of these intervals is not further presented in this study.

[1] varies 281±4.9 meaning calving in average happens between 276.1 and 285.9 days for the population of Danish Holstein cows

Richardson et. al, 1990 (22), provided CRL and weight gain curves alongside equations for a variety of measurements for purebred Jersey calves based on a study of 97 fetuses and 24 new-born Jersey calves conducted from 1974 to 1983. A study by Rexroad et. al, 1974 (23) contains linear regression models for CRL at 34-94 days (R^2 = 0.987) and another for 98-270 days (R^2 = 0.975), based on 229 samples collected from 1950 to 1971 for purebred Holstein-Friesian cows. Both studies are relatively old and lack information on current day cattle, or mixed breed issues.

Riding et.al, 2007 (24) measured head length and width and proved significant correlation to CRL (HL: R^2 = 0.99, HW: R^2 = 0.96), focus being on the 1st trimester of pregnancy for a total of 103 specimens. They state that using ultrasonography to measure fetal HL and HW can substitute CRL measurements in age prediction, because the fetal head is accessible during the majority of gestation. They also state, that due to the high correlation between HL and CRL, HL might be equally valid when predicting gestational age. The animals used in this study were of various beef breeds and Cross-breeds. Current day dairy production uses beef bulls in order to optimise production economy. Dairy cows, who do not contribute to genetic progress in the herd, are commonly inseminated with semen from beef cattle in order to achieve higher prices for the offspring at slaughter. This implies that the fetuses assessed at e.g. an abattoir are often of mixed dairy and beef breeds, making fetometry-based age-assessment less accurate. In Denmark, the most often used beef breeds are Danish Blue, Limousine and Simmental (25).

Assessing age of a fetus outside the cow, becomes relevant at times of slaughter and abortion, when an insemination date is not available or seemingly incorrect. Concerning abortions, it may not be possible to obtain the placenta with intact placentomes, and age assessment must be carried out on the fetus alone.

The insemination date is by far the most reliable source, when assessing age. Absence can however occur in natural breeding programmes where a number of females are served by one bull, and recording accurate dates of service can be challenging.

Age determination at abattoirs is mainly relevant when a fetus is presented, that appears to be in the last 10th of gestation. It is of interest to technicians and veterinarians to have methods that are accurate to avoid injustice to both animal and owner. If a case is filed, the matter in question, is the violation of transportation of animals in the last 10th of gestation. In this case it is the official veterinarian’s responsibility to report both the owner of the animal and those who transported the animal (9). As insemination dates are inaccessible to veterinary personnel at abattoirs, an on-site assessment of a fetus is the only way in which to prove there has been a violation, if a pregnant dairy cow does not show external signs of late pregnancy at arrival to the abattoir. These being udder oedema, enlarged abdominal circumference and palpably softened pelvic ligaments (26).

Veterinarians at abattoirs in Denmark do not have access to insemination dates of cows for slaughter, due to this information being considered private information, cf. ‘Lov om behandling af personoplysninger, LOV nr 429 af 31/05/2000’ (27).

Instead, they rely on tangible methods of age assessment, when a fetus is suspected of having exceeded the legal 252nd day age limit. First and foremost, their tools include the presence of erupted deciduous incisor teeth, stating that 6 out of 8 must have visibly erupted the gingival membrane (28), weight of the calf itself and relative to the dam, crown-rump length, gender, breed, placentome size and weight.
The eruption of at least 6 incisors is a main criterion when assessing a fetus suspected of having entered into the last 10th of gestation and this might suggest that fetuses who have exceeded the age limit, but lack 6 erupted incisors, are overlooked and disregarded when assessing at the viscera line at abattoirs. Therefore, expanding current criteria to statistically reliable methods is relevant.
The basis of this assessment derives from information obtained at The University of Copenhagen, Section of Veterinary Reproduction and Obstetrics 2009, where all fetuses over the age of 254 days had at least 6 erupted deciduous incisors (29). A recent Danish study states that there is a 97% probability that a fetus has passed the 252nd day of gestation when there are 6 incisors erupted incisors present (30).
Several studies have found that depending on biological variation, breed, nutritional status and overall management, at least two-to four deciduous incisor teeth have ruptured the gingival membrane at birth (31; 32; 30).
Determining age of a fetus at times of fetal death is relevant firstly to establish whether it is an abortion or stillbirth, death of an otherwise viable fetus after day 260. Embryonic death defined as being between days 0 and 42 of gestation, abortion from day 43 to the 260th day of gestation. Abortion being the expulsion of a non-viable fetus (15). Abortion can occur for both non-infectious and infectious reasons e.g. when the placenta is incapable of sufficient supply of nutrients to the fetus, and the fetus is expelled. Secondly due to various infectious agents and toxins being more likely to cause abortion in different stages of gestation than others e.g. *Listeria monocytogenes* or *Aspergillus fumigatus* (33) mainly causing abortion in the 3rd trimester, and *Neospora caninum* in the 2nd trimester.

When assessing age at different stages of gestation, other parameters than incisor evaluation are necessary to involve, being that the presence of incisors and their eruption, only provide age-relevant evidence in the later stages of gestation. The first incisors become evident radiographically around the 120th day of gestation (34). When they are in fact visible without radiographical assistance is unclear, but Evans and Sack, 1973 (35), state that there is tooth eruption by the 110th day, but do not further explain or specify what this implies, i.e. visibility of the tooth by inspection or radiographically, nor which teeth are in question, see Figure 1.

In Figure 1, Evans and Sack, 1973 (35), summarized results from 15 different studies conducted from 1909 to 1965. The populations were of varying size and breed, and most attention was payed to the earliest developmental stages. Obtaining all the original material to assess objectives and which breeds were used, was not possible. Furthermore, the genetic structure of current dairy breeds is assumed different to that of the studies applied by Evans and Sack, 1973, due to years of artificial genetic selection, possibly altering stages of fetal development as well. Changes that have occurred in the dairy cow due to genetic selection, amongst others, are the high milk yield as opposed to a natural yield related to feeding an offspring, and a reduction in reproductive performance (36) also related to high milk yield.

External Characteristics of Cow Embryos

Days of Gestation	
18	Primitive streak present; amnion complete
19	Neural folds and first somites forming
23	Neural tube closed; first branchial arch present; allantois crescent-shaped sac; optic and otic vesicles present
24	Three primary brain vesicles visible; second branchial arch present; *forelimb bud* present
25	Embryo C-shaped; third branchial arch present
26	Fourth branchial arch and mammary ridge present; *hind limb bud* forming
30	Olfactory pits forming; optic cup well formed; eyes pigmented; *hand plate* present
34	Facial clefts closed; olfactory pits deep; acoustic meatus present; grooves between forelimb digits
38	Eyelids forming; pinna present as ridge; genital tubercle present; grooves between hind limb digits
40	Pinna partly covers acoustic meatus
45	*Tactile hair follicles* on upper lip and above eyes; tongue visible; pinna covers acoustic meatus; digits separated distally
50	Eyelids begin to cover eyes
56	*Palate fused*
60	*Eyelids fused;* external genitals differentiated; hoofs forming; horn bud appears
76	Tactile hairs appear on face; hair follicles present on body
80	Pinna directed caudally exposing acoustic meatus with its epithelial plug; teats present; hoofs begin to cornify
83	Scrotum present
100	Acoustic meatus open again; hoofs becoming firm and opaque
110	Tooth eruption begins
150	Lower lip and chin covered with fine hair; eyelashes present; tactile hairs on chin; color markings appear; hoofs and dew claws become hard; teats well formed; descent of testes complete
182	Horn bud covered with hair; tail-tip hairs present
196	Eyelids separated
230	Body fully covered with hair
278—290	Birth

Figure 1: Traits in fetal development.

From Evans and Sack, 1973 **(35)** *summarising 15 studies conducted from 1909 to 1965. Based on information from study populations of varying size, breed and degree of detail.*

The one available study used in Evans and Sack’s summary is that of Winters et. al, 1942 (37), stating genetic differences related to breed account for only a small amount of variation, but do not affect the general trends. These variations were in hair, pigmentation, fetal size and fetal weight, without further specification as to what this implies.

Literature addressing macroscopic morphological external features is scarce and of older date, rendering the genetic progression of the dairy breeds unconsidered. In this literature, the level of detail of developmental features decreases as fetal age increases, and focus on fetuses in the final stages of gestation is relatively recent, possibly due to increased focus on animal welfare and welfare of the unborn in situations of slaughter.

Objectives

- To examine the relation between age of the bovine fetus and its developmental stage based on the following **macroscopic morphological external features**: presence of eyelids on embryos, gender and testicular descencus, presence of genital tubercle, tongue papillae, tactile hair on muzzle, eyebrow and eyelash, the opening of eyes, pigmentation and hair growth, presence and eruption of deciduous incisors 1-3 and canine teeth.
- To define a possible relation between gestational age in days and the objective **fetometry** measurements; head length, head width, fetal weight and crown-rump length. This both for the overall population and in accordance to breed of dam.
- To examine the hypothesis that presence of 6 erupted **incisors** mainly occurs within the final 10^{th} of gestation. To determine if lateral height of visible incisors is correlated to gestational age.

Limitations

This project is limited to the assessment of specific external features of bovine fetuses that based on literature and own evaluation, were considered to be correlated to stages of gestational age.
Collection, assessment and recording of fetuses at the abattoir procession line were done singlehandedly, therefore it was not possible to collect all fetuses and determine a prevalence of pregnancies in this study.
Given the extent of material collected, parameters measured and limited time, only descriptive and univariable analysis were carried out.
Regarding tooth height, only the lateral and medial heights of incisor 1 will be considered, though data exists on all present teeth, where both lateral and medial height was measured. This is due to limited time.

Materials and methods

Setting

Fetuses were collected at the cattle abattoir Danish Crown Beef in Aalborg. This abattoir has a weekly capacity of approximately 2400 cattle (38), these being both dairy-related and beef cattle. Fetuses were excised from pregnant uteri succeeding evisceration of the carcass approximately 45 minutes after culling of the dam. This taking place in the processing area of the abattoir. Within two hours of collection, fetuses were measured and registered to avoid deformation of tissue. Animals of interest in this study were dairy cattle, which comprise the largest part of the entire Danish cattle population.

Population and sample size

A total of 425 fetuses were collected and measured. Of these, 423 were collected at Danish Crown Beef, Aalborg and the remaining two were collected at The University of Copenhagen Large Animal Teaching Hospital in Taastrup, Denmark, from educational caesarean sections.

The initial population was comprised of every singleton pregnancy available, thereby no distinction was made between pregnant dairy cows and pregnant beef cattle at time of collection. Only two exclusion criteria were relevant at this point; the presence of multiple fetuses and macroscopically evident pathology e.g. hydroallantois, evidence of maceration or mummification.

Exclusion criteria for the final study population were; no insemination date available, gestational length over 350 days, incorrect recordings, no breed of dam available in the database, multiple pregnancies and obvious outliers i.e. large discrepancies between given age and measured size exceeding what can readily be explained by biological variation.

Study design and observational periods

The study was observational and conducted as a cross sectional type study.

The majority of data was collected over two separate periods, totalling approximately 4 weeks. The periods were March 20th through to 31st and April 18th through to 27th, 2017.

The two specimens excised by caesarean, were collected March 13th and May 2nd 2017 respectively.

Excising fetuses

All uteri were inspected and palpated to determine the presence of a fetus. The pregnant uteri were sectioned with a knife and fetuses excised manually, the umbilical cord was severed at the junction of the epidermis and the allantois. A label containing CKR-number of the dam was placed on the fetus to secure identification. This process was executed within the 60 seconds available during processing. When adequate numbers of fetuses were collected, they were moved from the processing area and measured in a separate room before being disposed of as waste product.

The two fetuses from the caesarean sections were both approximately three weeks prior term and considered viable. After removal from the uterus, the fetuses were immediately euthanised with an intravenous excess dose of phenobarbital, as according to current legislation on laboratory animals LBK nr 474 af 15/05/2014, 'Bekendtgørelse af lov om dyreforsøg' (39). Then they were measured and registered.

Macroscopic examination and recording of parameters

Gender and presence of a genital tubercle

Gender was determined by inspection and categorized as 'non-differentiated, female' or 'male', with or without genital tubercle. Presence of a genital tubercle and no other visible indication of gender gave rise to the classification of 'non-differentiated'. Transitional/partially differentiated specimens were classified as 'male' or 'female with genital tubercle'. Females had mammary swelling present and the positioning of the genital tubercle was differentiated as the clitoris. Presence of a scrotum was interpreted as 'male' gender. The scrotum was palpated and stages of testicular descencus determined, marked 'none, unilateral or full descencus'. 'Full descencus' was defined as two separate testicles palpable in the scrotum.

Tongue, inspection of papillae

Papillae of interest were: the large conical papillae situated on torus linguae, the large fungiform papillae and the smaller filiform papillae, both on the apex of the tongue. To inspect all specimens equally, both lateral oral commissures were cut with scissors to open the jaw and pull the tongue out. The categories chosen to characterise findings were: 'none, large back(conical), large front(fungiform), whole tongue (all papillae visible)'.

Eyelids and eyes

Presence of eyelids were visually inspected on all specimens. When visibly fused eyelids covering the bulbus occuli were present, it was recorded as 'eyelid present, yes'. If bulbus occuli was uncovered, 'eyelid present, no' was applied. All eyes were inspected, then palpated on every fetus. Fetuses eyes were classified as: 'open, partially open and closed'. 'Closed' was defined as eyelids being tightly shut/fused. 'Partially open' was noted as such when the eyes bilaterally were partially open from the medial canthus of the eye and structures of the eye e.g. the lacrimal caruncle, third eyelid, sclera or the iris were visible. Eyes were defined as 'open' when the eyelids were completely separated from the medial canthus to the lateral canthus bilaterally.

Tactile hair

Tactile hair examined visually was muzzle hair, tactile hair surrounding the eye area and eyelashes. For each parameter, there were three possible outcomes: 'none visible', 'hair follicles visible' or 'visible hair'.

Pigmentation

Pigmentation was assessed and recorded with 'yes' or 'no' according to location on the fetal body. Locations chosen were; muzzle, eyelids, lips, ears, legs, neck, tail and back. When all variables were recorded with 'yes', the assumption of 'fully pigmented' was made. Pigmentation was considered a 'no' when no visual evidence of pigmentation was present and the entire fetal skin was pink. Pigmentation was considered a 'yes' when the skin in respective locations showed signs of colour change e.g. lips or muzzle visually coloured. Breeds such as e.g. Danish Holstein with both pigmented and unpigmented areas (piebald) presented a challenge, here occurrence of pigmentation was assessed on the darker areas.

Hair

Only non-tactile hair growth was considered in this set of observations. Body hair was recorded as: 'yes' or 'no' according to chosen locations. The presence of hair, regardless of length, was recorded as 'yes'. There were two categories where location was not relevant, those being: 'no fur' and 'fully'. Table 1 gives an overview of how the various parameters were defined in this study. The percentage of hair dissemination was not considered in this study. Nor was hair length or conformation of hair considered.

Table 1. Definitions and concepts for description of fur

No fur	Thorough inspection of whole body, no hair growth found
Ear, at base	Transition between the external meatus and the auricle itself. To inspect all specimens uniformly, the skin covered tragus was cut longitudinally in order to inspect inside of the ear
Ear, inside	The entire ventral part of the external auditory meatus. Any visual hair gave rise to 'yes'
Eyelid	Non-tactile hair growth on the eyelid
Tail	'yes' when presence of visible hair, from anal fold to the tip of the tail, amount and extent not taken into account
Horn bud	Visible hair growth in horn pits
Coronary band, front	Any amount of hair present on the coronary band, ranging from a single visible hair just above the interdigital cleft to the entire circumference of the coronary band and pastern
Coronary band, hind	Any amount of hair present on the coronary band, ranging from a single visible hair just above the interdigital cleft to the entire circumference of the coronary band and pastern
Carpus	'yes' when the entire pastern is covered in hair and hair has extended dorsally just above the carpal joint
Tarsus	'yes' when the entire pastern is covered in hair and hair has extended on the plantar region of the tarsus to above calcaneus
Back	Present hair on the back region of the fetus
Body	Presence of hair on flanks, thorax and abdomen
Fur, bum	Fur around the region of tuber ischiadicum and distally to the popliteal region, around the anal and perineal areas
Fully	Hair covering the entire body, extent of development not taken into account

APPENDIX 4 contains pictures of some of the features presented in Table 1.

Fetometry

HL and HW

These were measured with digital callipers to a nearest 0.01 mm. HL was measured from philtrum rostrally to highest point in the region of the intercornual protruberance, i.e. nose-crown. HW was measured from the front as the widest distance between the zygomatic arches.

Weight

All specimens were weighed on digital scales and registered in kg. Specimens under 3kg were weighed on a small set of Funktion® scales with an accuracy of 0.001 kg. Specimens above 3kg were placed in a bucket and placed on a OBH® scale with an accuracy of 0.5kg.
Prior to weighing, remaining umbilical cord was severed on all specimens at the epidermal junction of umbilical cord and fetus.

CRL

CRL was measured from the crown of the forehead at the region of os frontale to tuber ischiadicum distally and measured in cm.
The fetus was placed in left lateral recumbency, the neck flexed leaving the forehead perpendicular to the dorsal line of the neck. The right hindleg was placed cranially to the left hind to prevent rotation of the pelvic bone. Specimens with a CRL under 15 cm were measured with digital callipers in cm with one decimal, positioned similarly to larger fetuses. Embryos were measured from the cephalic flexure to base of the tail.

Incisors

Teeth of interest in this study were the deciduous teeth; three incisors and one canine in each hemimandible. The oral cavity of every specimen was inspected and absence or presence of incisors noted. When incisors were present, they were touched with a metal object (e.g. blade of knife) to assess presence of eruption through the gingival membrane. Tooth eruption was considered true when metal and enamel produced a “clang” sound at contact. Bias in the form of digital palpation and manipulation of the gingival membranes, potentially provoking iatrogenic eruption, was sought avoided by using a metal object to verify straight away.

Height of incisors

The lateral and medial height of present tooth eruption i.e. bared enamel, was measured for all relevant incisors. This defined from the most lateral/medial highest part of the eruption to the boundary of the gingival membrane on the labial side of the tooth.
Heights of visible incisors were measured from the most lateral/medial tip of the tooth to the mucogingival transition for every visible tooth, with and without eruption, on the labial side of the tooth.

All measurements were carried out using digital callipers to nearest 0,1mm.

Sample information

All collected samples were looked up in DMS Dyreregistrering/"Kvægdatabasen" (Danish cattle database), based on the dam's CKR number in order to obtain insemination date and the CHR (Det Centrale Husdyrbrugs Register) to obtain information about breed using the given CKR number to identify the individuals. Animals with no insemination date, no available breed, beef cattle, wrong recordings, twins and abnormal gestational length were not involved in further calculations.

Descriptive statistics

Univariate analyses were carried out describing the total number of specimens in each group was determined along with minimum, maximum, first(q) and third quartile(q3) and median gestational age.
Correlations between age and continuous measurements in fetometry were illustrated graphically.
For tooth-related parameters a further division was made; specimens with erupted teeth were listed by age.

Analytical statistics

The Shapiro-Wilks test for normality was carried out for all relevant parameters. 95% confidence intervals describing the probability that a population mean is within the interval were calculated, the matching p-values to describe the statistical significance were calculated, via analysis of variance (ANOVA).
For continuous measurements, linear regression calculations for the whole study population and for individual breeds were performed. The coefficient of determination R^2 was determined for all equations.
The linear model: $y = ax + b$ was applied for continuous measurements.
95% confidence intervals were calculated for the DI1, DI2 and DI3 bilaterally. The probability for the presence of six erupted incisors present in the last 10^{th} of gestation was calculated. Correlation between lateral tooth height and gestational age in days was calculated.
A significance level was set at $p = 0.05$.
The programmes Microsoft Office Excel 2016 and R 3.3.2 were applied for both descriptive and analytical statistics.

Results
Population

The selection process, leading to the final study population is illustrated in Figure 2 in a flowchart displaying inclusion and exclusion criteria.

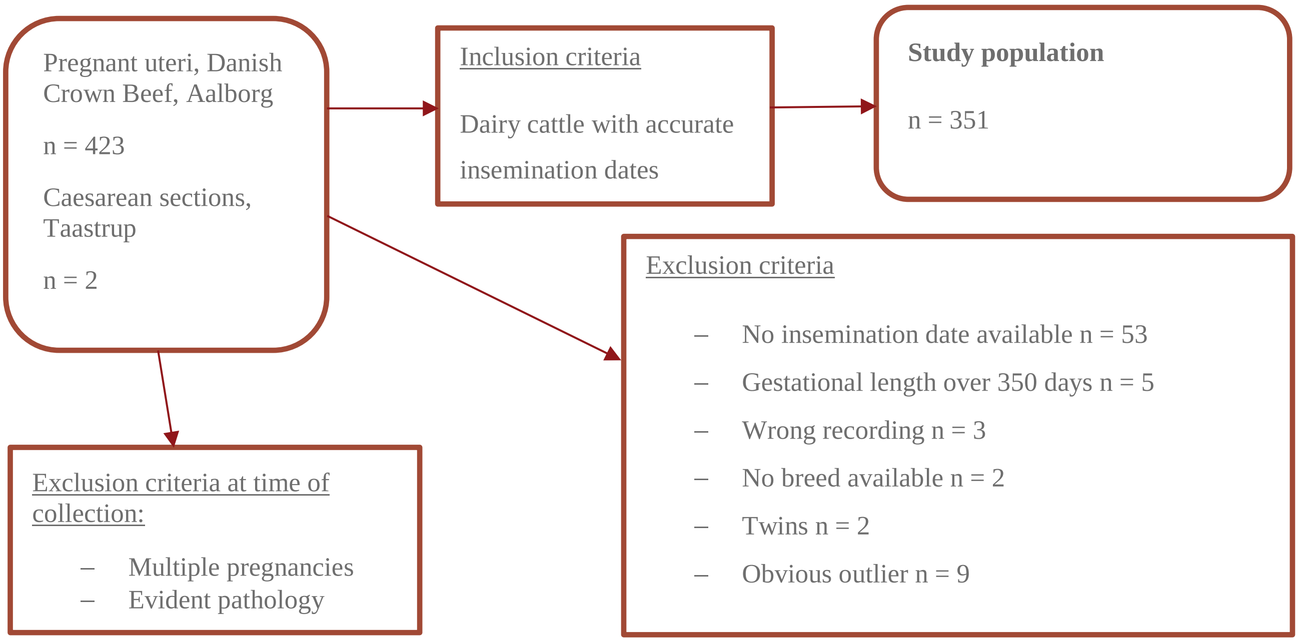

Figure 2. Flow chart of the selection process.
Display of inclusion and exclusion criteria for the final study population.

Among the excluded individuals, 53 dams did not have registered insemination dates. Of these, 42 were dairy cattle, six of these having erupted DI1 bilaterally. The consequence of excluding those is a lower degree of accuracy due to fewer specimens in the calculations regarding teeth.
Animals with an available insemination date, but a gestational age above 350 days were disregarded due to the prolonged gestation considered either pathological or inaccurate and unlikely.
Three specimens were recorded incorrectly at the time of collection and were removed from the final population. For two specimens, the breed of cow was not available in the database and placing them in the correct category was not possible. A set of twins was collected early on were disregarded due to the elimination of multiple pregnancies.
Finally, 9 obvious outliers were removed to minimize unfortunate influence on final results. They can be viewed in Table 2 and are compared to information in Table 3, from charts in the article by C. Richardson et. al, Figure 4, 1990 (22), displaying gestational intervals in relation to CRL and weight according to results achieved in their study for purebreed Jersey. In table 2 the equation for predicted CRL from Rexroad et. al, 1974 (23) for purebreed Holstein-Friesian cows is shown and specimens examined via this. Thus, a comparison to both small breed(Jersey) and large breed(Holstein-Fresian)can be made.

Fetus number 73 has a predicted CRL of 121.6 cm due to the prolonged gestation of 322 days influencing the equation.

Table 2. The obvious outliers.
These were considered to have discrepancies too large to be explained by biological variation. Putting them into the equation for CRL Holstein-Friesian cows **(23)**, *a value for an age-appropriate CRL is calculated and compared to the observed CRL in cm. Intervals for age related to CRL and weight are presented below.*

Fetus nr.	**Gestational age**	**CRL**	**Weight**	**CRL(mm)=-297.1+4.70days**	**Discrepancy**
275	109 days	63.6 cm	11.650	21.5 cm	Too large
191	128 days	65.0 cm	10.250	30.5 cm	Too large
8	132 days	10.4 cm	0.048	32.3 cm	Too small
193	145 days	68.5 cm	14.850	38.4 cm	Too large
16	150 days	15.5 cm	0.068	40.8 cm	Too small
32	234 days	36.7 cm	2.143	80.2 cm	Too small
280	250 days	20.0 cm	1.089	87.8 cm	Too small
411	273 days	49.7 cm	6.350	98.6 cm	Too small
73	322 days	40.7 cm	3.069	(121.6cm)	Too small

Table 3. Intervals of fetal CRL and weight for Jersey.
Reproduced from charts in the article by C. Richardson et. al, Figure 4, 1990 (22)

Gestational age in intervals	**CRL**	**Weight**
100 - 150 days	18 cm – 35 cm	0.5 kg – 3 kg
200 – 250 days	50 cm – 75 cm	5 kg – 23 kg
250 – 350 days	80 cm -	23 kg –

The breed composition of the final study population was 70.3% Danish Holstein, 13.7% Danish Jersey, 6.3% Danish Red and 9.7% Cross-breed.
They were distributed in trimesters as follows: 24% in 1st, 58% in 2nd, 18% in 3rd. Seven individuals were above 253 days of gestation, meaning they exceeded the legal limit of 252 days. The 2 specimens from educational caesarean sections were not subject to this limit, but the remaining 5 from Danish Crown Beef, Aalborg were. In two of the 5 cases, a police report was filed, due to both having 6 or more erupted incisors. Meaning 3 individuals were assessed as being below the limit of 252 days of gestation, due to not having 6 erupted incisors, two of them had 4 erupted and the last one 3.

Descriptive statistics; macroscopic morphological external features

Distributions of variables are displayed in Tables 4 to 9, described by gestational age and stratified by the parameter of interest. To illustrate the overall meaning of the results, **Example 1** will be explained throughout the remaining analysis on macroscopic morphological external features for the chosen categorical parameter 'tactile hair, muzzle; visible hair'. The interpretation of this example can be applied to all parameters described in gestational days.

Example 1: 'tactile hair, muzzle; visible hair'

Number of specimens in this category: **n = 222**

All specimens with visible tactile muzzle hair are grouped in this category, ranging from the first erupted hair to the fully developed fetus, being a dichotomous variable, with the values 'yes' or 'no'.

The **median** describing the central tendency and the middle observation in the study population of specimens with visible tactile muzzle hair: **169 days of gestational age**.

The youngest individual to have visible tactile muzzle hair was the **minimum 97 days** and the oldest the **maximum 291 days of gestational age**.

As comparison, 'hair follicles visible', being the stage prior to 'visible hair', the oldest 25% observed in this category range between 110 and 161 gestational days, and the youngest 25% to have 'visible hair' range between 97 days and 142 gestational days. These two observations illustrate the overlap between 'hair follicles visible' and 'visible hair'.
Correspondingly, there is an overlap present between various categories, these can be identified in Tables 4 to 9.
The first quartile, **q1** describes the youngest 25% of the population, where the oldest of them is **142 days of gestational age.** The third quartile, **q3** describes the youngest 75% of the population where the oldest is **194 days of gestational age**.

The interquartile range(IQR) between **q1 and q3**, describes the age-range of the middle 50% of the study population. In this case ranging from **142 days to 194 days of gestational age**, a total of 52 gestational days, describing how spread the data is within the middle 50%. This is a quantification of the amount by which the individual values vary, describing a large span for the category of 'visible hair'.

The p-value of **$p < 0.001$**, in this case describes how statistically significant this variable outcome is in comparison to the other two outcomes of this variable i.e. 'not visible' and 'hair follicle visible' with regards to effect on gestational age. A value below $p = 0.05$ indicates statistical significance and the hypothesis that there is no difference between the three possible outcomes' effect on age, is discarded.

An example of a high p-value of 0.98 can be seen in Table 4 under ‘Breed’, this describing that breed of cow does not influence gestational age.

Population composition, tongue papillae, tactile hair and opening of eyes

Table 4. Breed, gender, tongue papillae, tactile hair and opening of eyes.

The population composition is displayed in the upper two rows. All variables are in relation to gestational age in days.

	n	Minimum	q1	Median	q3	Maximum	p
Study population	351	25	96	136	177	291	
Breed:							
– Danish Jersey	48	43	93	126	166	271	
– Danish Holstein	247	25	98	134	176	291	
– Danish Red	22	52	73	151	182	252	
– Cross-breed	34	41	92	160	179	268	0.98
Gender All							
– Non-diff	31	37	44	47	53	61	
– Female	145	46	106	139	178	274	
– Male	175	25	114	150	182	291	<0.001
Papillae, tongue							
– None	51	25	45	53	61	124	
– Furthest back	34	28	77	80	88	124	
– Large front	48	86	99	106	117	161	
– Whole tongue	218	97	145	169	194	291	<0.001
Tactile, Muzzle							
– Not visible	46	25	45	50	60	82	
– Hair follicle visible	83	28	80	95	110	161	
– Visible hair[2]	222	97	142	169	194	291	<0.001
Tactile, around eye							
– Not visible	62	25	46	56	71	124	
– Hair follicle visible	67	28	88	100	111	161	
– Visible hair	222	97	142	169	194	291	<0.001
Tactile, eyelashes							
– Not visible	175	25	66	95	120	161	
– Hair follicle visible	35	97	126	135	147	166	
– Visible hair	141	138	172	185	211	291	<0.001
Eyes							
– Closed	296	25	86	124	161	207	
– Partially open	9	183	191	194	200	221	
– Open	46	137	211	222	243	291	<0.001

[2] Applied to exemplify and explain the descriptive data presented

Genital tubercle, testicular descencus and presence of eyelids

Table 5. Genital tubercle, testicular descencus, presence of eyelids in relation to gestational age in days

	n	Minimum	q1	median	q3	Maximum	p
Genital Tubercle							
– Yes	62	25	46	56	71	124	
– No	289	28	118	153	184	291	<0.001
Descencus, testicles							
– None	46	25	79	92	104	130	<0.001
– Unilateral	1	121	121	121	121	121	0.31
– Full	124	111	146	168	193	291	<0.001
Eyelids present							
– Yes	325	25	106	146	180	291	
– No	26	37	44	46	49	55	<0.001

Pigmentation, fur and incisors

Table 6. Pigmentation by location in relation to gestational age in days

	n	Minimum	q1	Median	q3	Maximum	p
Muzzle							
– No	70	25	47	60	73	70	
– Yes	281	79	121	155	185	281	<0.001
Lips							
– No	87	25	50	63	82	161	
– Yes	264	79	123	160	188	291	<0.001
Eyelids							
– No	93	25	50	69	83	127	
– Yes	258	87	127	161	188	291	<0.001
Ears							
– No	98	25	51	71	86	161	
– Yes	253	85	128	161	188	291	<0.001
Neck							
– No	140	25	60	83	107	164	
– Yes	211	97	146	172	196	291	<0.001
Legs*							
– No	143	25	60	84	109	162	
– Yes	208	97	147	172	196	291	<0.001
Tail							
– No	148	25	60	86	111	164	
– Yes	203	97	147	173	196	291	<0.001
Back							
– No	150	25	60	86	111	164	
– Yes	201	97	147	173	197	291	<0.001
Fully							
– No	153	25	61	87	113	164	
– Yes	198	97	147	173	197	291	<0.001

*****Leg pigmentation: a large number of animals have white/no pigmentation on the extremities, and when fur is not yet present, the skin will look unpigmented, though the rest of the animal is fully pigmented. However, fully pigmented animals are assumed to also have pigmentation on extremities in this study.

Table 7. Hair growth/fur by location in relation to gestational age in day.

	n	Minimum	q1	Median	q3	Maximum	p
Fur							
– Naked	216	25	74	106	128	219	
– Fur	135	150	173	185	212	291	<0.001
Ear, at base							
– No	214	25	74	106	128	171	
– Yes	137	151	173	187	212	291	<0.001
Tail							
– No	236	25	78	113	137	176	
– Yes	115	162	178	194	216	291	<0.001
Horn bud							
– No	234	25	78	113	136	193	
– Yes	117	150	177	193	215	291	<0.001
Eyelid							
– No	260	25	80	118	147	194	
– Yes	91	151	185	201	222	291	<0.001
Coronary band, F							
– No	264	25	81	118	149	183	
– Yes	87	177	188	202	222	291	<0.001
Coronary band, H							
– No	267	25	81	118	150	183	
– Yes	84	177	189	205	222	291	<0.001
Ear, inside							
– No	288	25	85	122	159	196	
– Yes	63	173	199	214	232	291	<0.001
Carpus							
– No	289	25	85	122	159	221	
– Yes	62	184	198	214	233	291	<0.001
Tarsus							
– No	308	25	88	126	162	221	
– Yes	43	194	214	222	244	291	<0.001
Back							
– No	318	25	88	128	167	217	
– Yes	33	211	221	228	252	291	<0.001
Body							
– No	315	25	88	127	166	215	
– Yes	36	194	220	226	250	291	<0.001
Fur bum							
– Yes	21	221	223	239	259	274	
– No, naked	330	25	91	130	173	291	<0.001
Fully							
– No	328	25	90	130	172	291	
– Yes	23	196	222	237	256	274	<0.001

Table 8. Teeth, deciduous incisors 1-3 and C, Right hemimandible in relation to gestational age in days

	n	Minimum	q1	Median	q3	Max	p
D-Incisor 1:							
– Not present	310	25	88	127	164	214	<0.001
– Present, not erupted	24	194	211	216	222	235	0.0091
– Present, erupted	17	223	239	252	264	291	<0.001
D-Incisor 2:							
– Not present	333*	25	92	131	173	291	<0.001
– Present, not erupted	9	219	223	237	244	262	0.32
– Present, erupted	9	237	252	259	268	274	<0.001
D-Incisor 3:							
– Not present	330**	25	91	130	173	228	<0.001
– Present, not erupted	15	217	225	237	252	291	0.4
– Present, erupted	6	249	252	260	270	274	<0.001
D-C							
– Not present	347***	25	94	135	176	291	<0.001
– Present, not erupted	3	252	260	268	271	274	0.92
– Present, erupted	1	271	271	271	271	271	0.014

*(23 with present I1, 3 with present I3)
**(19 with present I1, no I2)
***(36 with present I1, 14 with present I2, 17 with present I3)

Table 9. Teeth, deciduous incisors 1-3 and C, Left hemimandible in relation to gestational age in days

	n	Minimum	q1	Median	q3	Max	p
D-Incisor 1:							
– Not present	310	25	88	127	164	214	<0.001
– Present, not erupted	23	194	211	215	222	235	0.01
– Present, erupted	18	223	238	250	264	291	<0.001
D-Incisor 2:							
– Not present	333	25	92	131	173	291	<0.001
– Present, not erupted	11	219	225	237	244	262	0.32
– Present, erupted	7	249	252	264	270	274	<0.001
D-Incisor 3:							
– Not present	330	25	91	130	173	228	<0.001
– Present, not erupted	13	217	223	237	244	291	0.39
– Present, erupted	8	244	251	256	269	269	<0.001
D-C							
– Not present	347	25	95	135	176	291	0.014
– Present, not erupted	3	252	260	268	271	274	0.920
– Present, erupted	1	271	271	271	271	271	<0.001

Analytical statistics, macroscopic morphological external features

Alterations for further analyses

The variable ‘Gender’ was changed to combine ‘male’ and ‘female’ into ‘Differentiated’ while no statistical difference between the two groups was observed in a t-test.
‘Partially open’ and ‘open’ in the variable ‘Eyes’ were also merged, leaving the parameter with the values of ‘open’ and ‘closed’. This because ‘partially open’ only included 9 observations.
A single observation was made with unilateral testicular descencus, and therefore this observation was not included in further analytical calculations.
Each incisor was analysed independently with regards to presence and eruption status.

Macroscopic morphological external features

Using the Shapiro-Wilks test, it was determined that all parameters applied were normally distributed and for all relevant categorical variables, 95% confidence intervals are displayed in Tables 9 to 14, all stratified by age.
Again, **Example 1**, is used to illustrate ‘tactile hair muzzle, visible hair’:

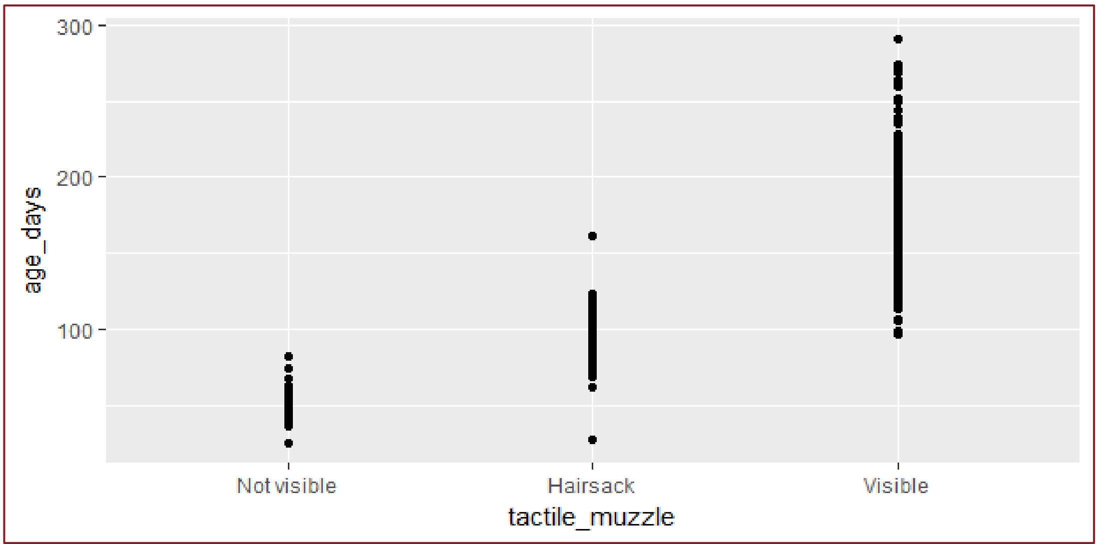

Figure 3. The three possible outcomes for 'tactile muzzle' in relation to gestational age in days
Illustration of the three possible outcomes; mean of ‘not visible’ 52.04, ‘hair follicle’ 95.08, ‘visible hair’ 171.13 gestational age in days, the latter on the y axis.

Example 1, continued: ‘tactile hair, muzzle’ from Table 10, all 3 possible outcomes included

The hypothesis tested is as follows:

Hypothesis: H_0: $\mu_{\text{not visible}} = \mu_{\text{hair follicle visible}} = \mu_{\text{visible hair}}$

```
lm(formula = age_days ~ 0 + tactile_muzzle, data = d)

Coefficients:
                          Estimate Std. Error t value Pr(>|t|)
tactile_muzzleNot visible   52.043      4.743   10.97   <2e-16
tactile_muzzleHairsack      95.084      3.531   26.93   <2e-16
tactile_muzzleVisible      171.131      2.159   79.26   <2e-16

Fit      lwrCI    uprCI
52.04    42.71    61.37
95.08    88.14    102.03
171.13   166.88   175.38
```

Figure 4. R-computing of simple linear regression.
The p-values indicate that the estimate is statistically significantly different from 0. Lower box: 95% CI for the three possible outcomes in the category 'tactile hair, muzzle'.

Number of specimens 'not visible': **n = 46**, at a **mean/estimate of 52.04 gestational days**
With a **standard error of the estimate, $SE_{\tilde{p}}$ = 4.74**, calculated:

$$SE_{\tilde{p}} = \sqrt{\frac{\tilde{p}\,(1-\tilde{p})}{n+4}}$$

95% confidence interval for a population proportion calculated:

$$\tilde{p} \pm 1.96\ SE_{\tilde{p}}$$

For 'not visible' this produces the **95% CI**: 52.04 ± (1.96* 4.74) = **42.71 – 61.37** gestational days, the IQR (middle 50% of the study population) ranging from 45 days to 60 days read.

Similarly, for the 2 remaining outcomes:
For 'hair follicle': **95% CI**: 95.08 ± (1.96***5.91**) = **88.14 – 102.03** gestational days with IQR ranging 80-110 days.
For 'visible hair': **95% CI**: 171.13 ± (1.96***5.21**) = **166.88 – 175.38** gestational days with IQR ranging 142-194 days.
All IQR for tactile hair are presented as the interval between q1 and q3 in Table 4.

The p-value being **$p < 0.001$** gives rise to H_0 being rejected, meaning that 'tactile hair, muzzle' has a statistically significant effect on the dependant variable 'gestational age in days'.
To determine if breed of dam was statistically significant for the development of tactile hair, an ANOVA was carried out, and p-values evaluated.
Only Danish Holstein (p = 0.036) was correlated to age. This can potentially be due to sample composition i.e. Danish Holstein contributing to around 70% of the study population. Further analysis of this is not carried out in this study.

Further notes on ‘tactile muzzle’:

The group ‘visible hair’ contains all individuals ranging from the very earliest evidence of visible tactile hair on the muzzle, to the completely developed fetus. Therefore, being dichotomous, this confidence interval represents the whole category of ‘visible hair’ and is not an indicator of when tactile muzzle hair becomes visible.

To exemplify: if one samples a group of fetuses as according to directions in this study, and finds visible tactile muzzle hair, one can be 95% sure that the mean of the population with ‘visible tactile hair’, will be between 167 and 175 gestational days old. This parameter cannot stand alone, and it is of importance to use multiple parameters to assess fetal age.

Table 10. Estimate, SE, p, 95% CI for eyelids, gender, tactile hair, tongue papillae, genital tubercle, testicular descencus and opening of eyes, stratified by earliest first

	n	Estimate	Std. Error	p-value	95% CI
Eyelids present					
– No	26	46.12	9.75	<0.001	26.95 – 65.28
– Yes	325	144.9	10.13	<0.001	139.43 – 150.3
Gender					
– Non-differentiated	31	48.55	8.74	<0.001	31.35 – 65.75
– Differentiated	321	146.16	9.16	<0.001	140.81 – 151.52
Tactile muzzle[3]					
– Not visible	46	52.04	4.74	<0.001	42.71 – 61.37
– Hair follicle visible	83	95.08	5.91	<0.001	88.14 – 102.03
– Visible hair	222	171.13	5.21	<0.001	166.88 – 175.38
Papillae, tongue					
– None	51	54.98	4.43	<0.001	46.27 – 63.69
– Furthest back	34	83.09	7.00	<0.001	72.43 – 93.75
– Large front	48	107.05	6.36	<0.001	98.59 – 116.54
– Whole tongue	218	171.95	4.92	<0.001	167.74 – 176.16
Tactile around eye					
– Not visible	62	58.92	4.10	<0.001	50.85 – 66.99
– Hair follicle visible	67	99.00	5.69	<0.001	91.24 – 106.76
– Visible hair	222	171.13	4.64	<0.001	166.87 – 175.40
Genital Tubercle					
– Yes	62	59.73	6.0	<0.001	49.03 – 70.43
– No	289	154.20	2.52	<0.001	149.2 – 159.19
Descencus, testicles					
– None	46	90.30	4.93	<0.001	80.56 – 100.0
– Full	124	171.20	5.78	<0.001	165.3 – 177.2
Tactile eyelash					
– Not visible	175	93.51	2.33	<0.001	88.90 – 98.10
– Hair follicle visible	35	136.17	5.71	<0.001	125.95 – 146.4
– Visible hair	141	192.52	3.49	<0.001	187.42 – 197.6
Eyes					
– Closed	296	121.97	2.49	<0.001	117.1 – 126.9
– Open	55	221.30	6.28	<0.001	209.9 – 232.7

[3] The data applied in Example 1

Table 11. Estimates, standard error, p-value and 95% CI for pigmentation on the fetal body

		n	Estimate	Std. Error	p-value	95% CI
Muzzle						
–	No	86	61.50	4.918	<0.001	51.83-71.17
–	Yes	339	156.48	5.496	<0.001	145.71-167.26
Lips						
–	No	109	69.34	4.291	<0.001	60.94-77.82
–	Yes	319	160.00	4.948	<0.001	155.16-164.85
Eyelids						
–	No	93	68.94	3.93	<0.001	61.21-76.66
–	Yes	258	162.27	4.58	<0.001	157.63-166.91
Ears						
–	No	98	71.71	3.85	<0.001	64.14-79.3
–	Yes	253	163.04	4.54	<0.001	158.32-167.75
Neck						
–	No	140	84.70	3.02	<0.001	78.76-90-64
–	Yes	211	172.60	3.90	<0.001	167.76-177.44
Legs						
–	No	143	84.89	2.935	<0.001	79.21-90.76
–	Yes	2088	173.67	3.81	<0.001	168.9-178.46
Tail						
–	No	148	86.61	2.9	<0.001	80.92-92.31
–	Yes	203	174.67	3.81	<0.001	169.81-179.5
Back						
–	No	150	87.30	2.89	<0.001	81.67-93.03
–	Yes	201	175.00	3.82	<0.001	170.09-179.91
Fully						
–	No	153	87.92	2.83	<0.001	82.35-93.49
–	Yes	198	175.89	3.77	<0.001	170.99-180.79

Table 12. Estimates, standard error, p-value and 95% CI for fur.

	n	Estimate	Std. error	p-value	95% CI
Fur					
– Naked	216	102.5	3.75	<0.001	97.95-107.1
– Fur	135	193.6	2.94	<0.001	187.8-199.4
Ear, at base					
– No	214	101.4	2.26	<0.001	97-106
– Yes	137	194.0	3.61	<0.001	188-199.6
Tail					
– No	236	106.9	2.26	<0.001	102.5-111.4
– Yes	115	200.3	3.96	<0.001	194.0-206.6
Horn bud					
– No	234	106.9	2.31	<0.001	102.3-111.4
– Yes	117	198.9	4.01	<0.001	192.4-205.3
Eyelids					
– No	260	113.3	2.36	<0.001	108.6-117.9
– Yes	91	207.0	4.63	<0.001	199.2-214.8
Coronary band, F					
– No	264	113.9	2.33	<0.001	109.3-118.4
– Yes	87	209.4	4.68	<0.001	201.4-217.4
Coronary band, H					
– No	267	114.6	2.34	<0.001	110.0-119.2
– Yes	84	210.5	4.77	<0.001	202.3-218.7
Ear, inside					
– No	288	119.8	2.42	<0.001	115.5-124.5
– Yes	63	218.8	5.72	<0.001	208.6-229.0
Carpus					
– No	289	120	2.43	<0.001	115.3-124.8
– Yes	62	219.1	5.78	<0.001	208.8-229.4
Tarsus					
– No	308	124.7	2.52	<0.001	119.7-129.6
– Yes	43	229.7	7.19	<0.001	216.4-242.9
Back					
– No	318	127.2	2.58	<0.001	122.2-132.3
– Yes	33	237.0	8.40	<0.001	221.3-252.7
Body					
– No	315	126.5	2.56	<0.001	121.4-131.5
– Yes	36	234.5	7.99	<0.001	219.6-249.4
Fur bum					
– No	330	130.9	11.1	<0.001	125.5-136.2
– Yes	21	242.6	10.8	<0.001	221.4-263.8
Fully					
– No	328	130.4	2.71	<0.001	125.1-135.7
– Yes	23	239.6	10.58	<0.001	219.6-259.7

Table 13. Estimates, standard error, p-value and 95% CI for the deciduous incisors 1,2 and 3 in the right hemimandible.

	n	Estimate	Std. Error	p-value	95% CI
D incisor 1					
– Not present	310	125.17	2.505	<0.001	120.24-130.1
– Present, not erupted	24	215.88	9.345	<0.001	198.17-233.58
– Present, erupted	17	252.53	10.99	<0.001	231.49-273.57
D incisor 2					
– Not present	333	131.6	2.736	<0.001	126.25-137.01
– Present, not erupted	9	235.2	16.865	<0.001	202.49-267-95
– Present, erupted	9	258.4	16.865	<0.001	225.71-291.18
D incisor 3					
– Not present	330	130.58	2.68	<0.001	125.3-135.9
– Present, not erupted	15	241.3	12.87	<0.001	216.6-266.1
– Present, erupted	6	261.0	20.09	<0.001	221.8-300.2
D-C					
– Not present	347	136.06	2.92	<0.001	130.3-141.8
– Present, not erupted*	3	264.67	31.40	<0.001	202.9-326.4
– Present, erupted*	1	271.00	54.39	<0.001	164.0-378.0

*not illustrated in Figure 5 due to few observations

Table 14. Estimates, standard error, p-value and 95% CI for the deciduous incisors 1,2 and 3 in the left hemimandible.

	n	Estimate	Std. Error	p-value	95% CI
D incisor 1					
– Not present	310	125.2	2.51	<0.001	120.25-130.1
– Present, not erupted	23	215.2	9.54	<0.001	197.3-233.4
– Present, erupted	18	251.2	10.70	<0.001	230.7-271.6
D incisor 2					
– Not present	333	131.6	2.7	<0.001	126.3-137.0
– Present, not erupted	11	237.5	15.3	<0.001	207.9-267.2
– Present, erupted	7	261.4	19.1	<0.001	224.3-298.5
D incisor 3					
– Not present	330	130.6	2.68	<0.001	125.3-135.9
– Present, not erupted	13	239.8	13.79	<0.001	213.2-266.4
– Present, erupted	8	258.6	17.45	<0.001	224.7-292.5
D-C					
– Not present	347	136.06	2.92	<0.001	130.3-141.8
– Present, not erupted*	3	264.67	31.4	<0.001	202.9-326.4
– Present, erupted*	1	271.0	54.39	<0.001	164.0-378.0

*not illustrated in Figure 5 due to few observations

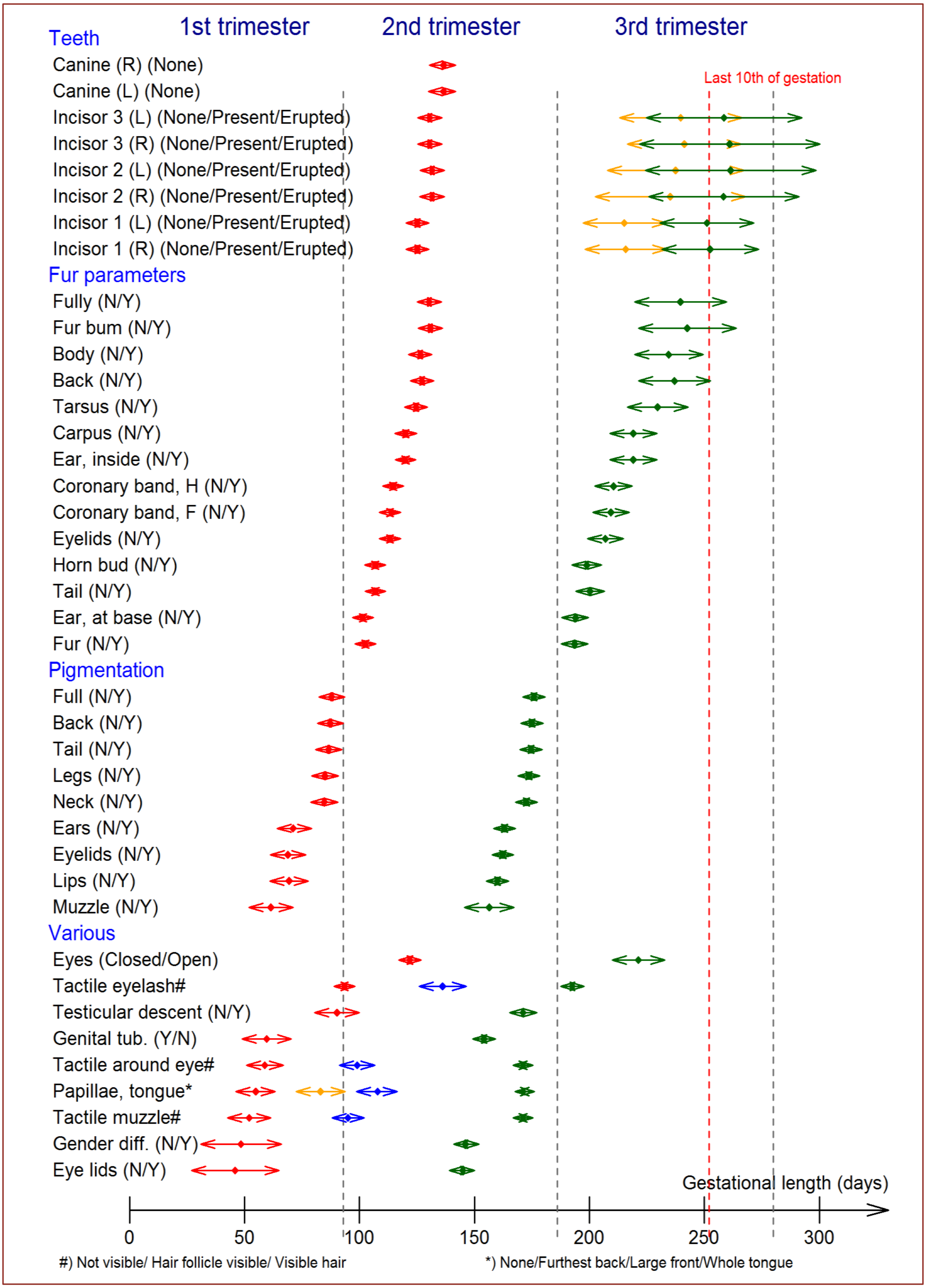
Teeth
1st trimester
2nd trimester
3rd trimester
Canine (R) (None)
Canine (L) (None)
Last 10th of gestation
Incisor 3 (L) (None/Present/Erupted)
Incisor 3 (R) (None/Present/Erupted)
Incisor 2 (L) (None/Present/Erupted)
Incisor 2 (R) (None/Present/Erupted)
Incisor 1 (L) (None/Present/Erupted)
Incisor 1 (R) (None/Present/Erupted)
Fur parameters
Fully (N/Y)
Fur bum (N/Y)
Body (N/Y)
Back (N/Y)
Tarsus (N/Y)
Carpus (N/Y)
Ear, inside (N/Y)
Coronary band, H (N/Y)
Coronary band, F (N/Y)
Eyelids (N/Y)
Horn bud (N/Y)
Tail (N/Y)
Ear, at base (N/Y)
Fur (N/Y)
Pigmentation
Full (N/Y)
Back (N/Y)
Tail (N/Y)
Legs (N/Y)
Neck (N/Y)
Ears (N/Y)
Eyelids (N/Y)
Lips (N/Y)
Muzzle (N/Y)
Various
Eyes (Closed/Open)
Tactile eyelash#
Testicular descent (N/Y)
Genital tub. (Y/N)
Tactile around eye#
Papillae, tongue*
Tactile muzzle#
Gender diff. (N/Y)
Eye lids (N/Y)
Gestational length (days)
0
50
100
150
200
250
300
#) Not visible/ Hair follicle visible/ Visible hair
*) None/Furthest back/Large front/Whole tongue

Figure 5. Summary of all relevant morphological external features depicted as 95% confidence intervals. Trimesters are indicated by grey dotted lines. The red dotted line indicates the 252nd gestational day. Intervals presented in red, represent fetuses who do not have the given feature, ***Example 1*** *'tactile muzzle' is represented with the interval for 'not visible' in red, 'hair follicle visible' in blue, and 'visible hair' in green. Few intervals are presented in yellow, for 'Papillae, tongue' owed to there being 4 outcomes, and* ***yellow*** *intervals where 'Incisors' are involved to better visualise the overlap between 'present' and 'erupted' intervals. For canine teeth, only the outcome 'none' is depicted because too few observations were made in the other two outcomes, see Tables 13 and 14. The varying width of the intervals illustrates the standard error*1.96.*

Summarising macroscopic morphological external features

The following summarises characteristics in the study population describing the youngest 25% to illustrate when a given characteristic is present at the earliest, and when it is present for 75% of the population. The latter to illustrate when a given characteristic is present for a majority of the population.
This is to assist in age assessment and should be viewed alongside the 95% CI visualised in Figure 5.

Characteristics present in the 1st trimester, gestational days 0-93 and 2nd trimester, gestational days 94-189:

- The ***genital tubercle*** is present for 75% of the study population until day 71, gradually differentiating to penis or clitoris. It has disappeared by the end of the 1st trimester
- The youngest 25% are ***gender differentiated*** before day 110. Gender is generally completely differentiated for 75% of the individuals by day 180
- ***Eyelids*** start to develop to cover the eye, and are present in the youngest 25% before day 106, and for 75% by day 180
- ***Eyelids are closed*** for 75% of the population until day 161
- The conical ***tongue papillae*** appear furthest back for the youngest 25% by day 77, and for 75% by day 88, where the large fungiform papillae start to appear and are present for 75% by day 117
- All papillae are present on the tongue for the youngest 25% by day 145 and for 75% by day 194.
- The earliest ***tactile hair follicles*** on the muzzle are present for the youngest 25% by day 80 and for 75% by day 110
- ***Visible tactile hair*** on muzzle and around the eye area appears for the youngest 25% by day 142 and for 75% by 194 days
- ***Eyelashes*** start to appear towards the end of the 2nd trimester, and are present in 25% by day 172 and in 75% by day 211
- ***Testis*** have descended into the scrotum for 75% of the males by day 193

- ***Pigmentation*** appears in the beginning of the 2nd trimester starting on lips, muzzle and eyelids, proceeding to ears. Pigmentation is completed for 75% of the study population by day 197

Characteristics present in the 3rd trimester, gestational days 190-280:

- At the transition from 2nd to 3rd trimester, the first ***fur*** starts appearing at the bottom of the ear for the youngest 25% by day 173 and for 75% by day 213.
- Hair was found to appear in the order: bottom of ear → tail + horn buds → eyelids and coronary bands → ventral part of external meatus of the ear + carpus → tarsus → whole body. The last place to be covered by fur is the rear end of the fetus
- For 75% of the study population, the entire body was ***covered with fur***, indicating completed fur growth by day 256
- ***Eyes are open*** for the youngest 25% by day 203 and for 75% of all individuals in the study population by day 237
- ***Incisor 1*** becomes visible through the gingival membrane without eruption for the youngest 25% by day 211 and later eruption becomes evident for the youngest 25% by day 239
- For 75%, presence of incisor 1 is evident by day 222, and eruption by day 264
- ***Incisor 2 and 3*** appear approximately at the same time without eruption for 75% by day 244 and 252 respectively.
- Eruption appears for 75% by day 268 for incisor 2, and 270 for incisor 3
- The ***canine*** tooth appears and erupts last.
- At the 252nd day of gestation, hair growth is complete and the incisors are present, some are erupted

Descriptive and analytical statistics: fetometry

Table 15 displays the continuous measurements HL(mm), HW(mm), weight(kg) and CRL(cm) in relation to breed of dam. The study population used were the 351 animals with accurate recordings.

The first measurement HL, is used in **Example 2**, to interpret results, and the following three measurements have been analysed accordingly.

Figures 6 to 15 illustrate the best fitted line for the four chosen continuous measurements, illustrated first for the entire study population and secondly with regard to breed. They grey areas surrounding all fitted lines are SE*1.96. The equations for each breed are presented in Tables 16 to 19.

Table 15. Univariate analysis: HL(mm), HW(mm), weight(kg) and CRL(cm)

	n	Mean	Minimum	q1	Median	q3	Maximum	p
HL(mm)								
– Cross-breed	34	110	7	53	123	151	224.3	
– Danish Red	22	110	17	35	119	155	223.1	
– Danish Holstein	247	103	7	58	101	145	239.0	
– Danish Jersey	48	92	8	44	84	122	230.0	p<0.001
HW(mm)								
– Cross-breed	34	65	6	39	74	85	124	
– Danish Red	22	65	10	23	70	90	137	
– Danish Holstein	247	62	4	41	63	83	152	
– Danish Jersey	48	58	7	35	55	77	199.7	p<0.001
Weight(kg)								
– Cross-breed	34	7	0.001	0.253	4	7	50.0	
– Danish Red	22	7	0.005	0.052	3	9	31.0	
– Danish Holstein	247	5	0.001	0.282	2	7	41.6	
– Danish Jersey	48	4	0.001	0.159	1	4	34.5	p<0.001
CRL(cm)								
– Cross-breed	34	41	2	17	45	56	96.3	
– Danish Red	22	41	4	11	40	60	90.5	
– Danish Holstein	247	38	2	20	35	55	101.3	
– Danish Jersey	48	35	2	16	28	42	93.6	p<0.001

Head length

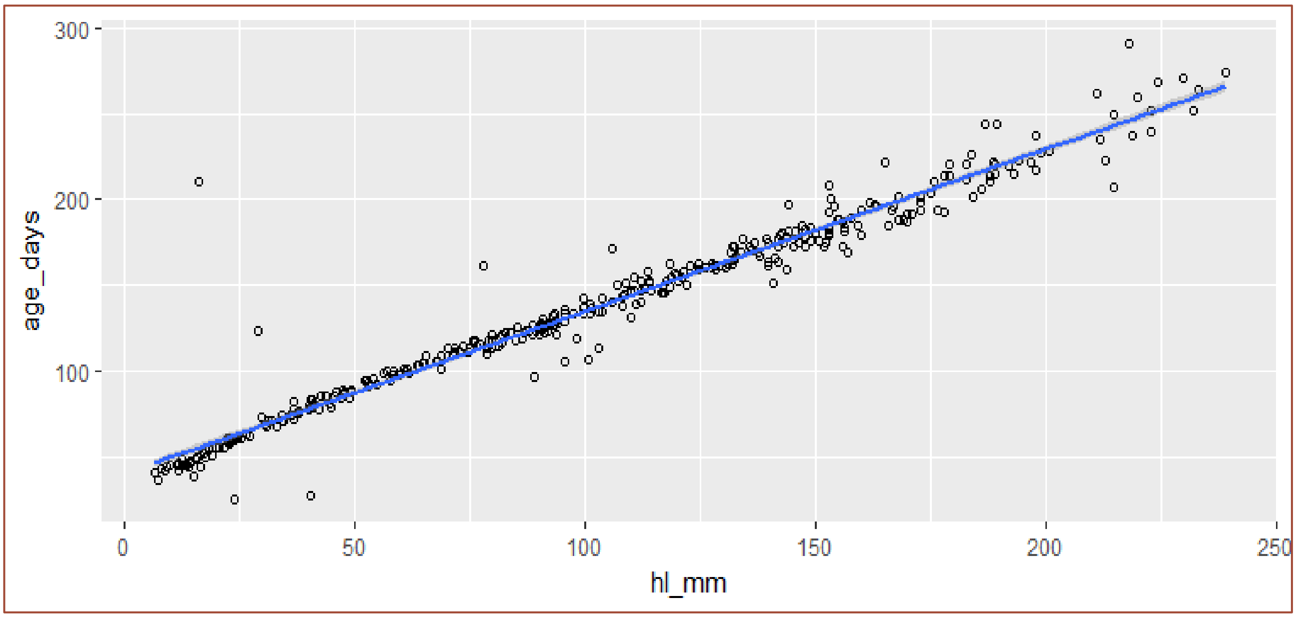

Figure 6. Best fitted line(blue) and outcomes for HL(mm) for all specimens, HL(mm) x age days, linear correlation.

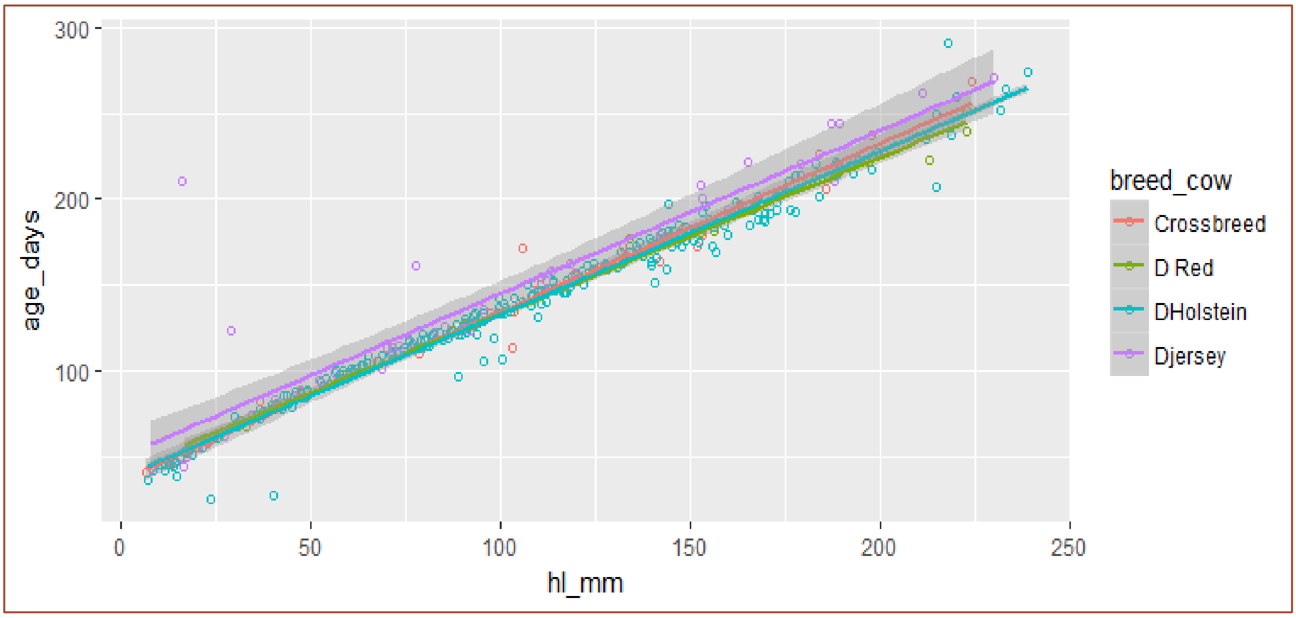

Figure 7. Best fitted lines and outcomes for HL(mm) according to breed of cow, HL x age days.

Example 2, Head length

Figure 6 illustrates the best fitted line of measured HL(mm) and gestational age in days, the equation found by performing ANOVA being as follows:

$$\boldsymbol{Age(days) \ = \ 40.22 \ + 0.95 * HL\ (mm)}$$

with an **R^2 value of 0.95** meaning that the model explains 95% of the variation within the study population. The p-value for the overall equation is **p< 0.001** indicates that the model fits the study population data well. To visualise assumed differences between breeds, Figure 7 was produced.

In Table 16, the equations for HL for the various breeds are shown:

Table 16. HL, breed-related equations with associated p-values

Danish Jersey	$Age(days) = (39.6 + 10.2) + 0.95 * HL(mm)$	$p < 0.001$
Danish Holstein	$Age(days) = (39.6 - 1.68) + 0.95 * HL(mm)$	$p = 0.44$
Danish Red	$Age(days) = (39.6 - 2.19) + 0.95 * HL(mm)$	$p = 0.50$
Cross-breed	$Age(days) = 39.6 + 0.95 * HL(mm)$	$p < 0.001$

p-values > 0.001: Danish Holstein and Danish Red are both of same stature and size and influence the overall model equally little, Danish Holstein being the main contributor with 247 observations, they set the overall standard, and therefore are minimally different from the overall equation.
p-values < 0.001: Danish Jersey: possibly explained by the smaller size of the Jersey fetus in general. Cross-breed: possibly owed to this group being of a heterogenic origin in regards to size and breed composition. They are statistically significant, and are to be considered as separate breeds.

Head width

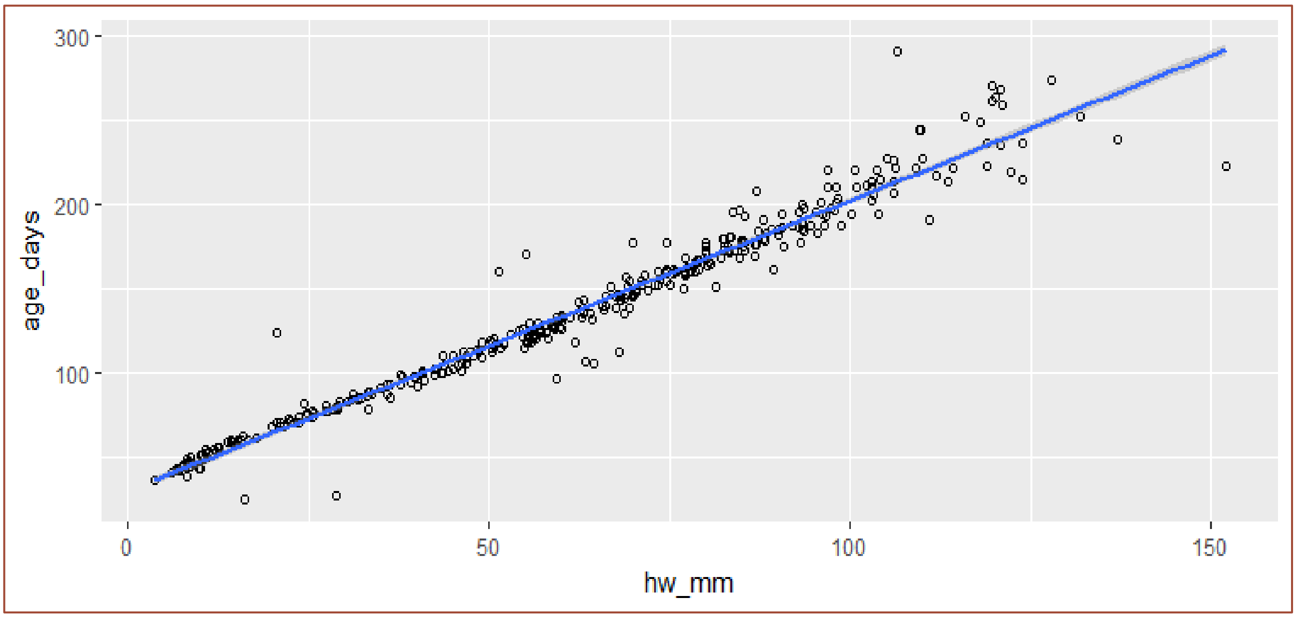

Figure 8. Best fitted line(blue) and outcomes for HW for all specimens, HW x ages days, linear correlation

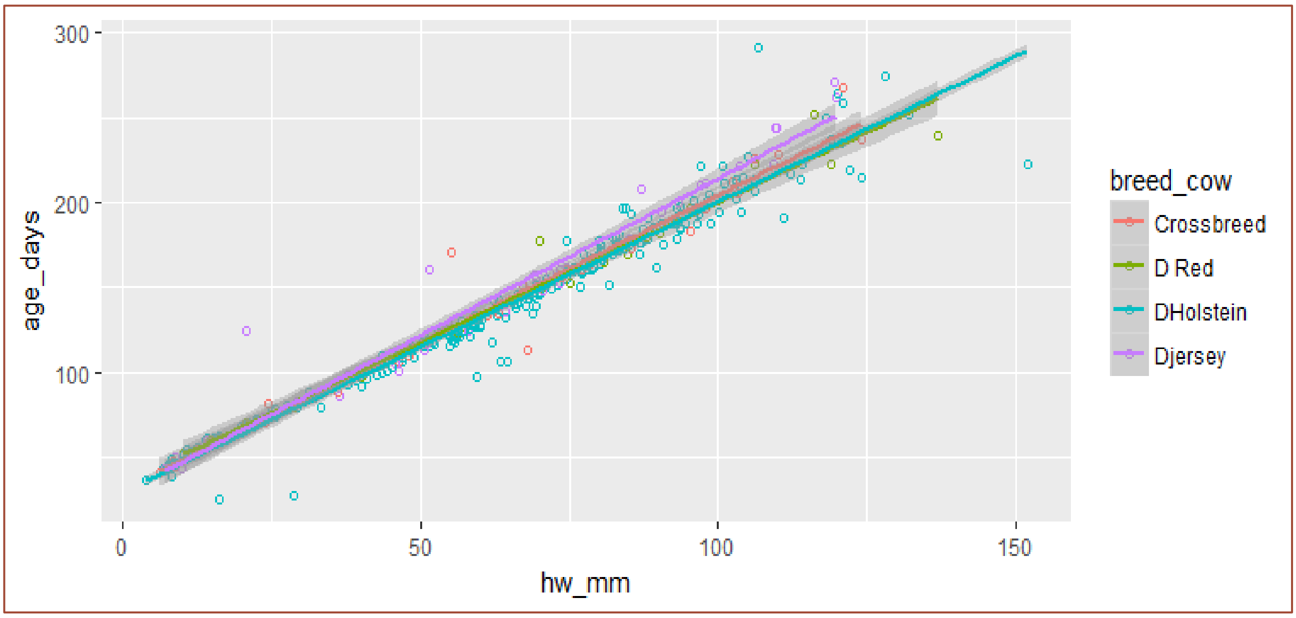

Figure 9. Best fitted lines and outcomes for HW separated by breed of cow, HW(mm) x ages days in accordance to breed of cow.

Equation for HW(mm) for the population:

$$\boldsymbol{Age(days) = 30.21 + 1.72 * HW(mm)}$$

With $R^2 = 0.956$ and $p < 0.001$

Table 17.Equations for HW(mm) for breed of cow.

Danish Jersey	$\mathrm{Age(days)} = (31.5 + 4.86) + 1.72 * \mathrm{HW(mm)}$	p = 0.06
Danish Holstein	$\mathrm{Age(days)} = (31.5 - 2.96) + 1.72 * \mathrm{HW(mm)}$	p = 0.16
Danish Red	$\mathrm{Age(days)} = (31.5 - 1.23) + 1.72 * \mathrm{HW(mm)}$	p = 0.68
Cross-breed	$\mathrm{Age(days)} = 31.5 + 1.72 * \mathrm{HW(mm)}$	p < 0.001

Weight

Figures 10 and 11 show the original data, Figures 12 and 13 show the data after being transformed to a logarithmic scale for a better fit.

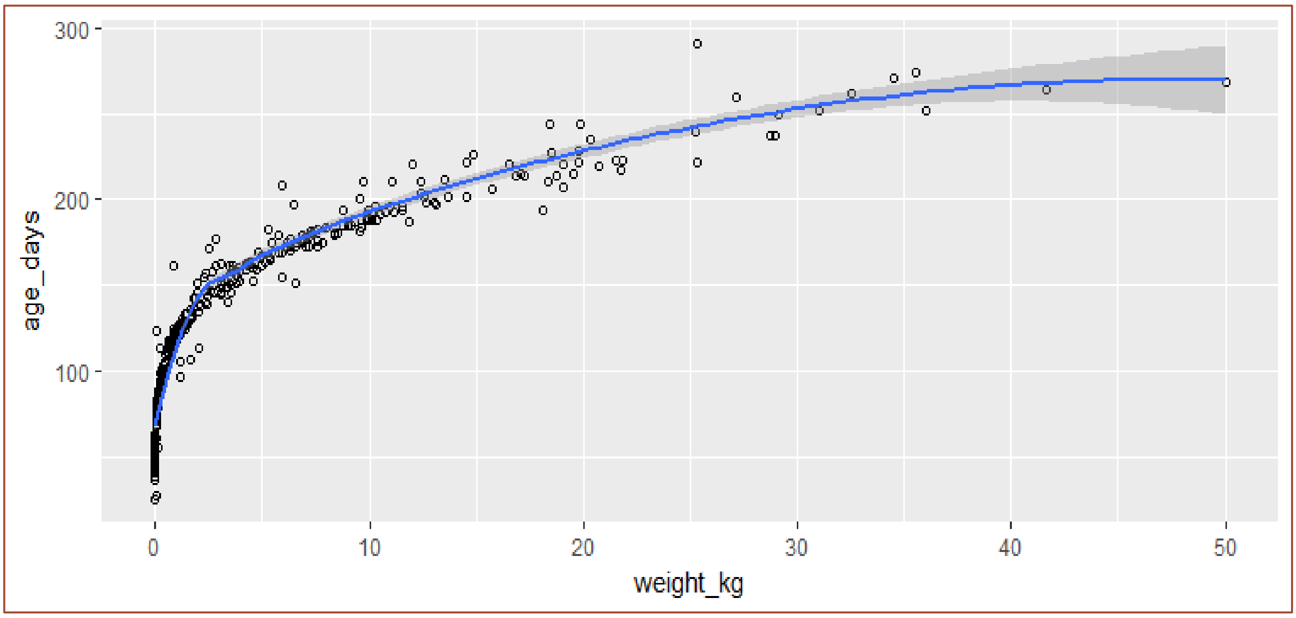

Figure 10. Best fitted line(blue) for Weight(kg) for all specimens, weight kg x age days, R2 0.68, with increasing SE.

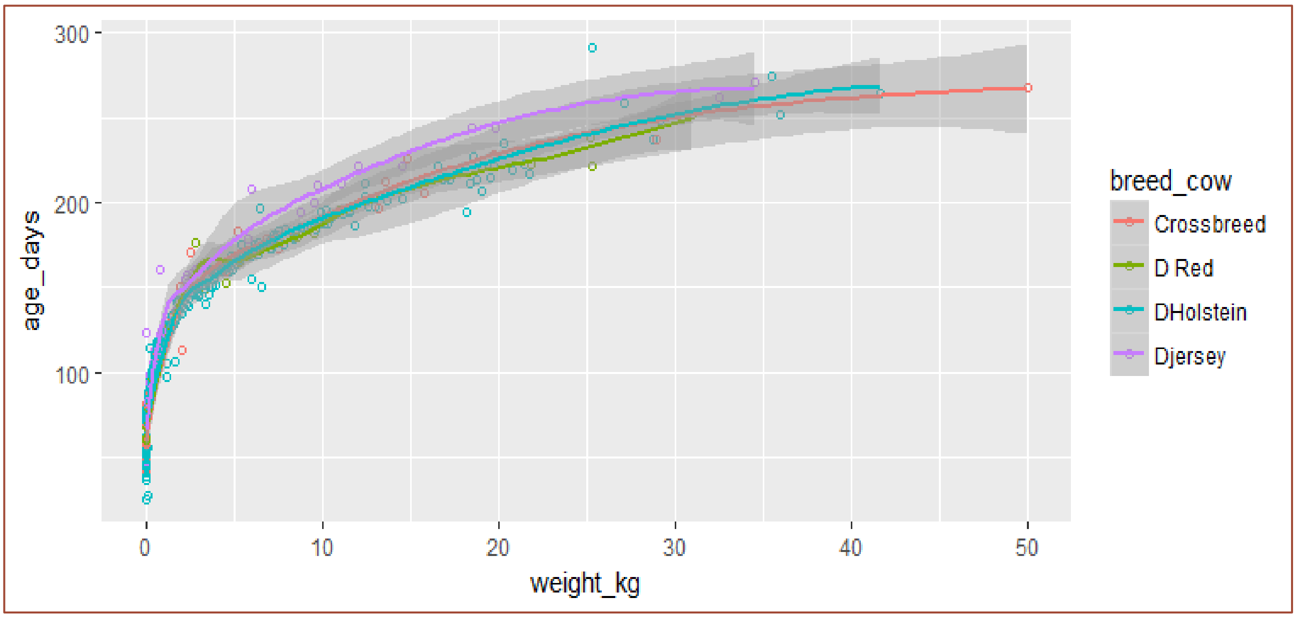

Figure 11. Best fitted lines for Weight(kg) according to breed of cow, weight(kg) x age days.

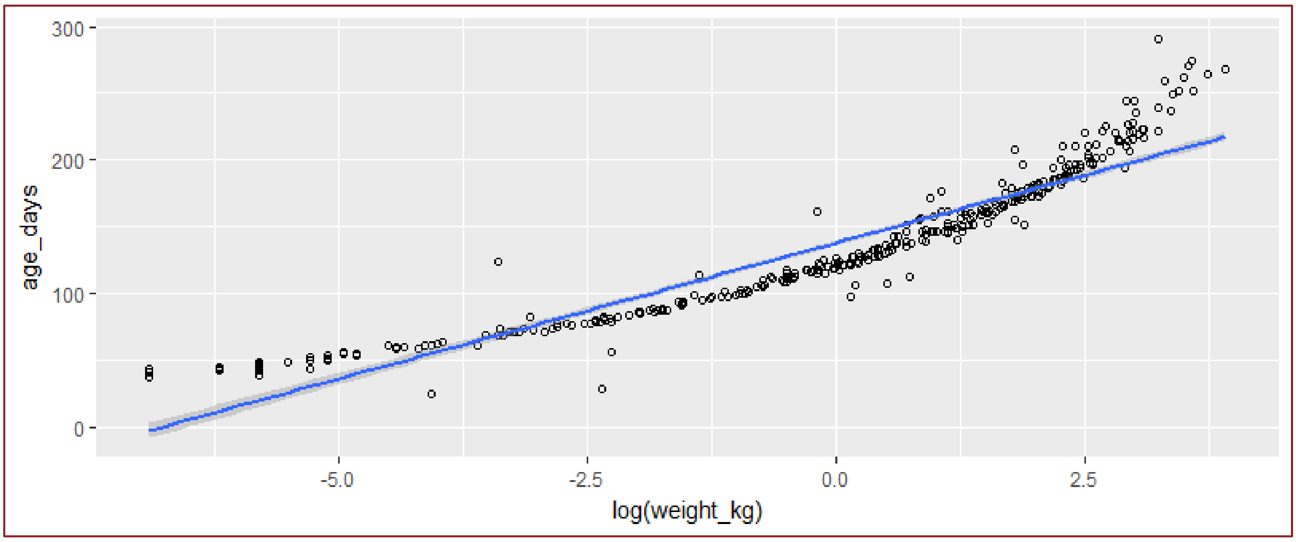

Figure 12. Best fitted line(blue) for Weight(kg), logarithmic scale, age days x log(weight (kg))

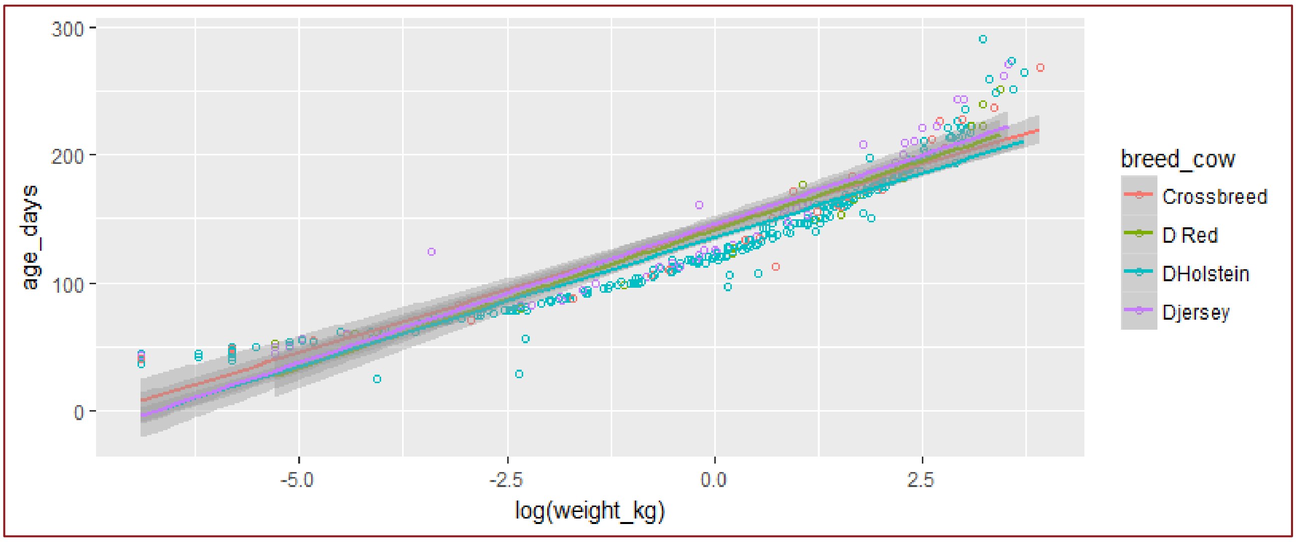

Figure 13. Best fitted lines for Weight(kg) on a logarithmic scale according to breed of cow, age days and log(weight (kg))

Equation for weight(kg) for the population:

$$\boldsymbol{Age\ (days) = 137.94 + 20.35 log(weight\ kg)}$$

With $R^2 = 0.88$ and $p < 0.00$

Table 18. Equations for breeds in relation to weight

Danish Jersey	$Age(days) = (143.4 + 1.89) + 20.41 log * (weight\ (kg))$	p = 0.66
Danish Holstein	$Age(days) = (143.4 - 7.9) + 20.41 log * (weight\ kg)$	p = 0.023
Danish Red	$Age(days) = (143.4 - 1.9) + 20.41 log * (weight\ kg)$	p = 0.711
Cross-breed	$Age(days) = 143.4 + 20.41 log * (weight\ kg)$	p < 0.001

CRL

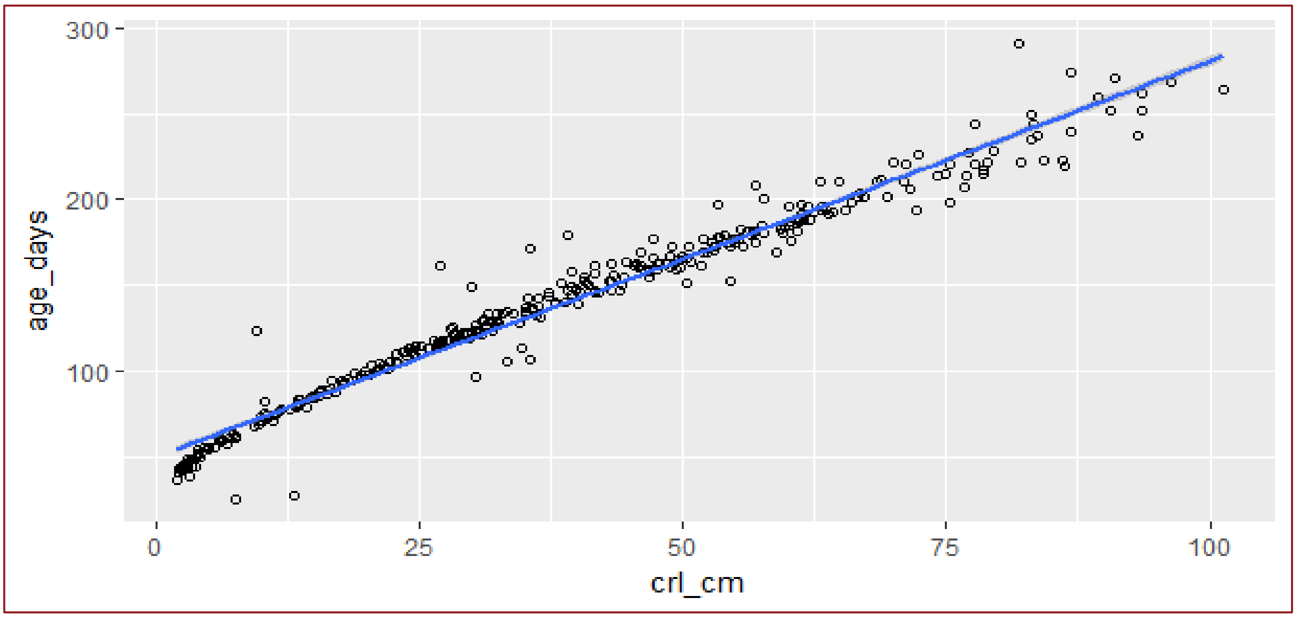

Figure 14. Best fitted line(blue) and outcomes for CRL all specimens, CRL(cm) x age days

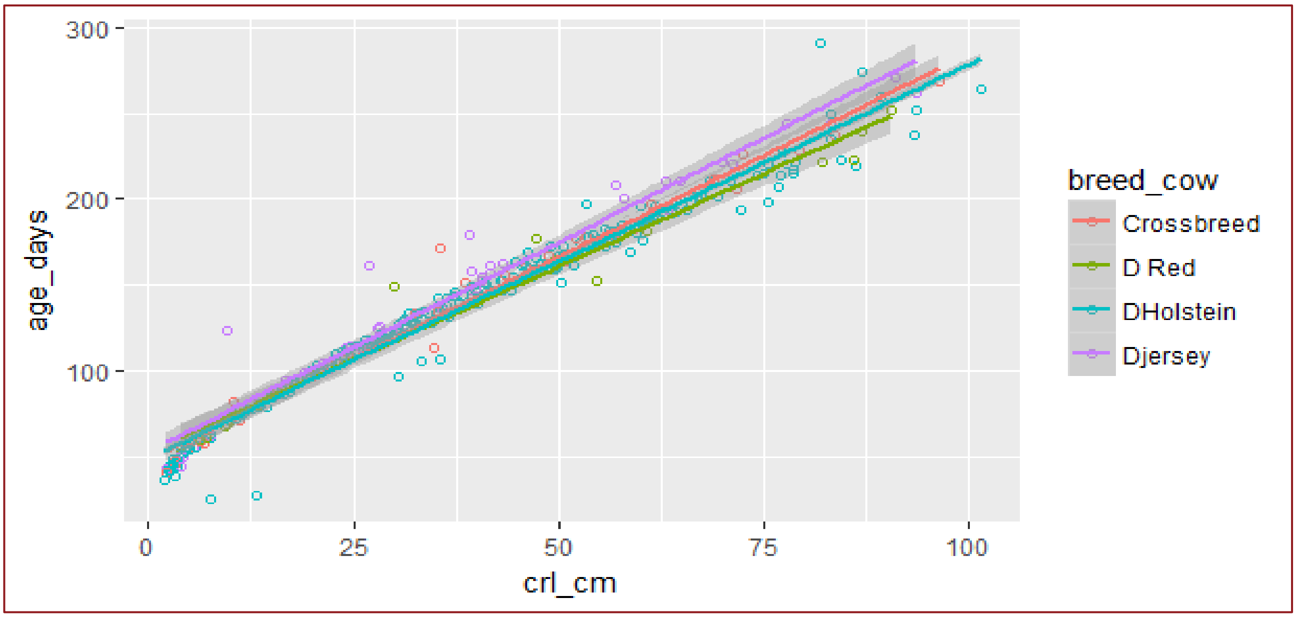

Figure 15. Best fitted lines for all specimens, CRL x age days in accordance to breed. R2 = 0.97

Equation for CRL(cm):

$$\boldsymbol{Age(days) = 50.01 + 2.3 * CRL(cm)}$$

With $R^2 = 0.96$ and $p < 0.001$

Table 19. The equations for specific breeds in relation to CRL(cm).

Danish Jersey	$\mathrm{Age(days)} = (50.4 + 7.01) + 2.31 * \mathrm{CRL(cm)}$	p = 0.0024
Danish Holstein	$\mathrm{Age(days)} = (50.4 - 1.92) + 2.31 * \mathrm{CRL(cm)}$	p = 0.30
Danish Red	$\mathrm{Age(days)} = (50.4 - 3.75) + 2.31 * \mathrm{CRL(cm)}$	p = 0.18
Cross-breed	$\mathrm{Age(days)} = 50.4 + 2.31 * \mathrm{CRL(cm)}$	$p < 0.001$

Summarising fetometry

In Table 20 all four equations for the fetometric data are summarised, describing tendencies for the study population. All models are statistically significant and explain variation well.

Table 20. Summary of linear regression models for fetometric measurements.

HL(mm)	$Age(days) = 40.22 + 0.95 * HL\ (mm)$	R^2 0.95
HW(mm)	$Age(days) = 30.21 + 1.72 * HW(mm)$	R^2 0.956
Weight(kg)	$Age\ (days) = 137.94 + 20.35\ log(weight\ kg)$	R^2 0.88
CRL(cm)	$Age(days) = 50.01 + 2.3 * CRL(cm)$	R^2 0.96

Incisors and the probability that presence of 6 erupted incisors indicating fetuses are in the last 10th of gestation.

The study population was tested by means of ANOVA to establish the effect of breed on incisor development. P-values were all larger than 0.05. The H_0 hypothesis that breed has no influence on eruption status could not be rejected. This meaning that breed of cow does not influence development of incisors (presence and eruption), in this study.

Table 22 in APPENDIX 1, displays all fetuses with present tooth eruption, n = 18. Of these, 6 fetuses had ‘at least 6’ erupted incisors. The remaining 12 specimens had ‘1-5 erupted’ incisors. Two of the fetuses with ‘at least 6 erupted’ incisors were 252 days of gestation and one 249 days of gestation, the remaining 3 were all above 252 days. The group of ‘1-5 erupted’ had 4 individuals that were above the 252nd day age limit.

Probability

To determine the probability that a specimen with ‘at least 6 erupted incisors’ is older than the 252nd gestational day the following is applied:

$pnorm(\frac{x-mean}{SD}, \text{lowertail} = \text{True}),$ where: mean = 261 and SD = 11.17

Pr{6 or more} = 0.7897 ≈ 79% probability that a specimen with ‘at least 6 erupted incisors’ is above 252 gestational days.

Correspondingly the probability that a specimen is older than 252 days of gestation and has ‘1-5 erupted incisors’ is:

Mean = 246.25 and SD = 19.55

Pr{1-5} = 0.384 ≈ 38%

A Welch Two Sample t-test was performed to determine if there is a statistical difference between the group means of ‘1-5 erupted incisors’ and ‘at least 6 erupted incisors’, testing the following hypothesis:

$$H_0: \mu_{1\text{-}5present} = \mu_{6\ present} \quad , \quad H_A: \mu_{1\text{-}5present} \neq \mu_{6\ present}$$

p =0.06 giving rise to a **failure to reject H_0**, meaning there is no statistical significant difference in the means between the two groups; having ‘1-5 erupted’ incisors and having ‘at least 6 erupted incisors’ in the study population of 18 specimens.

Lateral height of visible incisor DI in correlation to age

Lateral measurements of DI1 from right and left hemimandible were t-tested to determine presence of a statistically significant difference in values from the right side as opposed to the left, by means of a Welch Two Sample t-test.

$p = 0.35$ signifying no statistically significant difference in measurements between those of the right and left hemimandibles.

Therefore, in the following only the right hemimandible will be addressed.

Table 23 in APPENDIX 2 shows a total of 41 individuals having presence of DI1 in the right hemimandible. For calculations regarding tooth height, eruption status will not be taken into consideration and all individuals with presence of incisor 1 are presented as 1 group. Only the lateral height of right incisor 1 is taken into consideration.

The model is statistically significant and explains variation medium to strongly. The assumption being that tooth height will increase with increasing gestational age.

The p-values in Table 21, show that breed of cow is only statistically significant in relation to tooth-height for Cross-breeds, these contributing with only 4 specimens. Breed of fetus (beef-dairy-mix or purebred) is not taken into consideration in this study, and the possible influence of this is not accounted for.

Figures 16 and 17 illustrate the linear relation between lateral incisor height for the right DI1, for the entire study population and in breed of cow, respectively. The grey areas around the fitted lines represent SE*1.96. $R^2 = 0.68$ and $p < 0.001$.

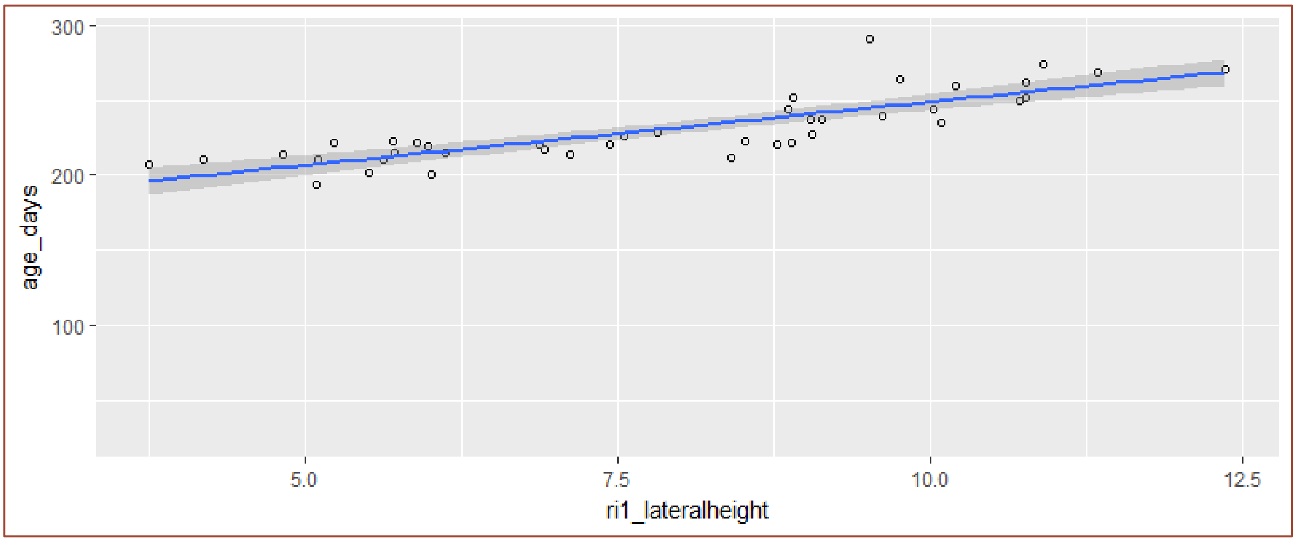

Figure 16. Lateral height of right DI1 in mm

Correlated to gestational age in days, best fitted line. 41 specimens.

The equation for lateral tooth height in mm for the RIGHT DI1:

$$Age(days) = 164.4 + 8.4 * lateral\ height(mm)$$

$R^2 = 0.67$ and $p < 0.001$

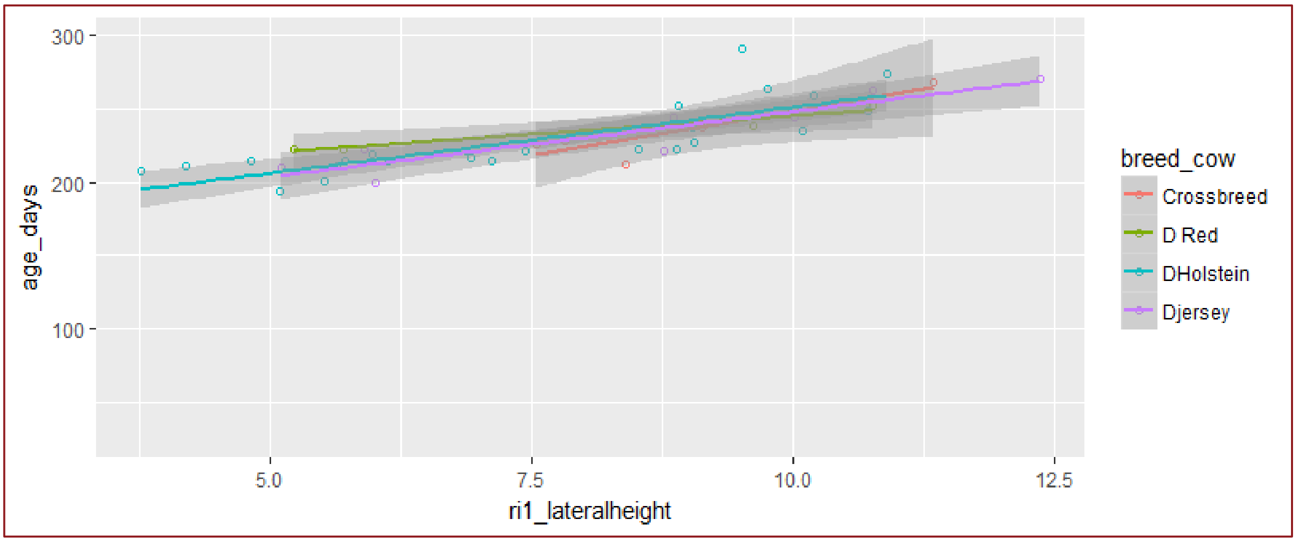

Figure 17. Lateral height of right DI1 in mm, for breed of cow.

Table 21. Lateral height of right incisor 1 in relation to breed differences.

Danish Jersey	$Age(days) = (157.7 + 3.3) + 8.65 * lateral\ height\ (mm)$	p = 0.66
Danish Holsten	$Age(days) = (157.7 + 6.1) + 8.65 * lateral\ height(mm)$	p = 0.34
Danish Red	$Age(days) = (157.7 + 8.3) + 8.65 * \text{lateral height}(mm)$	p = 0.34
Cross-breed	$Age(days) = 157.7 + 8.65 * lateral\ height(mm)$	p < 0.001

When repeating the analyses shown above for **medial height of DI1**, values of $p < 0.001$ and $R^2 = 0.47$, are calculated for the right side. This implying statistical significance of the presence of linear correlation, but the low R^2 value meaning that only 47 % of the variation in the data, is explained by the model produced by ANOVA.

Discussion

Summary of results

In this study, certain morphological features were proven present within the three trimesters, providing benchmarks with 95% confidence intervals for the developmental stage of these. In the 1st trimester, the large conical papillae appear on the torus linguae, in the 2nd trimester visible tactile hair appears on muzzle and around eyes. All papillae on the tongue are present and pigmentation is completed at the end of the 2nd trimester. Fur starts to appear at the beginning of the 3rd trimester starting in the ears, and is completed around the 260th day of gestation. Incisor 1 is visible through the gingival membrane between day 198 and 233 of gestation and erupts between day 231 and 273.

Fetometric data revealed strong correlation of gestational age in days with HW(R^2 0.95), HL(R^2 0.96), weight(R^2 0.88) and CRL(R^2 0.96), all $p < 0.001$ for all aforementioned parameters. Division in accordance to breed of cow gave equations to correct for breed related differences in size.

This study revealed the probability of a fetus being older than 252 gestational days and having 'at least 6 erupted incisors' to be 78%, and having '1-5 erupted incisors' to be 38%. No statistically significant difference was found between the groups having 'at least 6 erupted incisors' and having '1-5 erupted incisors' with regards to gestational age. In addition, a statistically significant correlation between lateral incisor height of the right incisor 1 and gestational age in days was found with R^2 0.67 and $p < 0.001$.

Bias and study limitation

The chosen study population is considered representative, with regard to the entire population of yield recorded dairy cows in Denmark. The composition of the study population corresponds well to that of the Danish dairy population in relation to breed with 70.3% Danish Holstein, 13.7% Danish Jersey, 6.3% Danish Red and 9.7% Cross-breed. In 2015, approximately 24% of the Danish dairy population was situated in North Jutland and Danish Crown Beef, Aalborg slaughtered 35% of all slaughtered cattle in Denmark (1). There might however, be variations throughout the country with regards to e.g. breed, gestational stages and nutritional status. Being that the study was conducted as a cross-sectional study, yearly variations in composition of the slaughtered animal population i.e. amount of beef cattle slaughtered, is not accounted for. In this study, 24% of fetuses were in the 1st trimester, 58% in the 2nd and 18% in the 3rd trimester. The 18% in the 3rd trimester being much greater than the 3% in the 3rd trimester estimated by EFSA, 2017 (6), but corresponding well to the normal distribution found in the recent Danish study by Nielsen and Andersen, 2016 (3), namely 25% in the 1st trimester, 53% in the 2nd and 22% in the 3rd trimester. Even when removing the two specimens collected from educational caesarean sections, the study population from the abattoir consisted of 17% of pregnancies in the 3rd trimester.

In general, parity of the cow was not taken into account meaning that heifers were included, potentially causing overall inaccuracies in population composition. This owed to the fact that heifers are not included as yield recorded animals until first lactation commences. Information on parity is obtainable, and can be implemented in existing data to determine if parity of the dam has an influence on any of the examined parameters in this study.

The greatest challenge of this study, was the vast amount of data to be processed and the need to choose relevant focus points. The implication of this has been that not all parameters have been examined equally thorough e.g. tooth height for all present incisors. Only univariable statistics were possible to carry out due to limitations in time. The main focus of this study was put on macroscopic morphological external features, and all parameters in this group acquired equal attention. Secondly, strictness of exclusion criteria and division of groups can be increased. It will be necessary to further scrutinize the current study population and recalculate to determine the remaining outliers' effect on e.g. correlation, and thereby decide whether to remove them from the overall dataset. In this study, breeds were only taken into consideration in fetometric context, due to breeds having little to no effect on the developing morphological features according to analyses carried out.

The literature available on detailed descriptions of morphological features in relation to gestational age was very limited, making it necessary to examine all collected variables on external features to determine importance in relation to age. Likewise, comparison to the findings of this study were limited to few available earlier studies. In this study, the development of tongue papillae, pigmentation, growth of fur and tactile hair that have not examined in large sample populations earlier, were examined here for the first time. Limitation was present when examining incisors for eruption status, in spite of the relatively large overall sample size, few specimens had erupted teeth, and only 6 had eruption of 'at least 6 incisors', this reducing the reliability of the outcome.

The risk of wrong identification of fetuses, was kept to a minimum by attaching a unique label to the fetus and keeping fetuses sufficiently separate immediately after collection. All recordings and measurements were executed by the same person, and continuity in recording methods was sought by applying the same set of digital callipers, and measuring tools throughout the whole data collection period. Selection bias were sought eliminated by examination of all uteri on the viscera line. All lesion-free singletons were collected regardless of breed or size. Breed of dam was not evident at the viscera line due to carcasses already being skinned at this stage. Nor was it possible to identify heifers. The age of the fetus was not obtained until ended collection of material and selection bias on certain age groups is therefore negligible.

Confounders in this study are potentially the lack of knowledge of nutritional status and relative size of the dam to the fetus. The information on size can be obtained along with the meat classification of the dam, however it was not taken into consideration in this study. All individuals were considered suitable for slaughter, leading to the assumption that a certain health standard was met in order to be approved by the abattoir veterinarians. This implying that unacceptably low nutritional status did not occur. Low nutritional status can possibly be influential regarding fetal development i.e. fetometric measurements and morphological features. Another confounder in this study, might be the breed of fetus i.e. mixed dairy-beef and purebred fetuses. This influencing fetometric measurements owed to beef breeds in general being larger than dairy cattle. For all fetuses, a sire was determined, but not applied in the final examination.

A future possibility could be to place purebred Danish Holstein and Danish Red in one group given their relatively equal size, and dairy-beef mixed fetuses of the same dams into another group. Then testing for significant statistical differences on relevant variables. These might be fetometric data and possibly also incisor development. Correspondingly, the same divisions could be applied when examining Danish Jersey, being relatively smaller in size in general. For Cross-breed, it is not possible to divide, and this group might be examined as one group, regardless of sire used, producing less reliable results due to heterogenicity.

If corrections are made to account for nutritional status and breed of fetus, the internal validity of this study could be increased.

Interpretation and generalisation

Assuming that the results in this study are not preliminary, it is important to understand that when interpreting the data set forth in this study, each variable cannot stand alone and is to be viewed in relation to other variables to age assess most accurately. When applied in age assessing, a single variable can act as a standing point and from there one may have an idea as to whether the given fetus is younger or older, based on a certain morphological trait.

When viewing single variables, it can also be with comparison to benchmarks in other studies in mind. For macroscopic morphological external features, few comparisons to literature were possible due to little information available in the 2nd and 3rd trimester in literature. The best comparison is made between Figure 1 by Evans and Sack, 1973 (35), and the overall results from the analysis of the information obtained in this study. The findings in this study are in agreement with the descriptions given by Evans and Sack, with few discrepancies, but the accuracy of given characteristics linked to gestational age, was only obtained as age-intervals and not specific age.

The largest discrepancy was found with regard to tactile hair where this study revealed a much later appearance of visual tactile hair on muzzle and surrounding eyes. Evans and Sack stating appearance at day 76, this study finding an interval between 167 – 175 days of age in average, the youngest individual to have visible tactile hair being 97 days of age. Also, pigmentation involved discrepancies, although the details of pigmentation in Evans and Sack are unknown and assumed to be what they refer to as ‘colour markings’ appearing on day 150. This study observed the earliest pigmentation to appear at day 80 and the entire pigmentation process seemed complete around day 175. They also state that the fetus’ body is fully covered in hair at day 230, this study found the wide interval of 220-260 days.

In this study, many variables were taken into consideration when wanting to assess age based on morphological macroscopic external features. If the study was to be reproduced, certain variables could be left out, and others should be scrutinised further. Variables to be left out are the ‘presence of genital

tubercle', mainly owed to it being present in early stages of pregnancy that have been examined in literature to a greater extent than features in the later stages. Presence of eyelids may be left out for the same reasons. The descencus of testicles is interesting, but only relevant for around 50% of the population, leaving it irrelevant in the overall picture. Another variable to be questioned is pigmentation. Given that light and piebald animals are unpigmented in various areas of the body it is difficult to create a method of recording that is valid and easy to operationalise.
Variables to be looked further into are mainly the development of fur and possibly tactile hair growth. To increase detail on fur growth, it would be relevant to assess the dissemination of fur more extensively at each relevant gestational stage. The conformation of fur can also be a factor to consider, being that the fur seems to grow in length, also after the whole fetal body is covered in fur. It is however, not readily operationalised due to subjectivity of the observer. Yet this study has provided insight into the gradual dissemination of fur on the fetal body and its development.

Fetometry, being widely applied in ultrasonography with regards to fetal age and age assessment in general, has received attention over time in literature, and comparisons are somewhat accessible, contrary to those of morphological features. In this study, correlations between HL(R^2 0.95), HW (R^2 0.96), weight (R^2 0.88), CRL (R^2 0.96) and gestational age in days were found.
If comparing to the study by Riding et.al, 2007 (24), it is important to note that they focused on the 1st trimester, and did not correlate age with fetometric measures, but the different measurements with each other; the correlation of HL and HW with CRL were found significant in their study; CRL: HL, R^2 0.99 and CRL: HW, R^2 0.96. The correlation between HL and HW being R^2 0.97.
Methods of measuring used in the current study are similar to that of Riding et.al, and to compare, the similar correlations for this study were produced:

- CRL: HL, R^2 0.96 with $p < 0.001$
- CRL: HW, R^2 0.96 with $p < 0.001$
- HW: HL, R^2 0.96 with $p < 0.001$

Similar tendencies in this study are evident compared to that of Riding et.al. 2007 (24), and the slightly lower R^2 might potentially be owed to the fact that the current study includes all 3 trimesters, potentially indicating that variation increases with age, making these correlations gradually less accurate. One must keep in mind that data presented in the current study, is to be further corrected to adjust for bias currently present.
With regards to fetal weight, it is of importance to consider large breed related differences as those between Jersey and Holstein cows. Therefore, to compare to available studies i.e. that of Richardson et.al, 1990 (22), only Jersey will be addressed. Richardson et al. examined purebred Jersey fetuses and calves.

Firstly, data in this study were transformed to logarithmic scale to establish if a linear correlation was acceptably present. Secondly, separation by breed was carried out. To establish if results in this study are consistent with that of Richardson et. al. an example will be used of a fetus weighing 15 kg:

- (Richardson et. al.) Age(days): $15kg^{(0.33)} + 0.817 / 0.0141$ = 231.3 days
- (Krog) Age(days): $(143.4 + 1.89) + 20.41(\log(15kg))$ = 169.3 days

The above shows a large discrepancy. This foremost due to incorrect handling of weight data in this study, and possibly the influence of a different composition of fetal breed. The weight data should have been handled as two separate linear models being that the illustration of the original data in Figure 10 indicates an exponential weight gain until around day 150 and from here a more linear weight gain. This would potentially improve the accuracy of the equations. Furthermore, the composition of the Danish Jersey population in this study, contains both purebred jersey fetuses and dairy-beef mixed fetuses, influencing weight gain. The weight equations in this study are not satisfactory in accurate age assessment. Weight gain may be the most sensitive to breed in comparison the other measurements in this study, and if data is handled correctly, this might become evident. Perhaps weight of the fetus relative to the dam could be more useful than weight alone when correcting for biological variation.
Correspondingly, when assessing CRL equations and comparing to that of Rexroad et. al. 1974 (23), it would have been beneficial for the accuracy of the outcome to also divide CRL into 2 separate equations to correct for the rapid growth occurring until approximately day 50 according to Figure 14. Different handling of data might reveal better approximations.
Comparing equations for CRL to that of Richardson et. al. 1990 (22) similarly to the example for weight gain with a view to Jersey, produces following results when a 60cm specimen is used:

- (Richardson et. al) Age(days): $65 + (2.3 * 60cm)$ = 203 days
- (Krog) Age(days): $(50.4 + 7.01) + 2.31*60cm$ = 196 days

The discrepancy between the two is minimal, and can be owed to the CRL data not being handled in the same way. It might also be a result of the Danish Jerseys encountered in this study carrying both fetuses of pure Jersey breed and mixed beef-dairy fetuses, possibly influencing fetal growth patterns.

This study revealed a probability of being above 252 days of gestation and having ‘at least 6 erupted’ incisors to be 78%. The study population was limited to 6 specimens having ‘at least 6 erupted’ incisors and 13 specimens having ‘1-5 erupted’ incisors. It also revealed that within the study population there is no statistical difference between the two groups, implying that age assessment on behalf of tooth eruption is not sufficiently accurate, due to differentiation between the two groups not being possible.
A probability of 38% for being over 252 days and having less than 6 erupted, i.e. ‘1-5 erupted’ incisors was shown, this not being insignificant, but in opposition to the study by Nielsen and Andersen, 2016 (30), that showed a statistical difference between the similar groupings. As in their study, this study does not provide a

sufficient sample size to accurately assess a potential difference between groups having 6 as opposed to 5 erupted and having 5 as opposed to 4 erupted incisors. It can be argued that the internal validity of this study is better than that of Nielsen and Andersen, based on a reduced amount of bias due to the study frame.
To further support the theory that eruption of ‘at least 6’ incisors is not an accurate tool for age assessment, it was only 2 out 5 fetuses in this study, older than 252 days at the abattoir, that were assessed as so and reported. For the three fetuses that were assessed to not having exceeded the age limit, the ages were 259, 262 and 268 days, having 4, 2 and 6 erupted incisors respectively. The latter bringing about another matter, namely that of defining eruption.
At the abattoir, it was experienced that the definition of ‘eruption of incisor teeth’ was not in agreement with the definition in this study. The definition of 6 erupted teeth at Danish Crown Beef, Aalborg, was ‘visible tooth without gingival membrane to an extent visible on camera i.e. approximately 1 mm for each relevant tooth’. This study’s definition of eruption was the ‘clang’ sound of a metal object touching enamel, this possibly implying that eruption of 6 incisors was considered true at an earlier gestational age than at the abattoir, assuming that incisors will be increasingly exposed as age increases.
This study has brought forth the arguments reducing the validity of current age assessment around the 252nd gestational day, showing that 6 erupted incisors in itself is not a statistically sound method of determining whether or not a fetus is in the last 10th of gestation. Also, awareness on definition of eruption is to be communicated and applied to certain standards that are agreed upon.
It might be possible to combine findings in this study with those of Nielsen and Andersen to reach a better sample size revealing more accuracy. However, it will be important to correct for observational, informational and selection bias.
To further apply teeth as a tool for age assessment, lateral tooth height for the right deciduous incisor was measured to determine if tooth height was correlated to age. The sample consisted of 41 specimens all with present right incisor 1, eruption status not taken into consideration, being that lateral height was measured from the highest point of the tooth to the mucogingival transition. The best fitted line was plotted and R^2 0.67 suggests a medium to strong correlation with $p < 0.001$ signifying the models’ statistical significance. The significance for breed of cow was tested and only Cross-breed had significant influence. Division into breeds was not optimal, due to very few observations for Cross-breeds and Danish Red. The relatively large variation presents challenges, and it would be beneficial to test for a possible difference in breeds. The assumption that tooth height increases with increasing age was met, and with further analysis, this could prove beneficial in age assessing.

Conclusion

Results produced in this study confirm that certain morphological external features are correlated to gestational age and trimesters. Features present in the 2nd trimester are tactile hair follicles followed by visible tactile hair surrounding eyes and on the muzzle and complete pigmentation towards the end. In the beginning of the 3rd trimester hair growth starts in ears, gradually developing to cover the entire body. Incisor teeth become evident through the gingival membrane and eruption happens gradually towards the final 10th of gestation. Correlations were found between fetometric measurements head length, head width, weight, crown-rump-length and gestational age. Head length, head width and CRL showing strongest linear correlations. The groups Danish Jersey and Cross-breed deviating most in relation to breed related size differences. Weight gain showed a weaker correlation to age.

A probability of 78% was found for fetuses being older than 252 days of gestation for ‘at least 6 erupted’ incisors, but no statistical difference as found between groups ‘at least 6 erupted’ and ‘1-5 erupted’ with regard to gestational age. This indicating that the two groups cannot be differentiated based on number of erupted teeth alone. A medium to strong correlation was found between lateral height of incisor 1 and gestational age, indicating that this could be applied to age assess in the final stages of pregnancy.

If all parameters in this study were used in combination, age assessment would become more precise. Further analysis of present data is required to refine findings of this study.

Perspectives

The information in this study represents that of current genetic status and provides an insight into distribution of gestational age at time of slaughter and into fetal development. Furthermore, it provides arguments for using the division into trimesters with regards to gestational age.

Evidently, it has not provided refined tools to age assess at current age limits at time of slaughter, but instead questioned the sensitivity of applied methods with regards to assessment of incisor teeth and their eruption. It does however, support current methods, but also points to problematics of fetuses being overlooked when not having 6 obviously erupted teeth.

If a decision is made to prohibit slaughter of pregnant cows in the 3rd trimester, this study has provided preliminary, but comprehensible tools to determine if a fetus has exceeded the age limit, in this study being 190 days, within reasonable doubt.

All chosen features in this study were of an easily accessible nature and might be operationalized to on-site assessment by educated personnel e.g. technicians at abattoirs.

The most obvious solution to a reduction in current problematics regarding age assessment at abattoirs, would be to grant veterinarians the right to access insemination dates. Allowing abattoirs access to insemination dates would possibly encourage farmers to be better informed regarding the reproduction status of cows sent for slaughter, and the abattoirs would have readily accessible tools to prove a fetus has exceeded the age limit. It might reveal a reduction in the number of cows in late gestation sent for slaughter.

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Appendices

APPENDIX 1

Table 22. 18 fetuses with tooth eruption.

Varying number of teeth, applied in determination of probability. The oldest fetus presented first.

Fetus number	CRL cm	Weight, kg	Breed cow	Gest. age in days	Erupted teeth	252nd day gestation*	6-8 teeth erupted
287	81.8	25.4	DHolstein	291	3	+	-
206	86.8	35.5	DHolstein	274	6	+	+
1	91.0	34.5	DJersey	271	8	+	+
183	96.3	50.0	Cross-breed	268	6	+	+
200	101.3	41.6	DHolstein	264	4	+	-
429	93.6	32.5	DJersey	262	2	+	-
182	89.3	36.0	DHolstein	259	4	+	-
235	93.5	36.0	DHolstein	252	6	+/-	+
307	90.5	31.0	D Red	252	6	+/-	+
199	83.0	29.1	DHolstein	249	6	-	+
416	77.8	18.5	DJersey	244	3	-	-
420	83.2	19.9	DJersey	244	2	-	-
142	86.9	25.3	D Red	239	2	-	-
130	93.2	28.8	DHolstein	237	3	-	-
319	83.2	29.0	Cross-breed	237	2	-	-
61	79.4	19.8	Cross-breed	228	1	-	-
217	77.2	18.6	DHolstein	227	2	-	-
194	84.3	21.5	DHolstein	223	2	-	-

*the maximum limit of legal slaughtering in gestational days, corresponding to the last 10th of the gestational period

APPENDIX 2

Table 23. Fetuses, lateral tooth height, right incisor 1.

Fetus number	**Breed of cow**	**Gestational age**	**Right Incisor 1**	**Lateral tooth height, mm**
428	Danish Holstein	194	Not erupted	5,09
427	Danish Jersey	200	Not erupted	6,01
181	Danish Holstein	201	Not erupted	5,51
14	Danish Holstein	207	Not erupted	3,76
62	Danish Jersey	210	Not erupted	5,10
63	Danish Holstein	211	Not erupted	5,63
135	Danish Holstein	211	Not erupted	4,19
215	Cross-breed	212	Not erupted	8,40
55	Danish Holstein	214	Not erupted	4,82
292	Danish Holstein	214	Not erupted	7,12
127	Danish Holstein	215	Not erupted	5,72
170	Danish Holstein	215	Not erupted	6,12
318	Danish Holstein	217	Not erupted	6,92
125	Danish Holstein	219	Not erupted	5,98
168	Danish Holstein	221	Not erupted	7,44
237	Danish Jersey	221	Not erupted	8,77
417	Danish Holstein	221	Not erupted	6,88
195	Danish Holstein	222	Not erupted	8,89
238	Danish Red	222	Not erupted	5,23
419	Danish Jersey	222	Not erupted	5,89
134	Danish Red	223	Not erupted	5,70
194	Danish Holstein	223	Erupted	8,52
105	Cross-breed	226	Not erupted	7,55
217	Danish Holstein	227	Erupted	9,06
61	Cross-breed	228	Not erupted	7,82
389	Danish Holstein	235	Not erupted	10,09
130	Danish Holstein	237	Erupted	9,04
319	Cross-breed	237	Erupted	9,13
142	Danish Red	239	Erupted	9,62
416	Danish Jersey	244	Erupted	8,86
420	Danish Jersey	244	Erupted	10,02
199	Danish Holstein	249	Erupted	10,71
235	Danish Holstein	252	Erupted	8,90
307	Danish Red	252	Erupted	10,76
182	Danish Holstein	259	Erupted	10,20
429	Danish Jersey	262	Erupted	10,76
200	Danish Holstein	264	Erupted	9,76
183	Cross-breed	268	Erupted	11,34
1	Danish Jersey	271	Erupted	12,36
206	Danish Holstein	274	Erupted	10,90
287	Danish Holstein	291	Erupted	9,51

APPENDIX 3

Registration scheme

CKR				Dato		
KONR				KALV Nr.		
CRL (mm)				**Vægt (gram)**		
Køn	**Kvie**		**Tyr**	**Ikke synligt**		
Testis, descencus	Fuld			Uni/ intermed	Ingen	
Head Width				**Head length**		

Tænder

	Højre side				**Venstre side**			
	I1	**I2**	**I3**	**C**	**I1**	**I2**	**I3**	**C**
Synlig uden gennembrud								
Synlig med gennembrud								
Størrelse af gen.brud lateralt								
Størrelse af gen.brud medialt								
Højde i mm lateralt								
Højde i mm medialt								

Hårlag og pigmentering

Lokalisation Hår	**Kron -rand fo**	**Kron- rand bag**	**Øre r Ind- side**	**Ører Bun d**	**Øjen-låg**	**Hal e**	**Ryg**	**Kro p**	**Fuldt behåre t**	**Fuld t**	**Uden pels**
Hår Beskriv											
Pigmenterin g	**Mule**	**Øjenomg i**	**Cav ori**	**Ører**	**Klov/be n**	**Hals**	**Hor n**	**Hale**	**Flanke**	**Ryg**	**Fuld t**
Ingen											

Øjenvipper og taktile hår, øjne

	Frembrudt	**Hårsække**	**Intet synligt**		**Åbne**	**Lukket**
Mule				**Øjne**		
Øjenomgivelser				Delvist		
Øjenvipper					ja	Nej
				smagsløg		
				Øjenlåg		
				Horn anlæg		

APPENDIX 4

Pictures of developing tactile hair and fur.

Picture 1. All tongue papillae present, 140 days old, purebred Danish Holstein

Picture 2. Tactile muzzle hair. Gestational age 150 days. Purebred Danish Holstein

Picture 3. Tactile hair surrounding eye area. Gestational age 159 days. Purebred Danish Holstein.

Picture 4. Eyelashes. Gestational age 201 days. Purebred Danish Holstein.

Picture 5. Base of the ear in a specimen with no hair growth on the inside of the ear, sectioned ventrally. Gestational age 150 days. Purebred Danish Holstein.

Picture 6.Base of the ear in a specimen with visible hair growth on the inside of the ear, sectioned ventrally. Gestational age 159 days. Purebred Danish Holstein.

Picture 7. Visible hair on the ventral part of the external meatus. Gestational age 201 days. Purebred Danish Holstein.

Picture 8. Hair on tail. Gestational age 201 days. Purebred Danish Holstein.

Picture 9. Hair on horn bud and eyelids. Gestational age 201 days. Purebred Danish Holstein.

Picture 10. Hair on the palmar side of the coronary band. Gestational age 201 days. Purebred Danish Holstein.

Picture 11. Hair on the dorsal side of the front leg, extending dorsally above the pastern. No hair present on the palmar side above the pastern. Gestational age 201 days. Purebred Danish Holstein.

Picture 12. Hair on the dorsal side of the carpus, extending just above the carpus. No hair is present on the palmar side. Gestational age 201 days. Purebred Danish Holstein.

Picture 13. The dorsal side of the rear coronary bands, with most hair distally, hair growth proceeding proximally. Gestational age 201 days. Purebred Danish Holstein.

Picture 14. Hair on the dorsal side of the tarsus. No hair present on the plantar side. Gestational age 201 days. Purebred Danish Holstein.