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Studies of fetal-placental development in Australian and Indonesian breeds of tropically adapted cattle

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Abstract

Fetal and placental development has been most comprehensively described in the Holstein-Friesian (HF) breed. However, there is a lack of published data on fetal and placental development in tropically adapted genotypes. The major objectives were to define fetal and placental development during approximately the first half of gestation in Droughtmaster (DM) cattle (the most numerous breed of tropically adapted beef cattle in Australia), and in the three most common breeds of Indonesian beef cattle (Bali, Ongole and Madura). Previous studies of the HF breed during the first gestational age was possible to measure fetal and placental development as reported by Ginther (1998). The major hypothesis investigated was that fetal and placental development during the first approximately 140 days of gestation is similar among these four breeds of cattle, and is similar to that reported for the HF breed.

Two ultrasonographic studies of fetal and placental development were conducted. The first study was conducted at The University of Queensland Pinjarra Hills Farm, Brisbane, Australia and involved twenty approximately 2-year old cycling DM heifers (mean liveweight±SD; 430 ± 27.35 kg). All heifers underwent treatment to synchronise ovulation followed by fixed-time AI and then AI to observed oestrus. The second study was conducted at the Beef Cattle Research Station, Grati-Pasuruan, East Java Indonesia. Ongole (n=20; mean liveweight±SD; 239 ± 37.04 kg), Bali (n=20; mean liveweight±SD; 179 ± 10.68 kg) and Madura cows/heifers (n=20; mean liveweight±SD; 193 ± 27.34 kg) were treated to synchronise oestrus, and then each group of females was mated to a single bull of the same breed. In each study, all females underwent transrectal ultrasonography of the reproductive tract commencing 4 weeks after AI/mating and then once a week until approximately day 140 of gestation. Depending on stage of development the following measurements were made; crown-rump length (CRL; mm), head length (HL; mm), trunk diameter (TD; mm), placentome diameter (PD; mm), hoof diameter (HD; mm) and eye diameter (ED; mm). Measures of fetal development {trunk diameter (TD) and eye diameter (ED), head length (HL)} in HF cattle were derived using a modification of published equations. Fetal and placental morphometric variables were initially summarised using means and standard deviations. Student's *t*-test was then used to compare the equality of the mean between morphometric variables reported for each breed of cattle examined and those derived from published equations of development for the HF breed.

To compare fetal and placental development in the breeds of cattle studied for each of the morphometric variables, a separate linear mixed effects model was fitted with heifers/cows as a random effect and a first order autoregressive function for the residuals.

Morphometric data on fetal and placental data was derived from examination of 12 DM heifers and 5 Ongole cows, 5 Bali heifers and 15 Madura heifers. Between weeks 5 and 12 HL and HD of DM fetuses increased 12 and 11 fold, respectively, PD increased 12 fold between weeks 6 and 12, but ED increased only 2.5 fold between weeks 9 and 12. Generally the size of HF fetuses (as defined by CRL and HL) was greater than DM fetuses during the period week 4 to 15 of gestation. However, the differences were generally only significant during the period week 4 to 7.

The pattern of fetal and placental development as defined by the measures used were similar across the 3 Indonesian breeds. Although the CRL and ED for Bali fetuses was greater than that for Madura and Ongole the other fetal measurements were greater for Madura fetuses than the Bali and Ongole. Only CRL was consistently greater for the HF fetuses compared to the Indonesian breed fetuses, although for HL and TD differences occurred sporadically during the period of monitoring.

Overall the pattern of fetal and placental development during approximately the first half of gestation was similar among the breeds of tropically adapted cattle and with that reported for the HF. For the 2 breeds (HF and DM) which have typical calf birth weights at least double that of the 3 Indonesian breeds CRL was significantly greater but for the other measures of fetal and placental development differences were not consistently different.

Declaration by author

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Contributions by others to the thesis

List the significant and substantial inputs made by :

- Prof. Michael McGowan : work and writing represented; conception and design of the project; non-routine technical work; analysis and interpretation of research data; critical review of drafts of thesis.
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Key words

Fetal-placental development, tropically adapted cattle, Droughtmaster, Ongole, Madura, Bali cattle

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List of Abbreviations

AI	Artificial insemination
BCS	Body condition score
CRL	Crown rump length
DM	Droughtmaster
E_1S	Estrone sulphate
ED	Eye diameter
FTAI	Fixed time artificial insemination
HD	Hoof diameter
HF	Holstein-Friesian
HL	Head length
IFN-λ	Interferon tau
IPRD	Intravaginal progesterone releasing device
ODB	Oestradiol benzoate
PCA	Production and Companion Animal
PD	Placentome diameter
PGF2α	Prostaglandin F-2 alpha
PSPB	Pregnancy specific protein B
TD	Trunk diameter

Chapter 1 Introduction

In ruminants, the conceptus consists of the fetus, fetal fluids and characteristic cotyledonary placenta [1]. Studies of the bovine conceptus have been conducted in both dairy and beef cattle during early-, mid- and late-gestation to define the growth and differentiation of the fetus and placenta to enable development of guidelines for estimating gestational age, and to esimate when fetal death and subsequent abortion has occurred [2-5]. There are only limited studies examining the relationship between measures of fetal and placental development and weight of the bovine neonate. A study in 30 Angus cross heifers in the United States concluded that there was a positive correlation between the weight of the calf immediately after birth and total placentome weight at calving [6]. Similar results were also reported for dairy cattle [7] and another beef cross breed [8]. Therefore, there is some evidence that gestational measures of placental and fetal development may be able to be used to predict the weight of the bovine neonate (between 24hrs and a week after calving).

Measurement of fetal characteristics such as crown-rump length, trunk, head, limb/hoof and eye diameter have been conducted [9] to enable monitoring of conceptus development in *Bos taurus* breeds of cattle. However, similar studies have not been performed in Indonesian beef cattle breeds, such as Bali, Madura and Ongole cattle. These are all tropically adapted genotypes[10], of varying *Bos javanicus* and *Bos indicus* content. Recent research has demonstrated that Madura cattle are genetically distinct from other native breeds in Indonesia [11].

In 2004, Ongole cattle were the most prevalent of these native breeds in Indonesia, followed by Bali cattle, which accounted for nearly 30% [12] of the whole cattle population. By 2011, this trend had reversed, with Bali cattle more prevalent than Ongole [13]. Madura cattle is the least dominant of these cattle breeds [14]. The majority of Indonesian beef cattle production is derived from small holder farms, which is often associated with low productivity due to a multitude of factors inherent in such production systems [13,15]. Previous studies in Bali cattle have demonstrated a high calf death rate, up to 48% [16]. Similarly, in the extensive tropical arid production system in northern Australia losses between confirmed pregnancy and weaning are unacceptably high with for example heifers experiencing mean losses of approximately 15%[17].

The overall aim of the research presented in this thesis was to address the lack of information about fetal and placental development in tropically adapted beef cattle varying in mature body weight. The major objectives were to define fetal and placental development during approximately the first half of gestation in DM cattle (the most numerous breed of tropically adapted beef cattle in Australia), and in the three most common tropically adapted breeds of Indonesian beef cattle (Bali, Ongole and Madura). The major hypothesis investigated was that fetal and placental development during the first approximately 140 days of gestation is similar among these four breeds of cattle, and is similar to that reported for the HF breed.

This thesis is structured as follows:

- Chapter two, the literature review, provides a summary of research undertaken to date on fetal and placental development, focusing on assessment of the reproductive tract by transrectal ultrasonography.
- Chapters three presents the results of ultrasonography of fetal and placental development in Australian DM cattle and compares the findings to that observed in HF cattle.
- Chapters four presents the results of ultrasonography of fetal and placental development in the three main Indonesian native cattle breeds (Bali, Madura and Ongole) and compares the findings to that observed in HF cattle.
- Chapters five presents the findings of the comparison of fetal and placental development in DM, Madura, Bali and Ongole cattle.
- Chapter six discusses the overall findings of the research and presents the major conclusions.

Chapter 2 Literature Review

2.1 Placental and fetal development in cattle

2.1.1 Development of the placenta

At the blastocyst stage of embryo development (approximately day 7 after mating), the embryo has a balloon shape, with a fluid filled central component (the ‘blastocoele’), an outer layer of cells lining the zona pellucida (the ‘trophectoderm’), and the embryo proper nestled within the trophectoderm. The trophoblast cells will ultimately become part of the chorion [1]. As the embryo begins to elongate, a layer of cells grow up from around the embryo to form the amnion, with the amniotic fluid and fetus inside. As the early fetus develops, an out-pocketing of the hindgut extends from the fetus to ultimately become the allantois. Subsequently, the allantois will fuse with the chorion to form the chorioallantois (Figure 1). The amnion progressively becomes attached to the chorioallantois except at the extremities of the conceptus [1].

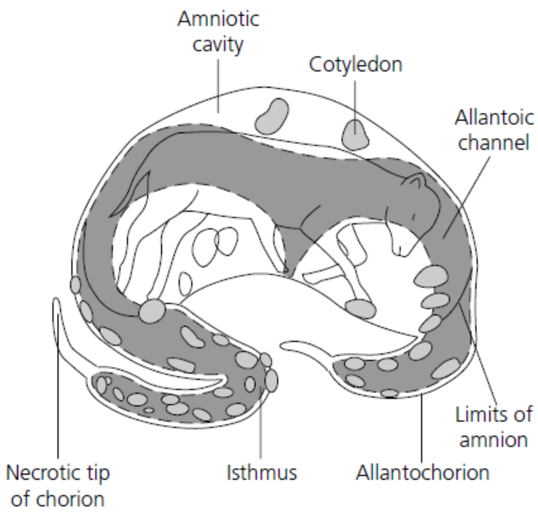


Figure 1. Organization of fetal membranes in the cow (from Noakes et al after Zietzschmann,1924)

The chorionic vesicle with the centrally located fetus progressively fills with amniotic and allantoic fluid; this first begins to distend the gravid cornu at about 35 days (Figure 1). At this time, the allanto-chorion already extends into the non-gravid horn; its length is about

40 cm and at its widest part in the dependent portion of the gravid horn is 4–5 cm in diameter.

In cattle the chorioallantois attaches to the endometrium at approximately 4 weeks of gestation, particularly over the caruncles. Progressively, these sites of attachment become recognized as placentomes consisting of the fetal cotyledon and the maternal caruncle. There are approximately 120 placentomes [18] arranged in rows along both cornu, with the largest diameter placentomes in the uterine cornu containing the fetus. The placentomes significantly decrease in size towards the tip of each cornu. The diameter of placentomes that can be readily determined per rectum and are routinely used to estimate fetal age in cattle (Table 1). However, it should be noted that the guidelines presented in Table 1 are derived from the findings of multiple anatomical studies, whereas the data presented in Figure 3 is from a single anatomical study and thus there are some differences between the two in placentome size at a given gestational age.

Table 1. Fetal age and placentome diameter

Fetal age	**Placentome diameter**
2.5 months	0.5-1.0 cm
3 months	1.0-1.5 cm
4 months	1.5-2.5 cm
5 months	2.5-4.0 cm
6 months	4.0-5.0 cm
7 months	5.0-7.5 cm
8 months	6.0-9.0 cm

*Adapted from ‘Pregnancy testing in cattle’ (Jephcott and Norman, 2004) Published by the Australian Association of Cattle Veterinarians

Amniotic fluid is produced by flows of fluid starting from the respiratory tract, which passes into the amnion cavity, and also from the fetal skin. After 240 days of gestation, the fetus urinates directly into the amniotic cavity. Prior to this, urine from the fetus is excreted into the vesica urinaria, and passes through the canal within the umbilical cord into the allantoic cavity [1]. The total quantity of fetal fluid in bovine conceptuses increases progressively throughout pregnancy; it averages about 5 litres at 5 months and 20 litres at parturition. Sharp increases in the total quantity occur between 40 and 65 days, 3 and 4 months, and 6½ and 7½ months, as shown in Figure 2 [18].

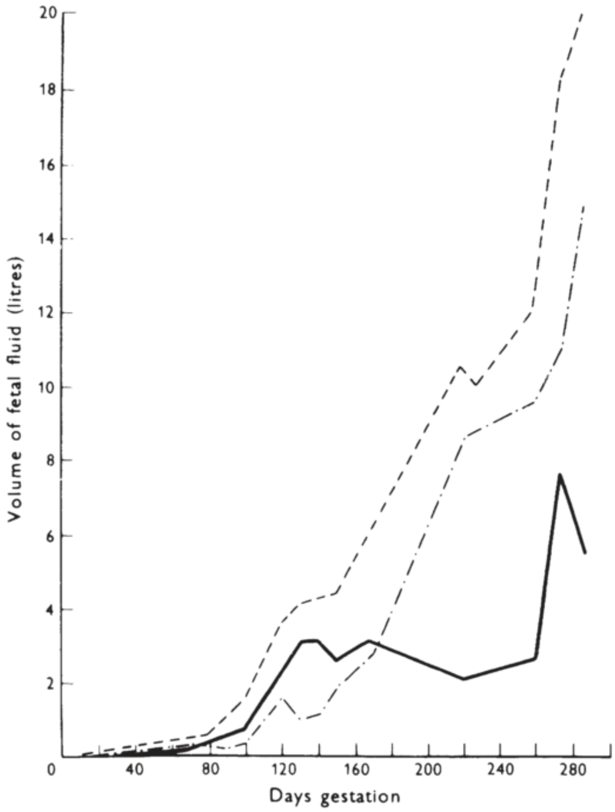


Figure 2. Volume of fetal fluid (--- total; -·-·- allantoic and ___ amniotic) at the successive stages of pregnancy cow (from Arthur, 1969)

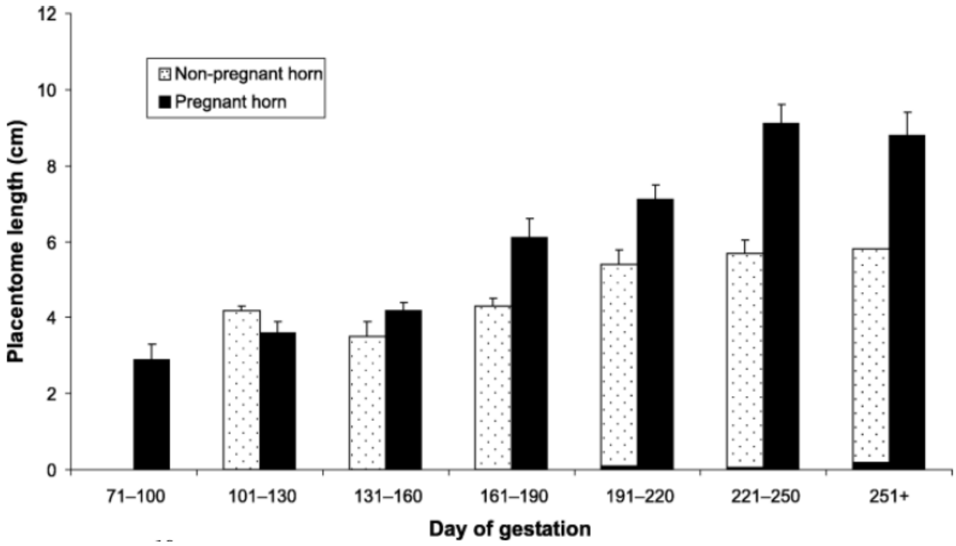


Figure 3. Placentome development in pregnant and non pregnant cornu (from Laven and Peters, 2001)

Compared to the placentomes in the pregnant cornu, placentomes in the non-pregnant cornu are smaller (Fig. 3; Laven and Peters, 2001), except between 101-130 days of gestation. After 191-220 days of gestation, placentome size in the non-pregnant cornu remains stable (4 to 6 cm in length), but in the pregnant cornu they increase on average from about 8 cm to nearly 10 cm (Fig. 3) [19].

2.1.2 Development of the fetus

The development of the conceptus from fertilisation on day 1 up to day 40 after ovulation is known as the embryonic period. Bazer et al [20] demonstrated that growth and development of the conceptus was controlled by the amount of glucose and fructose in the lumen of the uterus. The actions of these nutrients can promote cell signaling, cell proliferation and production of glycosaminoglycan, which is vital to development at this stage [20]. The bovine embryo itself, although differentiating fast, elongates slowly compared to the chorion, and at a month after mating it is only just over 1 cm long [18].

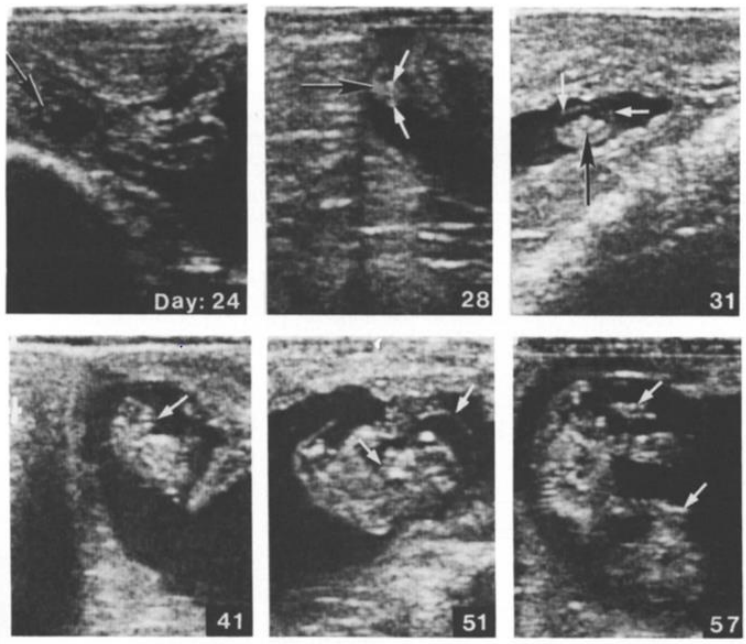


Figure 4: Bovine conceptus development from day 24 up to 57 of gestation. Day 24 = C shaped embryo (black arrow); Day 28 = forelimb buds (white arrows); Day 31 = amnion (white arrows); Day 41 = oropharynx (white arrow); Day 51= oropharynx and amnion (white arrows); Day 57 = limbs (white arrows) (from Curran et al, 1986)[21].

Using trans-rectal ultrasonography of the uterus, Curran et al (1986) reported that between 24 and 57 days of gestation, the 'C' shaped embryo proper/fetus increased in length from 9.5 mm to 53 mm, with the forelimbs and hindlimbs, and cranial and trunk sections easy to define, as shown in Figure 4. At 60 days of gestation the fetus is nearly 70 mm long and fine details of cranial and trunk structures, including ossified features [21] can be visualised. Fetal development between 60 and 170 days of gestation includes establishment of the genital tubercle and external genitalia, the fore-stomachs, the vascular system and differentiation of the brain [9].

Further study during late pregnancy until term, fetal and placental development can drive to abnormal progression that can lead to several causes of stillbirth in the bovine conceptus include abnormalities of the amniotic and allantoic fluids and placental membranes [22]. Other authors have suggested additional causes of declining fetal weight gain during gestation and fetal loss, including the function of placentomes and hormonal factors produced by the fetus and/or the placentome itself [23-25].

2.1.3 Relationship between development of placenta and fetus

Both beef and dairy cattle have similar placentomes, which are a combination of fetal placentomes (namely cotyledons) and maternal placentomes, or caruncles. The placentomes' size can be used as an indicator of fetal age. Post-mortem observations indicate that the size of the placentome and gestational age are positively correlated [19]. Transrectal ultrasonography can easily detect placentomes from as early as 33 days of pregnancy, so fetal age could be estimated from measurements of placentome size, provided the correlation between placentome size and fetal age is high.

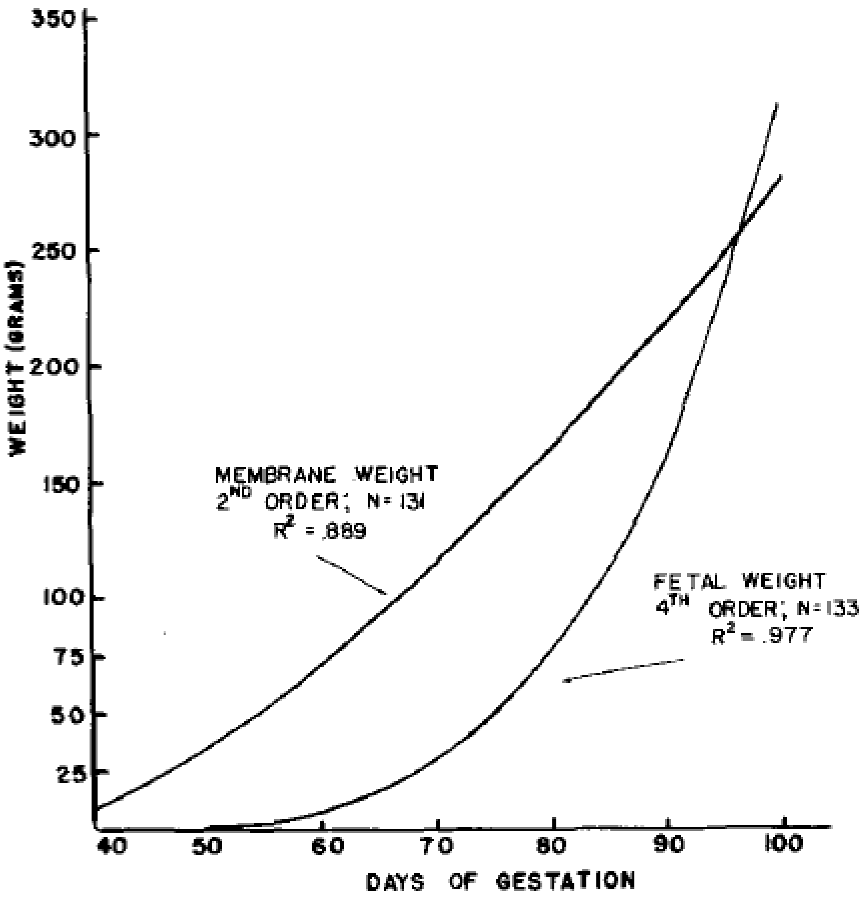


Figure 5. Predicted development of the placenta and fetus between 40 to 100 days of gestation based on gross examination of 133 bovine conceptuses (from Eley et al, 1978)

Another study on gross examination of bovine fetuses indicated that fetal weight increased significantly over membrane weight just before 100 days of gestation [26], as shown in Figure 5. However, the placentomes may be less capable of maintaining the aggressive growth of the fetus during the late pregnancy period, which may result in slower fetal growth rate [25].

2.1.4 Relationship between measurement of fetal and placental development and anatomical measurements of the neonatal calf

Riding et al (2008) concluded that both foetometric and amniotic data could be important for research and clinical purposes [27]. More specifically, physical measurements of the head and crown-rump-lengths correlate closely to those measured ultrasonographically. Head and crown-rump lengths were also more reliable indicators of fetal age during the first trimester of pregnancy in beef cattle than measures of allantoic and corpus luteum sizes, which are not recommended as indicators of fetal development [28].

One study on placentome development, which used abbatoir-derived pregnant uteruses at varying stages of pregnancy, concluded that during gestation the number and weight of placentomes increase significanly until term. However, there was no correlation between the mean number of placentomes present and the mean total weight of placentomes; this was partially due to the large variation observed between animals in the size, weight and shape of the placentomes [19].

For clinical purposes the use of ultrasonography prenatally can be useful for early identification of retardation or for predicting gestational age and assessing the aggresiveness of fetal growth. Identifying a decrease in fetal growth during late pregnancy may be useful to predict calf death before calving or stillbirth [29]. From the mid-last trimester of pregnancy, the retarded calf may be identified by observing the size of the placentomes in a pregnant cow. Previous findings in sheep have concluded that intra uterine growth restriction can be detected by ultrasonographic examination of placentome size [30].

2.2 Use of transrectal ultrasonography to assess development of the bovine placenta and fetus

2.2.1 Techniques used

Trans-rectal ultrasonographic examination of the uterus and conceptus has been used since the 1980's in large animals [31,32] and is commonly used for early pregnancy diagnosis in cattle and horses. Ginther (1995) has described in detail the technique of trans-rectal ultrasonography in cattle including how a range of fetal and placental measurements should be done. The ultrasound frequency may be varied according to stage of gestation [9].

There is a significant linear correlation between fetal age and physical or ultrasound measurements of fetal crown-rump length (CRL) and crown nose length [28]. Kahn (1989) also observed that as cranial measurements of the fetus are commonly assessed and easily measured by transrectal ultrasonography, they may be useful parameters to predict fetal age [33]. Figure 6 to 9 derived from scanning pregnant DM heifers (chapter) display how CRL, trunk diameter, head length and eye diameter are measured.

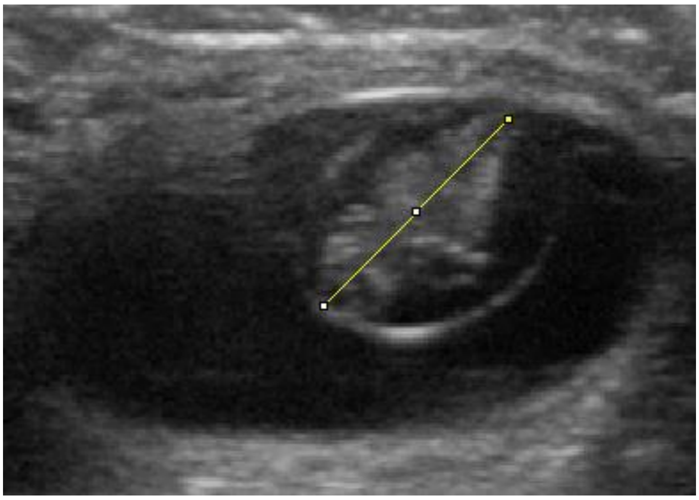


Figure 6. CRL (crown rump length) measurement in yellow line.

Figure 7. Trunk diameter measurement in yellow line.

Figure 8. Head length measurement in yellow line.

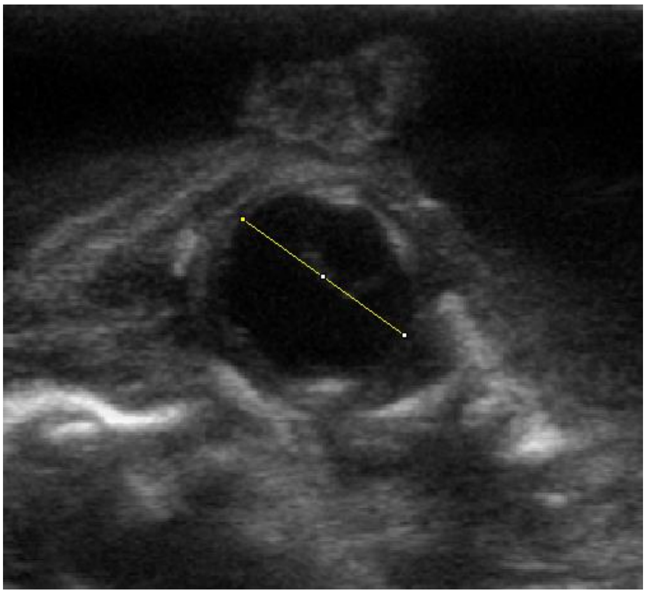


Figure 9. Eye diameter measurement in yellow line.

2.2.2 Ultrasonographic guidelines for estimating fetal age in *Bos taurus* cattle

Studies using embryos and fetuses recovered at slaughter or necropsy have provided guidelines for estimating the gestational age of the embryo / fetus based on body length measurements, as shown in Table 2.

The utilisation of real time trans-rectal ultrasonography can enable visualization of the embryo from the early stages of development through to late gestation. A study by Curran et al (1986) reported the different structures first detected by ultrasonography from day 19 of gestation (Table 4.) [34].

This technology can therefore be helpful to track embryo development and identify mortality and the risk of abortion. Given that embryo mortality is predicted to be as high as 30% in cattle raised in the humid tropics, the application of this technology may provide useful information for analysing the time of embryo death and understanding the underlying causes. Previous studies have shown that pregnancies can be confirmed by ultrasound at approximately 30 days after mating. Trans-rectal ultrasonography may therefore also have application in beef cattle situations where few reproductive records are available, to identify pregnant cows soon after they are joined with the bulls.

The fetal head in approximately 80% of cases can be detected by ultrasonography, and Kahn (1989) recommended the use of head size and crown rump length to estimate gestational age in cattle. In some circumstances, crown nose length can also be used to estimate gestational age, if it is not possible to measure CRL [28].

Table 2. Observed fetal body length (crown-rump length) measurements throughout gestation (from Hammond, 1927 and Winters et al, 1942)

Fetal age (months)	Fetal body length (cm)
1	0.8
2	6
3	15
4	28
5	40
6	52
7	70
8	80
9	90

Table 3. Ultrasonic appearance of embryonic and fetal structures by days of gestation (from Curran et al, 1986)

Characteristic	Earliest mean (days)	Range (days)
Embryo proper	20.3	19-24
Heartbeat	20.9	19-24
Allantois	23.2	22-25
Spinal cord	29.1	26-33
Forelimb buds	29.1	28-31
Amnion	29.5	28-33
Eye diameter	30.2	29-33
Hindlimb buds	30.1	30-33
Placentomes	35.2	33-38
Split hooves	44.6	42-49
Fetal movement	44.8	42-50
Ribs	52.8	51-55

2.3 Other methods of assessing conceptus survival and development in cattle

2.3.1 Observation/detection of signs of estrus greater than 4 weeks after mating

In normal conditions, oestrus can be detected from the distinctive behaviours and physical signs of female cattle, namely standing to be mounted by other cows, teasing actively or aggressively, mucus discharge from the vulva, swollen and reddish vulval mucosa, and sometimes decreased feed intake. As long as there is no fertilisation after mating, oestrus signs will be detected again between 18-24 days after the last mounting.

2.3.2 Measurement of changes in progesterone concentration

Progesterone is vital for establishing and maintaining pregnancy and also ensuring proper embryo development. Progesterone can be analysed from blood samples [35] and/or milk [36]. Higher blood concentrations of progesterone in the early mating period have been associated with higher pregnancy rates and higher embryonic growth rates [37,38]. The progesterone concentration in the serum must be adequate to maintain pregnancy, otherwise embryo loss will occur. If the progesterone concentration is less than 2 ng/ml one week after mating, then the likelihood of conceptus survival is low; by contrast, if the concentration is ≥2ng/ml then the likelihood of conceptus survival is high. Additionally, between weeks two and six after mating, progesterone (P4) levels should be more than 5 ng/ml to ensure continued development of the conceptus [39]. There is a trend for lower blood progesterone during dioestrus in cattle that have not conceived [40].

The period of greatest embryonic loss in cattle occurs between blastocyst formation and the initiation of elongation (days 7–16 after fertilization). During this period, the embryo undergoes a dramatic increase in size and protein content. High concentrations of circulating progesterone, both before and during this period, are strongly associated with an increase in conceptus length.

2.3.3 Measurement of oestrone sulphate

Estrone sulphate (E_1SO_4 or E_1S) is a sulfated oestrone ester and estrogen also known as conjugated estrogen, which is produced by the placentomes in cattle [41,42]. Previous research in dairy cattle has shown that E_1S concentration in blood plasma and milk can be used to estimate foeto-placental development [43-45]. The period of greatest E_1S

production is from the second trimester up to parturition [46], and high concentrations of this hormone in blood from cattle in the third trimester have been associated with calf birth [43]. However, another study both in beef and dairy cattle, the concentration of this hormone can not be used to estimate calf birth weight and fetal health in late pregnancy, although it may be a useful sign of the incidence of retained placenta [47].

Further research has identified interesting differences in blood E_1S concentrations between single and twin pregnancies, as illustrated in Figure 11. Blood E_1S concentrations increase steeply at approximately 50 days before calving; this trend continues until parturition for twin births, but declines rapidly just before parturition in single births [48].

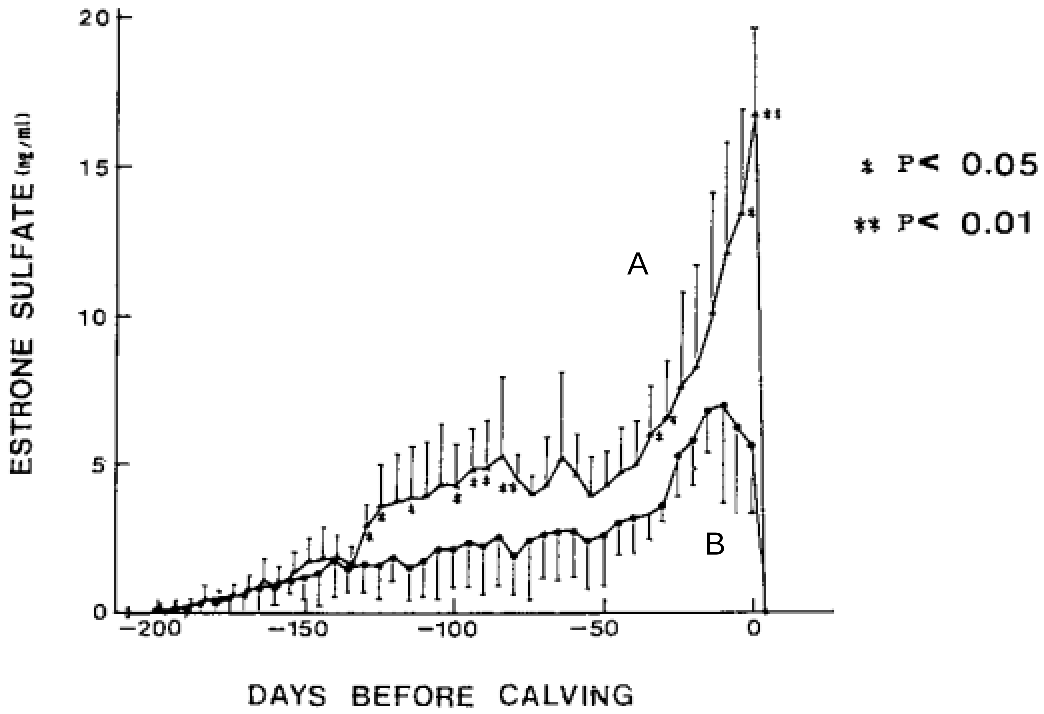


Figure 10. Mean of oestrone sulfate (E_1S) in singleton (B) and twin (A) birth (from Takahashi et al,1996).

2.3.4 Measurement of changes in pregnancy specific-protein B

Pregnancy specific protein B (PSPB) is synthesized by binucleate cells of the trophoblastic ectoderm of pregnant cows and remains in the blood circulation for several weeks after calving. PSPB can be detected in the serum of pregnant cows as early as two or three weeks after artificial insemination, but reliable results of the measurement are identified approximately 28-30 days after conception [49,50].

Similar findings have confirmed the accuracy of blood PSPB for pregnancy diagnosis from day 28 to 35 after insemination [51]. False negative results may occur in pregnant cows with low levels of PSPB and false positive results may occur due to persistence of PSPB from the previous pregnancy or in cases of embryo-fetal loss. PSPB analysis and trans-rectal ultrasonography represent two methods of confirming pregnancy diagnosis in the field.

2.3.5 Palpation per rectum of the uterus

Per-rectal palpation of the uterus is commonly used approximately 35 days after ovulation to determine pregnancy status [52]. Manual palpation of the uterus to determine pregnancy status between 5 to 6 weeks after mating has no detrimental effect on the viability of the fetus when the membrane slip technique is used[53]. Other palpable changes indicative of early pregnancy include asymmetry of the cornu and fluctuance in the gravid horn[54].

2.4 Use of transrectal ultrasonography to improve reproductive management of beef cattle

Embryonic and fetal loss mainly occurs in the first trimester of gestation, and can cause economic loss for both beef and dairy producers. Identification of these losses is therefore important [3]. The use of trans-rectal methods (manual palpation and ultrasound) to diagnose pregnancy and determine fetal age can help improve reproductive management of beef cattle, including identifying embryonic or fetal loss [55,56]. Both methods have advantages and disadvantages according to their application in the field, as outlined below.

Ginther (1995) has reported that trans-rectal ultrasonography can be used to detect the presence of the conceptus as early as approximately 20 days after mating in cattle, and can be used to monitor fetal development through to parturition. It is possible to observe the heartbeat, define sexual identity, and also diagnose any abnormalities of the calf before calving. The other advantages of ultrasound compared to rectal palpation are confirmation of pregnancy at an earlier time, assessment of fetal viability, and also minimising misdiagnosis [57]. However, ultrasound equipment is expensive and great skill is required to operate the equipment safely. In many situations rectal palpation is more affordable and easier to conduct. According to Franco et al (1987), pregnancy can be reliably diagnosed by rectal palpation from 42 days post mating, however due to ongoing losses the proportion of cows subsequently diagnosed pregnant approximately 90 days after mating is often significantly lower [58].

Chapter 3 Fetal and placental development in DM cattle and comparison with development in HF cattle

3.1 Introduction

Fetal and placental development has been most comprehensively described in the Holstein-Friesian (HF) breed. However, there is a lack of published data on fetal and placental development in tropically adapted genotypes. This chapter will describe the first study of fetal and placental development conducted in DM cattle. DM are a tropically adapted breed of beef cattle developed in northern Australia. They are approximately 50% *Bos taurus* and 50% *Bos indicus* and were derived from matings of primarily Shorthorn and Brahman breed cattle. The mature cow weight is approximately 450kg and birthweight is typically 30-35kg. Under tropical arid conditions they can achieve annual pregnancy rates of 73.9 to 93.8%[59].

Studies of fetal and placental development in HF cattle have been reported by White et al (1985), Kahn (1989) and Ginther (1995). Ginther (1995) has developed a series of equations defining fetal development for HF cattle. A comparison between reported mean measurements is presented in Table 4. However, there is little or no data comparing fetal development between HF cattle and other breeds of cattle especially tropically adapted cattle.

Table 4. Comparison of reported* trunk and eye diameter of HF fetuses

days of gestation	trunk diameter (mm)				eye diameter (mm)
	a	b	c	mean**	b
30			4	**4**	
40	11	8	8	**9**	
50	15	14	13	**14**	
60	20	22	17	**20**	4
70	25	29	24	**26**	6
80	31	37	31	**33**	8
90	37	44	38	**40**	10
100	47	52	47	**49**	12
110	57	61	58	**59**	14
120	71	70	69	**70**	16
130		79	81	**80**	18
140		89	92	**91**	19
150		99		**99**	20
160					22
170					23
180					24
190					25
200					25
210					26
220					26
230					27
240					27

*a=(White et al, 1985); b=(Kahn 1989); c=(Ginther 1995); ** mean of 'a', 'b' & 'c' ((a+b+c)/3)

The hypothesis of this study is that there is no difference in fetal and placental development during approximately the first half of gestation between DM cattle (most common tropically adapted beef breed of cattle in northern Australia) and HF breed cattle (most common *Bos taurus* dairy cattle breed in the world).

3.2 **Materials**

3.2.1 Animal ethics

The experiment was approved by the Production and Companion Animal (PCA) Animal Ethics Committee of The University of Queensland (Approval number SVS/457/14/ACIAR).

3.2.2 Location and time of experiments

Study 1 was conducted at The University of Queensland's Pinjarra Hills farm in south-east Queensland, Australia (27°32‟12S 152°55‟19E) between January and June 2015.

3.2.3 Animal selection and management

Twenty approximately 2-year old DM (*Bos indicus* crossbred) heifers with a mean (+ SD) weight of 430 ± 27.35 kg and body condition score of (BCS 1 to 5 scale) 4 ± 0.38 were enrolled in this study. Live weight (LW) and health were recorded at the beginning (January 2015) and at the end of the study (June 2015). Prior to the commencement of the study all heifers were confirmed to be non-pregnant and had normal reproductive tracts by transrectal ultrasonography of the reproductive tract. The heifers grazed improved and natural pastures consisting predominantly of Callide Rhodes grass (*Chloris gayana*) with smaller areas of Green Panic (*Panicum maximum var. trichoglume*) and Creeping Bluegrass (*Bothriochloa insculpta)* as a single mob in a paddock and had access to drinking water at all times.

3.3 **Methods**

On day 0, live weight and body condition score were recorded and each heifer underwent transrectal ultasonography of the reproductive tract. The ovulation synchronization protocol used was based on the findings of Butler *et al* (2011). All heifers received an intravaginal progesterone releasing device (IPRD 0.78g progesterone; Cue-Mate®, Bioniche Animal Health Australia/Asia) and 1 mg of oestradiol benzoate im. (ODB; Bomerol™, Bayer Australia, Sydney, Australia). On day 8, the IPRD was removed and all heifers received 500 µg cloprostenol im. (Ovuprost™, Bayer Sydney, Australia). On day 9, all heifers received 1 mg oestradiol benzoate im. All heifers were inseminated 52 to 56 h after IPRD removal using frozen-thawed semen from a purebred DM bull.

3.3.1 Transrectal ultrasonography

All enrolled cattle were first examined 3 weeks after FTAI. Thereafter, cattle were scanned once a week up to approximately week 20, unless they were observed to have undergone embryonic/fetal loss. The number of cattle enrolled (pregnant) after FTAI were 12 cattle. Based on the published literature this was considered a sufficient number of pregnant animals to enable definition of placental and fetal development.

Ultrasonography monitoring was done using one of two B-Mode real-time Sonosite M-turbo, Fujifilm ultrasound scanner with L52X/ 10 to 5 MHz linear array transrectal transducer (Sonosite Inc., Bothel, WA, USA). The size of structures observed could be estimated if required by using the scale (1 centimetre increments) on the right hand side of the ultrasound image (Figure 11).

Wherever possible the following parameters were measured and recorded for each pregnant female at each scanning: fetal crown-to-rump length (CRL; mm), fetal head length (mm), fetal trunk diameter (mm), placentome diameter (mm), fetal hoof diameter (mm) and fetal eye (bony orbit) diameter (mm). All trans-rectal ultrasound images were analysed by image J software. The method of measuring each parameter is shown in Figure 122 to 16.

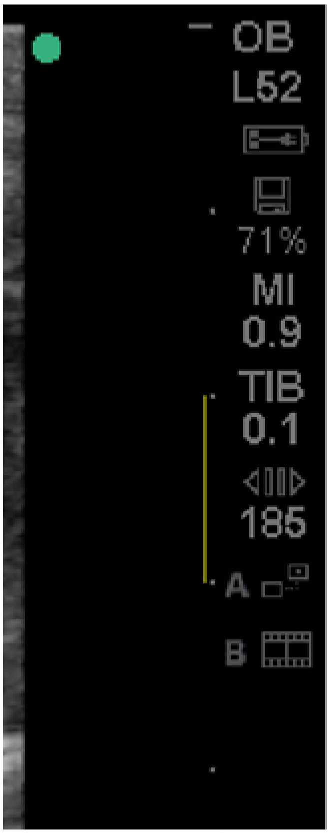


Figure 11. Yellow line scale in centimetres on the ultrasound image.

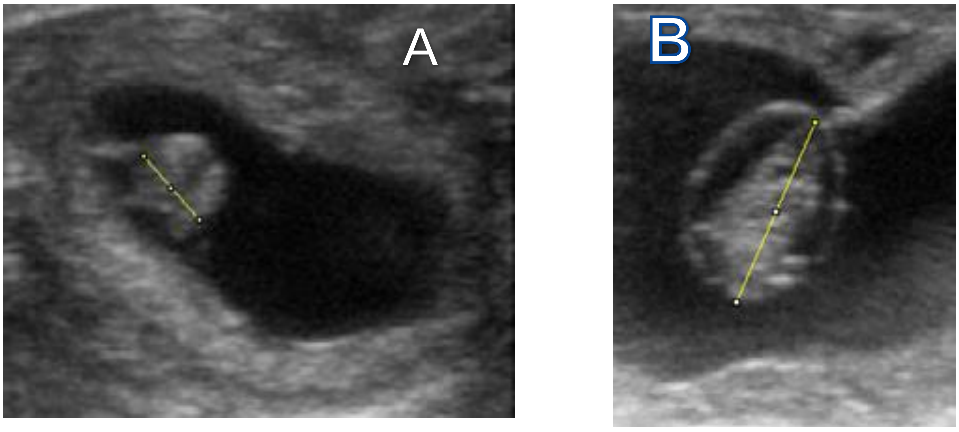


Figure 12. Yellow line was how to measure CRL on A (27 days of gestation); B (34 days of gestation)

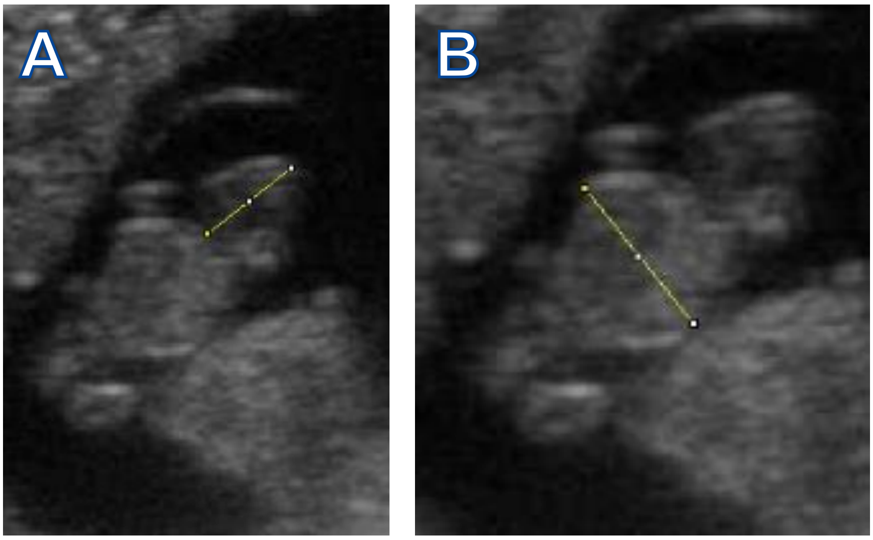


Figure 13. Yellow line was how to measure head length on A (36 days of gestation); and trunk diameter on B (36 days of gestation)

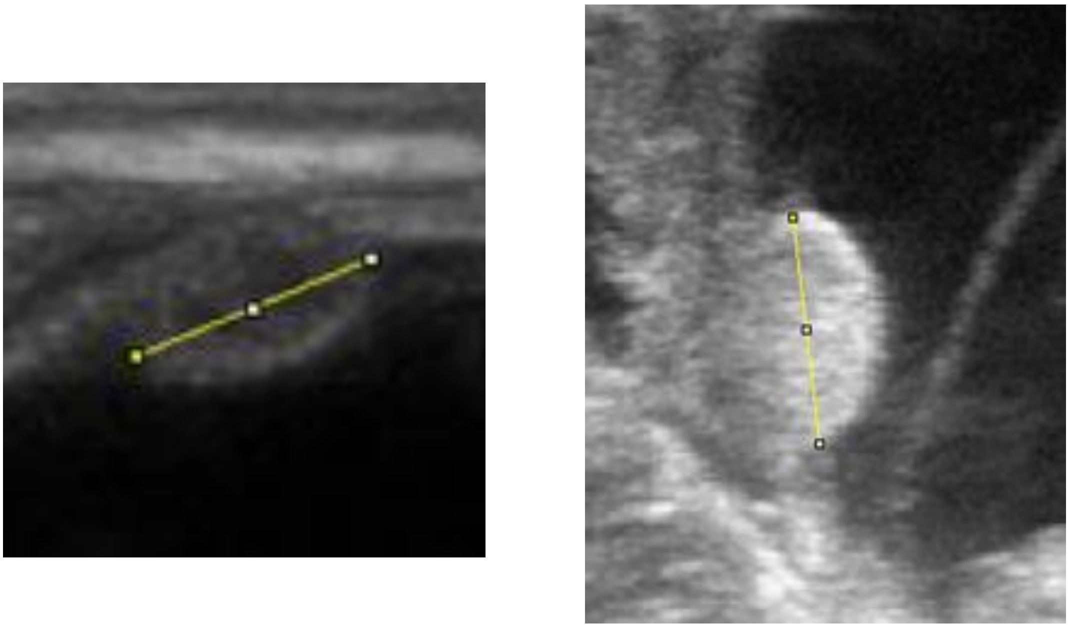


Figure 14. Yellow line was how to measure placentome diameter at 53 days of gestation

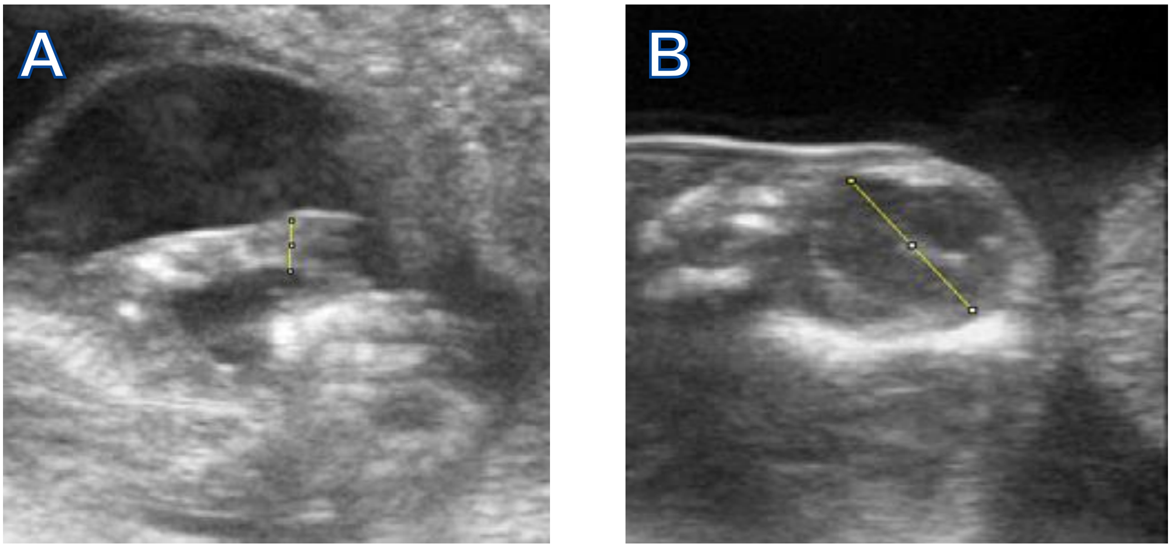


Figure 15. Yellow line was how to measure hoof diameter on A (53 days of gestation); and eye diameter on B (84 days of gestation)

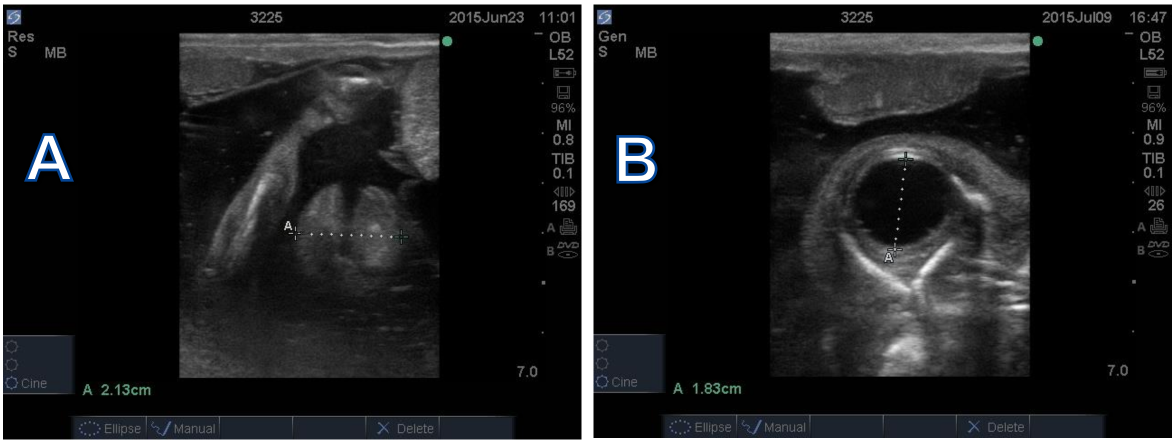


Figure 16. The dotted line was how to measure hoof diameter on A (123 days of gestation); and eye diameter on B (139 days of gestation)

3.3.2 Comparison of fetal development in DM and HF cattle

Fetal ultrasonographic measurements from the current study, where possible, were compared to those reported in the literature for HF cattle[9,33,60]. Ginther 1995 reported mathematical equations to estimate day of gestation based on ultrasonographic measurements of CRL, fetal head length (HL) and trunk diameter (TD)[9]. These equations were rearranged to allow generation of mean CRL, HL and TD for each day of gestation measurements were obtained from the DM conceptuses. The formulae are presented below:

$$CRL\ length = (e^{\frac{day\ of\ gestation\ (days) - 27.5}{16.73}})$$ Equation 1

$$Fetal\ head\ length = (e^{\frac{day\ of\ gestation\ (days) - 25.7}{40.38}})$$ Equation 2

$$Fetal\ trunk\ diameter = (e^{\frac{day\ of\ gestation\ (days) - 39.7}{37.21}})$$ Equation 3

DM data were collected from Image J measurement and using the formulae above we were able to generate values for the 3 parameters for each week of gestation for HF cattle. The CRL, HL and TD were expressed in mm.

3.3.3 Statistical analysis

Fetal and placental morphometric variables were summarised using means, standard deviation (SD) and count of animals scanned. Student's *t*-test was then used to compare the equality of the mean between morphometric variables reported in this study and those estimated using Equations 1-3 for HF fetuses. Reported P-values were corrected using Bonferroni adjustment to account for multiple hypothesis tests being conducted on the same variable within the same dataset. Statistical significance was declared at an alpha level of 0.05 or less. All statistical analysis were carried out using R[61].

3.4 Results

3.4.1 Summary of reproductive performance

The reproductive performance of the DM heifers is summarized in Table 5. Most (85%) heifers had a corpus luteum at the commencement of the study and thus it is likely that all heifers were cycling. In January, the pregnancy rate achieved to FTAI was as expected, although the proportion becoming pregnant to the second AI conducted on heifers which failed to conceive to FTAI was lower than expected.

Table 5. Summary of reproductive performance of DM heifers (n= 20) throughout the study period.

Parameters	**Calendar month**		
Day of study (month)	**January**	**April**	**June**
Mean weight (kg ± SD)	429.75 ± 27.35	448.65 ± 15.65	N/A
Mean body condition score (+-SD)	3.8 ± 0.38	3.8 + 0.34	N/A
Proportion with a corpus luteum at commencement (%)	85	N/A	N/A
Proportion detected in oestrus after oestrous synchronisation (%)	85	N/A	N/A
Cumulative proportion AI/observed mated (%)	100	N/A	N/A
Cumulative proportion pregnant (%)	45 (n=9)	55 (n=11)	50 (n=10)

SD=Standard deviation; N/A=Not available/not measurable

3.4.2 Fetal and placental development

Twelve heifers were confirmed pregnant at week 5 of gestation, however this declined to nine by week 17 of gestation due to spontaneous abortions (Table 6) which occurred between the 9^{th} and 16^{th} week of gestation.

CRL was able to be determined between weeks 5 to 9 of gestation, HL and TD between weeks 5-15, HD between weeks 5-19, ED between weeks 10-19 and PD between weeks 6-19. (Table 7).

3.4.3 Comparison of fetal development in DM and HF cattle

Table 8. shows mean CRL measurement (±SD) of DM cattle from week four to week eight and estimated CRL measurements for HF fetuses in the same period of gestation. Observed CRL of DM fetuses were different for gestation week four (7.45mm; ±1.18; P = <0.001), five (13.99mm, ±0.59; P = <0.001) and eight (44.58mm, ±4.73; P = 0.01) compared to those for HF fetuses (10.30mm, 15.70mm, 54.90mm, respectively). Compared to HF fetuses HL was lower for DM fetuses in gestation weeks five (5.33mm, ±0.71; P = <0.001), six (10.78mm, ±0.85; P = <0.001), and seven (16.25mm, ±0.94; P = <0.001) as reported at Table **9.**9. While TD tended to follow a similar trend to that observed for CRL and HD, DM TD were greater (P = 0.01) than those of HF fetuses between gestation weeks 12-14 (Table 10.).

Table 6. Mean days of gestation (range) and total number of pregnant DM heifers scanned throughout the study period.

Gestation week (range)	Total number of pregnant animals scanned
5(4-5)	12
6	12
7(7-8)	12
9(8-9)	12
10(10-11)	12
12(12-13)	10
14	10
15	10
16	10
17	9
18	9
19	9
20	9

Table 7. Mean (±SD) fetal and placentome development between week 5 and 19 of gestation in DM heifers

Variable		**Week of gestation**											
		5	6	7	9	10	12	14	15	16	17	118	19
CRL (mm)	Mean (±SD)	10.18(3.40)	24.04(2.00)	36.76(2.99)	50.80(2.36)	--ζ	--	--	--	--	--	--	--
	No. animals	12	12	11	6	--ζ	--	--	--	--	--	--	--
HL (mm)	Mean (±SD)	5.33(0.71)	10.78(0.85)	16.88(1.38)	23.84(2.51)	33.74(1.71)	45.21(3.14)	53.26(2.65)	60.54(0.43)	--	--	--	--
	No. animals	7	11	11	12	12	8	3	2	--	--	--	--
TD (mm)	Mean (±SD)	7.61(0.72)	11.46(1.06)	14.19(1.14)	18.50(1.75)	25.20(2.5)	40.58(0.15)	45.71(3.14)	54.97(1.60)	--	--	--	--
	No. animals	7	11	12	9	8	5	6	5	--	--	--	--
HD (mm)	Mean (±SD)	1.35(0.63)	2.21(0.73)	2.94(0.26)	4.53(0.42)	5.83(1.50)	7.75(1.58)	12.09(0.71)	14.02(0.90)	15.56(0.89)	17.65(0.90)	18.50(1.13)	22.10(1.48)
	No. animals	6	11	12	12	12	10	6	5	7	8	8	7
ED (mm)	Mean (±SD)	--	--	--	--	7.06(0.77)	7.94(1.96)	13.81(0.69)	15.02(0.44)	17.71(0.60)	18.90(0.33)	19.70(0.49)	21.54(0.22)
	No. animals	--	--	--	--	12	4	5	6	7	8	6	6
Placentome diameter (mm)	Mean (±SD)	--	2.42(0.22)	8.53(2.95)	11.53(1.76)	13.08(2.80)	21.04(2.68)	24.24(1.79)	27.21(0.75)	30.98(1.19)	34.70(0.91)	38.33(0.52)	40.45(1.47)
	No. animals	--	11	2	3	6	5	7	7	7	8	7	4

Key: CRL=Crown rump length; HL=Head length, TD=Trunk diameter; HD=Hoof diameter; ED=Eye diameter; mm=millimeter, SD= Standard deviation.
ζ not measured or could not be measured.

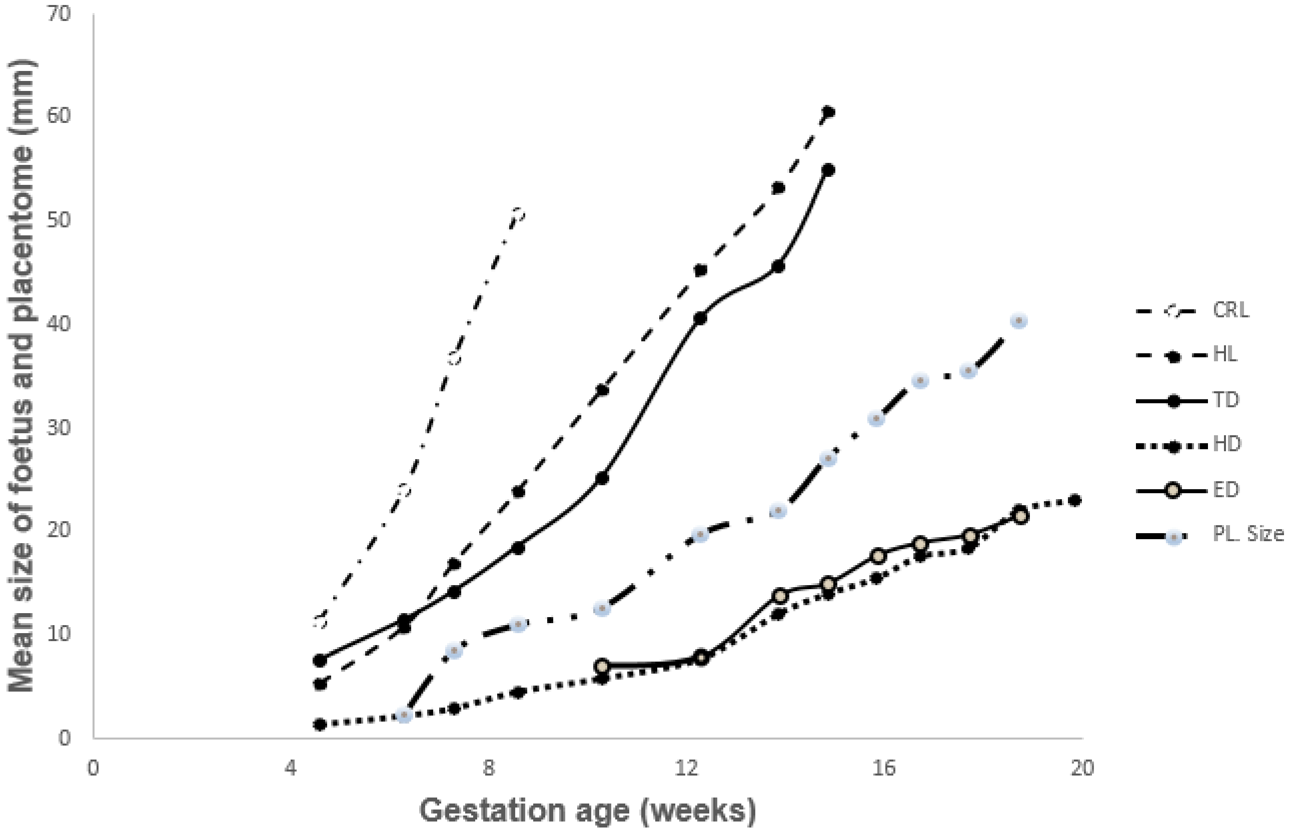


Figure 17. Mean morphometric measurements of fetal and placental development in DM heifers.

From Figure 18, it is clearly noticeable that between week 4 and 20 of gestational age, CRL, HL and TD increased in size more rapidly than HD, ED and placentome diameter. All the parameters were first time detected and measured in similar gestational age which is in week 5.

Table 8. Comparison of CRL between DM and HF fetuses

Gestation week	Crown-rump length (CRL; mm)			P value**
	DM		HF	
	mean(SD)	Number of Animal	Mean*	
4	7.45 (1.18)	12	10.30	<0.001
5	13.99 (0.59)	7	15.70	<0.001
6	24.21 (1.73)	12	23.80	1.00
7	34.01 (2.21)	7	36.20	0.20
8	44.58 (4.73)	7	54.90	0.01

DM = Droughtmasters; HF = Holstein Friesian
*mean measurements were estimated using Equation 1
**Bonferroni adjusted P values

Table 9. Comparison of HL between DM and HF fetuses

Gestation week	Head Length (HL; mm)			P value**
	DM		HF	
	mean(SD)	Number of Animal	Mean*	
5	5.33 (0.71)	7	12.60	<0.001
6	10.78 (0.85)	12	15.00	<0.001
7	16.25 (0.94)	7	17.80	0.01
8	20.45 (2.22)	12	21.20	1.00
9	25.43 (2.02)	10	25.20	1.00
10	31.36 (2.55)	5	3<0.001	1.00
11	37.41 (5.56)	5	35.60	1.00
12	43.80 (1.68)	3	42.40	1.00
13	47.73 (3.11)	6	50.40	0.99
14	54.67 (3.55)	4	59.90	0.66
15	63.25 (4.71)	3	71.30	1.00

DM = Droughtmasters; HF = Holstein-Friesian
*mean measurements were estimated using Equation 2
**Bonferroni adjusted P value

Table 10. Comparison of TD between DM and HF fetuses

Gestation week	Trunk diameter (TD; mm)			P value**
	DM		HF	
	mean(SD)	Number of Animal	Mean*	
5	7.61 (0.72)	7	8.80	0.05
6	11.46 (1.06)	12	10.60	0.17
7	14.35 (0.75)	8	12.80	0.01
8	16.42 (2.33)	12	15.50	1.00
9	19.96 (2.62)	9	18.70	1.00
10	23.24 (0.78)	6	22.60	1.00
11	27.04 (1.23)	8	27.20	1.00
12	40.60 (0.13)	4	32.9	0.01
13	44.48 (1.03)	5	39.70	0.01
14	54.97 (1.60)	5	47.90	0.01

DM = Droughtmasters; HF = Holstein-Friesian
*mean measurements were estimated using Equation 3
**Bonferroni adjusted P values

3.5 Discussion

The DM heifers were all well grown and most if not all were cycling, and the pregnancy rate to FTAI was as expected. Between weeks 5 and 8 CRL increased approximately 5 fold. Between weeks 5 and 12 HL, HD and TD increased 12 ,10 and 7 fold, respectively, PD increased 11 fold between weeks 6 and 12, but ED increased only 2 fold between weeks 9 and 12. The findings for HL,HD and PD are consistent with the reported[15] positive correlation between placentome size and fetal size.

Generally, the size of HF fetuses (as defined by CRL and HL) was greater than DM fetuses during the period week 4 to 15 of gestation. However, the differences were generally only significant during the period week 4 to 7. The first seen fetus from this study was older in gestational age compared to the result from Curran et al (1986) and Pierson and Ginther (1984).

A major limitation of this study was that it was not possible to obtain sufficiently good quality images for measurement of all morphometric parameters at each scanning, especially after day 70 of gestation. This was due to the marked increase in size of the fetus and difficulty in being able to localize specific fetal structures due to fetal movement. Further, with measurements such as TD it was difficult to be able to consistently take measurements in the same plane and position along the trunk of the fetus.

Overall the findings of this study support the hypothesis that early development of the conceptus is similar across distinctly different genotypes. This is a useful finding as the guidelines for ultrasongoraphic aging of conceptuses is primarily based on HF data.

Chapter 4 Fetal and placental development in three Indonesian tropically adapted beef cattle breeds, and comparison with development in HF cattle

4.1 Introduction

This chapter describes the first ever ultrasonographic study of fetal and placental development conducted in tropically adapted Indonesian beef cattle. Three breeds of cattle, Madura, Bali and Ongole cattle were available for study. The puberty weight for Madura cattle was between 170.4±17.4 kg and 225.2±23.9 kg, while mature weight was between 211±18.4 kg and 303.3 ± 4.9 kg[16]. The primary hypothesis of this study was that there is no difference in fetal and placental development during early gestation between Indonesian beef breed cattle (Bali, Ongole and Madura cattle). The secondary hypothesis was that there was no difference in early fetal development between Indonesian breed fetuses and HF fetuses, despite the fact that the birthweight of the former is at least half that of the latter.

4.2 Materials

4.2.1 Animal ethics

The experiment was approved by the Production and Companion Animal (PCA) Animal Ethics Committee of The University of Queensland (Approval number SVS/457/14/ACIAR).

4.2.2 Location and time of experiments

The study was conducted at the Beef Cattle Research Station, Grati-Pasuruan, East Java Indonesia (7°30‟S 113°30‟E) between June, 2015 and January 2016.

4.2.3 Animal selection and management

Twenty non-lactating Ongole (*Bos indicus*) cows (239 $\pm$ 37.04 kg LW, 3 $\pm$ 0.25 BCS), 20 Bali (*Bos javanicus*) heifers (179 $\pm$ 10.68 kg LW, 3 $\pm$ 0.26 BCS, 48 months of age) and 20 Madura (*Bos indicus* x *Bos javanicus*) heifers (193 $\pm$ 27.34 kg LW, 3 $\pm$ 0.28 BCS, 48 months of age) were available for use in this study. Live weight (LW) and health were recorded at the beginning (July 2015), in the middle (October 2015) and at the end of the study (January 2016). Body condition score was defined using the same method as in Chapter 3 (McGowan, 2014). Prior to the commencement of the study all heifers were confirmed to be non-pregnant and had normal reproductive tracts by transrectal ultrasonography of the reproductive tract. All females were treated with kalbazen (Kalbazen®-SG-Albendazole 19 mg/mL, PT. Kalbe Farma, Bekasi, Indonesia) orally to eliminate internal parasites prior to the experiment.

The cattle were held in three open-sided, covered pens with one breed per pen, and remained in the same pen throughout the study. The cattle were offered the same diet which consisted of 5.7 kg (as fed) concentrate (32% rice bran, 23% palm kernel cake, 20% cassava flour, 10% corn waste, 7% potato flour, 7% molasses and 1% of limestone) per head per day, 8.5 kg (as fed) elephant grass (*Pennisetum purpurpeum*) per head per day and rice straw *ad libitum*. The concentrate was offered as a single portion at 0800 h and the elephant grass was offered in two equal portions at 0800 and 1200 h. Drinking water was available at all times.

4.3 Methods

The study was commenced by breed group with a 2 day interval between each breed group in the day scheduled procedures were performed.

On day 0, prior to feeding, live weight and BCS were recorded and each female underwent transrectal ultrasonography of the reproductive tract. All animals were also treated with PGF2α 25 mg/animal (*Dinoprost tromethamine*, Lutalyse™, Pfizer New York, USA) i.m. It took approximately 3 hours to process all animals after which they were returned to their pens. On day 14, a second dose of PGF2α (25 mg/animal) and 15 to 20 cm of paint was applied to the tail head and the animals were then returned to their pens. On the same day a bull of the same breed as the females was introduced to each pen for a period of 7 days. From days 14 to 20, all cattle were under continuous observation for signs of oestrus (mounting, discharge, teasing, tail paint rubbing, and swollen vulva) and matings were recorded. On day 20, all bulls were removed from each pen. On day 31, tail paint was reapplied to all females and each bull was reintroduce to each pen. Again heat detection was conducted twice daily to record matings. On day 38, all bulls were removed from each pen. On day 40 all females were scanned for the first time. Because only a low percentage of each breed group were seen to be mated by Day 38 it was decided to reintroduce the bull to each group for the duration of the study and to continue twice daily observation for signs of oestrus or mating. Each female was scanned once a week for a further approximately 14 weeks. This enabled early detection of pregnant females and date of conception was either determined from observed mating or where mating had not been observed week of conception was estimated from CRL.

4.3.1 Transrectal ultrasonography

Ultrasonographic monitoring of fetal and placental development was conducted using the same methods as chapter 3.The same parameters as measured in chapter 3 were also measured in chapter 4.

4.3.2 Statistical analysis

As in Chapter 3 the ultrasonographic data were measured using image J software before being analysed with R[61]. The data were comprised of morphometric variables (CRL, Head, Hoof, Eye, Trunk, Pl size). Data were summarised and presented as the mean and standard deviation for each parameter. To allow for a visual comparison of different fetal measurements across the Indonesian breeds, a nonparametric cubic spline regression model was used for each of the fetal morphometric variables to generate a smoothed curve as a function of gestation week. The cubic spline was part of the regression analysis method to visualise the parameter of the fetal placental measurement and the gestational age in a curve.

To compare fetal morphometric measurements (outcomes) between Indonesian breeds, each outcome measure was assessed for normality. Any variable which did not appear to be normally distributed was transformed (log transformation was used). After transformation, the transformed variable was assessed to ensure that transformation was successful. For each of the outcome variables, a separate linear mixed effects model was fitted with cows as a random effect and a first order autoregressive function for the residuals. The significance of each of the explanatory variables was assessed using the Likelihood Ratio Test with an alpha level set at 0.2 for the univariable analysis and 0.05 or less for the multivariable analysis. Wald test statistics was used to assess the significance of categorized variables in the univariable and multivariable models. Alpha was set at 0.05 or less. Finally, as described in Chapter 3 above, Student's *t*-test was used to compare the equality of the mean between morphometric variables reported in this study and those estimated using Equations 1-3. Reported P-values were corrected using Bonferroni adjustment to account for multiple hypothesis tests being conducted on the same variable within the same dataset.

4.4 Results

4.4.1 Summary of reproductive performance

The reproductive performance of each pen of females is summarized in Tables 11,12 and 13. All females gained weight and improved body condition during the study. However, only the Madura cattle achieved a satisfactory pregnancy rate consistent with them having a high proportion (75%) of females which had a CL present at the commencement of the study, compared to the Ongole and Bali females (45% and 50%, respectively). Further, it is important to note that there was a marked difference between the proportion of Ongole and Bali females observed in oestrus after the second PGF2α treatment compared to the proportion which were observed to have been mated. This may indicate possible errors in heat detection, some females showing involuntary signs of oestrus but not standing to be mounted and a bull mating ability/libido problem. The net result was that for the Ongole and Bali cattle only a small number of females were available for monitoring of fetal and placental development and the gestational period of monitoring was reduced because it took longer to achieve conception in these females.

Table 11. Summary of reproductive performance Madura cattle

Parameters	Calendar month		
Day of study (month)	**June**	**September**	**December**
Mean weight (kg+SD)	193.40 + 27.34 (n=20)	225.08 + 27.61 (n=20)	255.83 + 32.13 (n=20)
Mean body condition score (BCS+SD)	2.6 + 0.28 (n=20)	3.1 + 0.24 (n=20)	4.2 + 0.37 (n=20)
Proportion with a corpus luteum at commencement (%)	75 (n=15)	N/A	N/A
Proportion detected in oestrus after oestrous synchronisation (%)	65 (n=13)	N/A	N/A
Cumulative proportion observed mated (%)	30 (n=6)	45 (n=9)	45 (n=9)
Cumulative proportion detected pregnant (%)	20 (n=4)	70 (n=14)	75 (n=15)

SD=Standard deviation; N/A=Not available/not measurable

Table 12. Summary of reproductive performance of Bali cattle

Parameters	Calendar month		
Day of study (month)	**June**	**September**	**December**
Mean weight (kg±SD)	179.20 ± 10.68 (n=20)	198.70 ± 14.19 (n=20)	220.35 ± 17.12 (n=20)
Mean body condition score (BCS ± SD)	2.8 ± 0.26 (n=20)	2.9 ± 0.18 (n=20)	3.1 ± 0.22 (n=20)
Proportion with a corpus luteum at commencement (%)	50 (n=10)	N/A	N/A
Proportion detected in oestrus after oestrous synchronisation (%)	55 (n=11)	N/A	N/A
Cumulative proportion observed mated (%)	15 (n=3)	15 (n=3)	15 (n=3)
Cumulative proportion detected pregnant (%)	0	25 (n=5)	25 (n=5)

SD=Standard deviation; N/A=Not available/not measurable

Table 13. Summary of reproductive performance of Ongole cattle

Parameters	Calendar month		
Day of study (month)	**June**	**September**	**December**
Mean weight (kg+SD)	238.74 + 37.04 (n=20)	254.88 + 36.76 (n=17)	280.40 + 38.52 (n=17)
Mean body condition score (BCS+SD)	2.7 + 0.25 (n=20)	3.1 + 0.20 (n=17)	3.6 + 0.41 (n=17)
Proportion with a corpus luteum at commencement (%)	45 (n=9)	N/A	N/A
Proportion detected in oestrus after oestrous synchronisation (%)	55 (n=11)	N/A	N/A
Cumulative proportion observed mated (%)	10 (n=2)	35 (n=6)	35 (n=6)
Cumulative proportion detected pregnant (%)	0	18 (n=3)	25 (n=5)

SD=Standard deviation; N/A=Not available/not measurable

4.4.2 Fetal and placental development in Madura, Bali and Ongole

The observed fetal and placental development in Ongole, Bali and Madura cattle is summarized in Table 14.4-16. Retrospective examination of the data demonstrated that at week 4 the fetus was detected in 40%, 75% and 100% of pregnant Madura, Bali and Ongole females, respectively. The reasons for being unable to detect the conceptus at week 4 in all pregnant females was mainly due to difficulties in initially scanning some females which had only ever been scanned once before. By week 5 the fetus could be detected in all pregnant females enabling measurement of CRL. However, to measure each fetal parameter at each scanning requires a good quality image which was not always possible to obtain due to the temperament of some females and positioning of the conceptus in tightly coiled uterine horns. Bubble plots of the pattern of change in CRL, HD, HL, TD, ED and PD are presented in Figures 19 and 20. The patterns of fetal and placental development were very similar across breeds. Also the trajectory of development of each measure was similar (primarily linear) except for ED.

Table 14. Fetal and placental development in Madura cattle.

Week of gestation	Total number of pregnant animals scanned	Mean fetal measurement (n)					Mean diameter of placentome (mm)
		CRL (mm)	HL (mm)	TD (mm)	HD (mm)	ED (mm)	
4	6	5.54 ± 1.36 (n=6)	NA	NA	NA	NA	NA
5	15	12.66 ± 3.21 (n=15)	NA	NA	NA	NA	NA
6	15	24.78 ± 6.56 (n=14)	13.53 ± 3.12 (n=9)	11.81 ± 1.98 (n=10)	2.92 ± 0.55 (n=7)	NA	8.26 ± 2.11 (n=8)
8	15	41.09 ± 7.11 (n=12)	19.73 ± 3.15 (n=13)	14.67 ± 2.74 (n=13)	3.51 ± 0.70 (n=11)	4.73 ± 1.08 (n=2)	10.98 ± 2.19 (n=11)
9	15	NA	27.01 ± 3.33 (n=12)	19.35 ± 2.89 (n=12)	4.70 ± 0.84 (n=11)	5.26 ± 0.59 (n=9)	15.21 ± 2.72 (n=13)
11	15	NA	34.80 ± 3.66 (n=9)	24.69 ± 3.99 (n=10)	5.97 ± 0.88 (n=8)	8.11 ± 1.43 (n=10)	24.06 ± 3.20 (n=12)
12	15	NA	40.30 ± 6.05 (n=10)	31.14 ± 4.90 (n=11)	7.28 ± 1.02 (n=12)	10.09 ± 1.29 (n=11)	20.38 ± 3.69 (n=10)
13	15	NA	50.11 ± 5.55 (n=9)	35.56 ± 3.27 (n=6)	9.60 ± 1.97 (n=8)	12.61 ± 1.56 (n=11)	25.37 ± 2.11 (n=8)
15	15	NA	NA	NA	12.69 ± 2.27 (n=6)	15.99 ± 2.12 (n=5)	28.79 ± 6.50 (n=5)
16	15	NA	NA	NA	14.37 ± 3.31 (n=6)	17.46 ± 1.32 (n=4)	26.77 ± 5.52 (n=7)
18	15	NA	NA	NA	12.34 ± 1.47 (n=5)	17.01 ± 3.04 (n=5)	32.83 ± 3.20 (n=7)
19	15	NA	NA	NA	15.85 ± 0.67 (n=5)	19.09 ± 0.26 (n=2)	37.01 ± 3.03 (n=4)
20	15	NA	NA	NA	17.95 ± 0.07 (n=2)	NA	40.57 ± 8.76 (n=3)

Key: CRL=Crown rump length; HL=Head length, TD=Trunk diameter; HD=Hoof diameter; ED=Eye diameter; mm=millimeter.

Table 15. Fetal and placental development in Bali cattle

Week of gestation	Total number of pregnant animals scanned	Mean fetal of measurement(n)					Mean diameter of placentome
		CRL (mm)	HL (mm)	TD (mm)	HD (mm)	ED (mm)	
4	3	7.18 ± 1.95 (n=3)	NA	NA	NA	NA	NA
5	5	13.23 ± 2.09 (n=5)	4.75 ± 0.77 (n=5)	6.83 ± 1.56 (n=5)	NA	NA	NA
6	5	25.83 ± 6.86 (n=5)	12.65 ± 2.73 (n=5)	11.23 ± 1.23 (n=5)	2.14 ± 0.54 (n=5)	NA	NA
7	5	36.41 ± 5.10 (n=4)	17.62 ± 1.93 (n=4)	13.85 ± 1.65 (n=4)	3.04 ± 0.64 (n=4)	NA	7.01 ± 0.02 (n=4)
9	5	NA	24.65 ± 2.67 (n=5)	18.55 ± 3.57 (n=5)	4.97 ± 0.71 (n=5)	5.10 ± 1.89 (n=3)	11.16 ± 2.12 (n=5)
11	5	NA	33.79 ± 3.79 (n=3)	25.44 ± 3.11 (n=3)	5.94 ± 0.45 (n=3)	7.42 ± 1.31 (n=3)	16.82 ± 2.62 (n=3)
12	5	NA	36.75 ± 7.82 (n=4)	30.37 ± 3.72 (n=5)	8.12 ± 0.80 (n=5)	10.07 ± 1.50 (n=5)	20.83 ± 3.19 (n=5)
13	5	NA	NA	32.78 ± 5.14 (n=4)	9.15 ± 0.99 (n=4)	13.33 ± 2.08 (n=3)	25.70 ± 2.18 (n=5)
15	5	NA	NA	NA	11.6 ± 0.42 (n=4)	NA	NA
16	5	NA	NA	NA	12.00 ± 0.75 (n=3)	15.93 ± 0.73 (n=3)	35.36 ± 1.68 (n=3)

Key: CRL=Crown rump length; HL=Head length, TD=Trunk diameter; HD=Hoof diameter; ED=Eye diameter; mm=millimeter.

Table 16. Fetal and placental development in Ongole cattle

Week of gestation	Total number of pregnant animals scanned	Mean fetal measurement (n)					Mean diameter of placentome
		CRL (mm)	HL (mm)	TD (mm)	HD (mm)	ED (mm)	
4	4	4.47 ± 0.28 (n=4)	NA	NA	NA	NA	NA
5	5	15.91 ± 2.50 (n=5)	9.19 ± 1.34 (n=5)	8.76 ± 1.16 (n=3)	1.89 ± 0.52 (n=3)	NA	6.18 ± 2.76 (n=2)
6	5	23.41 ± 5.68 (n=5)	11.97 ± 2.13 (n=5)	10.43 ± 1.10 (n=5)	2.24 ± 0.48 (n=5)	NA	6.70 ± 2.16 (n=4)
8	5	38.60 ± 7.05 (n=4)	18.82 ± 3.41 (n=5)	15.00 ± 1.84 (n=5)	3.08 ± 0.4 (n=5)	5.52 ± 1.05 (n=2)	8.47 ± 1.94 (n=4)
9	5	NA	27.01 ± 2.78 (n=4)	20.07 ± 1.96 (n=4)	4.76 ± 0.67 (n=4)	6.73 ± 0.25 (n=2)	11.11 ± 1.12 (n=2)
10	5	NA	33.10 ± 0.71 (n=2)	24.58 ± 0.31 (n=2)	NA	8.15 ± 0.50 (n=2)	12.46 ± 1.25 (n=2)

Key: CRL=Crown rump length; HL=Head length, TD=Trunk diameter; HD=Hoof diameter; ED=Eye diameter; mm=millimeter.

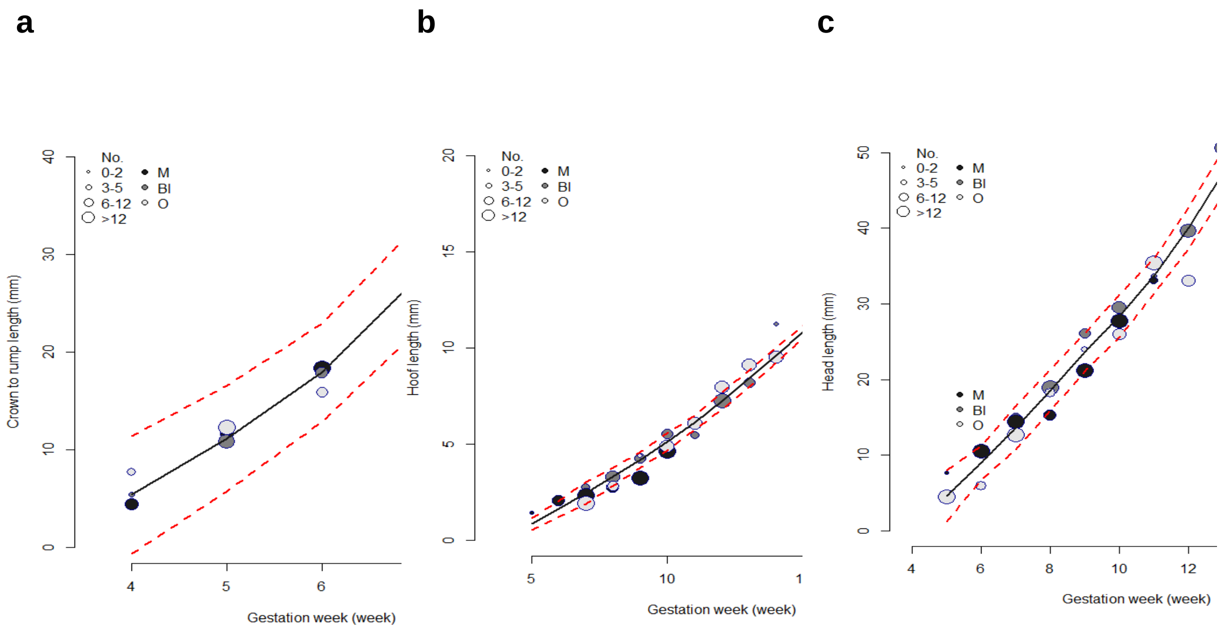
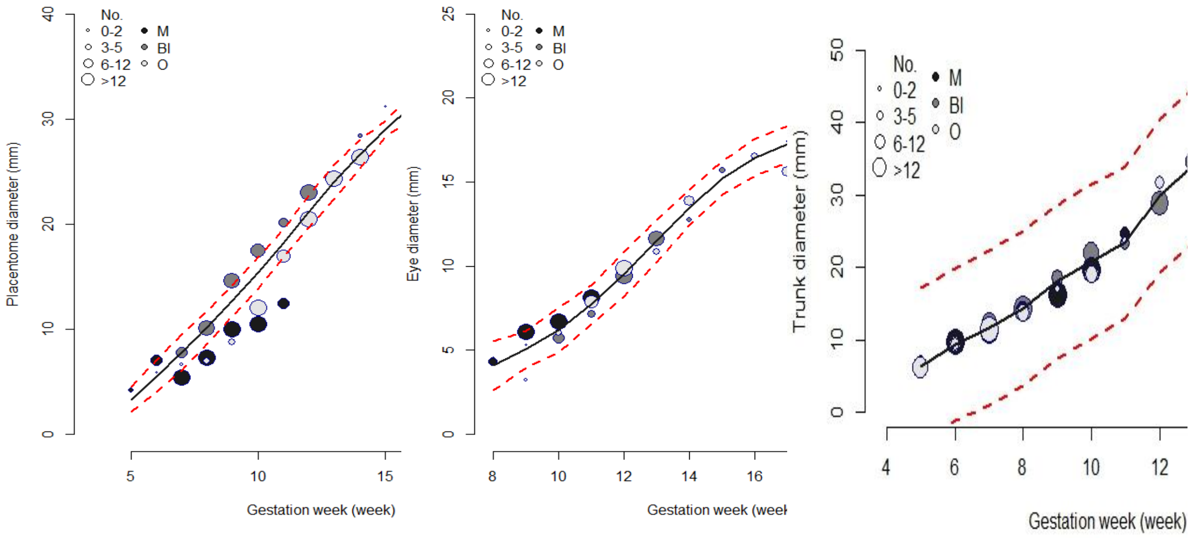


Figure 18. Bubble plots of a) mean CRL b) hoof diameter and c) head length as a function of gestation week for Madura, Bali and Ongole fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalized cubic regression line fitted to the data for each measure. The dashed lines represent the lower and upper 95% predictions.

a b



c

Figure 19. Bubble plots of a) placentome diameter, b) eye diameter and c) trunk diameter as a function of gestation week for Madura, Bali and Ongole fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the data for each measure. The dashed lines represent the lower and upper 95% predictions.

Results from the multivariable linear mixed models are shown in Table 17. to 22. For CRL (Table 17.), after controlling for the effect of breed, mean CRL approximately doubled (0.48; 95% CI 0.44 to 0.52, P value <0.001) each week between the 4th and 8th week of gestation. CRL measurement of Bali fetuses tended to be on average 10% (0.10 95% CI -0.11 to 0.30, P value 0.34) greater than that of Madura fetuses, while Ongole CRL measurements tended to be on average 1% (-0.01; 95% CI -0.20 to 0.19, P value 0.95) lower than Madura fetuses . The rate of increase per week of gestation in CRL for Madura fetuses was 6% and 3% less, respectively, compared to Bali (-0.06; 95% CI -0.13 to 0.02, P value 0.12) and Ongole fetuses (-0.03; 95% CI -0.09 to 0.04, P value 0.40). However, this difference in rate of increase was not significant.

Similarly, mean hoof diameter (Table 18.) increased approximately 7% (0.07; 95% CI 0.06 to 8.41, P value <0.001) between the 5th and 20th week of gestation. Hoof diameter measurement of Madura fetuses tended to be on average 14% (0.14; 95% CI 0.03 to 2.48, P value 0.01) greater than that of Bali fetuses , while Ongole hoof diameter measurements tended to be on average 9% lower than Bali fetuses (-0.09; 95% CI -0.24 to 5.76, P value 0.21). The rate of increase per week of gestation in hoof length for Bali fetuses was approximately 99% higher compared to Madura (-0.01; 95% CI -0.02 to 8.98, P value 0.05) and 98% less compared to Ongole fetuses (0.02; 95% CI -0.01 to 4.58, P value 0.17). The difference in rate of increase was not significant in gestation week between breeds.

Results for head length analysis are presented in Table 19.. After controlling for the effect of breed, mean head length increased approximately 8% (0.08; 95% CI 0.07 to 0.10, P value <0.001) per week between the 4th and 16th week of gestation. Head length measurement of Bali and Ongole fetuses tended to be on average lower than that of Madura fetuses by 23% (-0.23; 95% CI -0.34 to -0.12, P value <0.001) and 12% (-0.12; 95% CI -0.27 to 0.02, P value 0.08), respectively. For placentome diameter (Table 20.), Bali cattle and Ongole tended to be on average lower than that of Madura by 11% (-0.11; 95% CI -0.27 to 0.04, P value 0.15) and 26% (-0.62; 95% CI -0.44 to -0.09, P value 0.01), respectively.

Analysis of changes in eye and trunk diameter are presented in Table 21. and 22, respectively. Eye diameter increased by 6% (0.06; 95% CI 0.05 to 0.07, P value <0.001) each week between the 8th and the 22nd week of gestation. Compared to Madura fetuses the increase per week in eye diameter in Bali cattle was 2% higher (0.02; 95% CI -0.14 to 0.17, P value 0.82), while for Ongole fetuses it was 10% lower (-0.10; 95% CI -0.47 to 0.27, P value 0.58). Trunk diameter increased by approximately 8% (0.08; 95% CI 0.07 to 0.08, P value <0.001) between the 4th and the 16th week of gestation. The overall development in trunk diameter of Madura fetuses was greater than that in Bali fetuses but not in Ongole fetuses. The weekly increase in trunk diameter of Bali fetuses was 9% lower (-0.09; 95% CI -0.17 to 0.01, P value 0.03) and in Ongole fetuses 5% lower (-0.05; 95% CI -0.15 to 0.04, P value 0.27) than that for Madura fetuses.

Table 17. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing change in CRL in Bali, Madura and Ongole cattle between week four to eight (inclusive) of gestation

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	1.85 (0.05)	1.75 to 1.96	<0.001
Gestation week	0.48 (0.02)	0.44 to 0.52	<0.001
Breed			
Madura	**Reference**	-	-
Bali	0.10 (0.10)	-0.11 to 0.30	0.34
Ongole	-0.01 (0.01)	-0.20 to 0.19	0.95
Gestation week × Breed			
Madura	**Reference**		
Bali	-0.06 (0.04)	-0.13 to 0.02	0.12
Ongole	-0.03 (0.03)	-0.09 to 0.04	0.40

Key: SE (standard error)

Table 18. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing hoof diameter in Bali, Madura and Ongole cattle between week five to twenty (inclusive) of gestation.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.17 (0.04)	0.09 to 2.57	<0.001
Gestation Week	0.07 (<0.001)	0.06 to 8.41	<0.001
Breed			
Bali*	**Reference**	-	-
Madura	0.14 (0.05)	0.03 to 2.48	0.01
Ongole	-0.09 (0.07)	-0.24 to 5.76	0.21
Gestation week × Breed			
Bali	**Reference**		
Madura	-0.01 (0.01)	-0.02 to 8.98	0.05
Ongole	0.02 (0.01)	-0.01 to 4.58	0.17

Key: SE (standard error); * the reference breed was changed to allow the statistical model to converge

Table 19. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing head length in Bali, Madura and Ongole cattle between week four to fourteen (inclusive) of gestation.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.93 (0.04)	0.85 to 0.10	<0.001
Gestation Week	0.08 (0.01)	0.07 to 0.10	<0.001
Breed :			
Madura	**Reference**	-	-
Bali	-0.23 (0.05)	-0.34 to -0.12	<0.001
Ongole	-0.12(0.07)	-0.27 to 0.02	0.08
Gestation week × Breed =			
Madura	**Reference**		
Bali	0.02 (0.01)	<0.001 to 0.04	0.02
Ongole	0.02 (0.01)	-0.01 to 0.05	0.17

Key: SE (standard error)

Table 20. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing placentome diameter in Bali, Madura and Ongole cattle between week five to twenty (inclusive) of gestation.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.86 (0.04)	0.79 to 0.93	<0.001
Gestation Week	0.05 (<0.001)	0.04 to 0.06	<0.001
Breed :			
Madura	**Reference**		
Bali	-0.11 (0.08)	-0.27 to 0.04	0.15
Ongole	-0.26 (0.09)	-0.44 to -0.09	0.01
Gestation week × Breed =			
Madura	**Reference**		
Bali	0.01 (0.01)	-0.01 to 0.02	0.33
Ongole	0.02 (0.02)	-0.01 to 0.05	0.25

Key: SE (standard error)

Table 21. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing eye diameter in Bali, Madura and Ongole cattle between week eight to twenty-two (inclusive) of gestation.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.48 (0.04)	0.39 to 0.56	<0.001
Gestation Week	0.06 (<0.001)	0.05 to 0.07	<0.001
Breed :			
Madura	**Reference**		
Bali	0.02 (0.0)	-0.14 to 0.17	0.82
Ongole	-0.10 (-0.1)	-0.47 to 0.27	0.58
Gestation week × Breed =			
Madura	**Reference**		
Bali	-<0.001 (0.01)	-0.02 to 0.08	0.54
Ongole	0.02 (0.03)	-0.04 to 0.08	0.55

Key: SE (standard error)

Table 22. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing trunk diameter in Bali, Madura and Ongole cattle between week four to twelve (inclusive) of gestation.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.85 (0.03)	0.80 to 0.90	<0.001
Gestation Week	0.08 (<0.001)	0.07 to 0.08	<0.001
Breed :			
Madura	**Reference**		
Bali	-0.09 (0.04)	-0.17 to 0.01	0.03
Ongole	-0.05 (0.05)	-0.15 to 0.04	0.27
Gestation week × Breed =			
Madura	**Reference**		
Bali	0.01 (0.01)	<0.001 to 0.02	0.19
Ongole	0.01 (0.01)	-0.01 to 0.03	0.40

Key: SE (standard error)

4.4.3 Comparison of fetal development in Madura, Bali and Ongole cattle and HF cattle

Table 23. to 31 summarise the findings of comparisons of fetal development between each Indonesian breed and that predicted for the HF breed. The P value was a significance prediction of the comparison between breed from the equation. With few exceptions, the CRL of HF fetuses was greater than that for Madura, Bali and Ongole fetuses. However, for head length and trunk diameter only for a few individual gestation weeks were there any significant differences between the Indonesian breed fetuses and the HF fetuses.

Table 23. Comparison between changes in CRL between Madura cattle and HF cattle

Gestation week	Crown-rump length (CRL; mm)		P value**
	Madura mean(SD)	HF mean*	
4	5.54 (1.36)	10.30	<0.001
5	9.26 (1.07)	15.70	<0.001
6	17.67 (3.68)	23.80	<0.001
7	25.43 (3.06)	36.20	<0.001
8	40.01 (2.72)	54.90	<0.001

*mean measurements were estimated using Equation 1
**Bonferroni adjusted P values

Table 24. Comparison between changes in HL in Madura and HF fetuses

Gestation week	Head length (HL; mm)		P value**
	Madura mean(SD)	HF mean*	
7	13.53 (3.12)	17.80	0.02
8	19.08 (2.74)	21.20	0.82
9	26.60 (3.37)	25.20	1.00
10	29.66 (3.19)	30.00	1.00
11	33.26 (2.88)	35.60	1.00
12	40.09 (4.34)	42.40	0.64
13	50.65 (2.79)	50.40	1.00

*mean measurements were estimated using Equation 2
**Bonferroni adjusted P values

Table 25. Comparison between changes in TD in Madura and HF fetuses

Gestation week	Trunk diameter (TD; mm)		P value**
	Madura mean(SD)	HF mean*	
7	11.81 (1.98)	12.80	1.00
8	16.42 (2.33)	15.50	1.00
9	19.96 (2.62)	18.70	1.00
10	23.24 (0.78)	22.60	1.00
11	27.04 (1.23)	27.20	0.18
12	40.60 (0.13)	32.9	0.06
13	44.48 (1.03)	39.70	0.01
14	54.97 (1.60)	47.90	0.83

*mean measurements were estimated using Equation 3
**Bonferroni adjusted P values

Table 26. Comparison between changes in CRL between Bali cattle and HF cattle

Gestation week	Crown-rump length (CRL; mm)		P value**
	Bali mean(SD)	HF mean*	
4	6.70 (1.98)	10.30	0.44
5	11.55 (2.48)	15.70	0.05
6	16.02 (0.73)	23.80	<0.001
7	23.42 (2.45)	36.20	0.06
8	34.10 (2.64)	54.90	0.03

*mean measurements were estimated using Equation 1
**Bonferroni adjusted P values

Table 27. Comparison between changes in HL in Bali and HF fetuses

Gestation week	Head length (HL; mm)		P value**
	Bali mean(SD)	HF mean*	
5	4.37 (0.48)	12.60	<0.001
6	6.91 (2.13)	15.00	0.16
7	13.31 (2.45)	17.80	0.11
8	17.62 (1.93)	21.20	0.60
9	23.92 (1.92)	25.20	1.00
10	26.49 (1.43)	3<0.001	0.36
11	36.52 (0.95)	35.60	0.05

*mean measurements were estimated using Equation 2
**Bonferroni adjusted P values

Table 28. Comparison between changes in TD in Bali and HF fetuses

Gestation week	Trunk diameter (TD; mm)		P value**
	Bali mean(SD)	HF mean*	
5	5.99 (0.68)	8.80	0.07
6	9.27 (0.79)	10.60	0.90
7	11.44 (1.20)	12.80	0.35
8	13.85 (1.65)	15.50	1.00
9	16.94 (2.88)	18.70	1.00
10	22.66 (1.68)	22.60	1.00
11	25.44 (3.11)	27.20	1.00
12	31.63 (2.78)	32.9	1.00
13	35.92 (2.10)	39.70	1.00

*mean measurements were estimated using Equation 3
**Bonferroni adjusted P values

Table 29. Comparison between changes in CRL between Ongole cattle and HF cattle.

Gestation week	Crown-rump length (CRL; mm)		P value**
	Ongole mean(SD)	HF mean*	
4	4.47 (0.28)	10.30	<0.001
5	12.46 (2.95)	15.70	0.46
6	18.19 (2.49)	23.80	0.03
7	25.72 (0.97)	36.20	0.01

*mean measurements were estimated using Equation 1
**Bonferroni adjusted P values

Table 30. Comparison between changes in HL in Ongole and HF fetuses

Gestation week	Head length (HL; mm)		P value**
	Ongole mean(SD)	HF mean*	
6	10.46 (0.68)	15.00	0.01
7	13.47 (2.03)	17.80	0.14
8	15.50 (1.34)	21.20	0.11
9	21.30 (1.68)	25.20	0.11
10	27.01 (2.78)	30.00	0.73
11	33.10 (0.71)	35.60	0.75

*mean measurements were estimated using Equation 2
**Bonferroni adjusted P values

Table 31. Comparison between changes in TD in Ongole and HF fetuses

Gestation week	Trunk diameter (TD; mm)		P value**
	Ongole mean(SD)	HF mean*	
6	9.52 (0.44)	10.60	0.10
7	11.34 (0.65)	12.80	0.12
8	13.47 (1.38)	15.50	1.00
9	16.16 (1.18)	18.70	0.14
10	20.07 (1.96)	22.60	0.49
11	24.58 (0.31)	27.20	0.01

*mean measurements were estimated using Equation 3
**Bonferroni adjusted P values

4.5 Discussion

Although the cows enrolled in this study were not lactating and both heifers and cows were in adequate body condition only the reproductive performance of the Madura cattle could be considered satisfactory. In the case of the Bali heifers the poorer than expected performance was partly due to the fact that the mean live weight was sub-optimal and thus some heifers had not reached puberty. Unfortunately, although each of the bulls used to mate the Ongole and Bali females had histories of being proven breeders, both were subsequently shown to be subfertile and had to be replaced.

The pattern of fetal and placental development as defined by the measures used in this study were similar across the 3 Indonesian breeds. Between weeks 4 to 8 CRL increased 7.5, 5 and 8.6 fold respectively for Madura, Bali and Ongole fetuses. For the other measures the increase in size over the period week 5 to 13 of gestation ranged from 2 to 4 fold. Although the CRL and eye diameter for Bali fetuses was greater than that for Madura and Ongole the other fetal measurements (hoof diameter, head length, trunk diameter and placentome diameter) were greater for Madura fetuses than Bali and Ongole.

For the measures of fetal development analysed only mean CRL was greater for the HF fetuses compared to the Indonesian breed fetuses (especially the Madura). For HL and TD significant differences between the Indonesian breed fetuses and HF fetuses only occurred at a few time points during the period of monitoring. Collectively these are very interesting findings as there are very marked differences in the birthweight and mature cow weight and size of HF cattle compared to the Indonesian breed cattle.

The major limitation of this study was the low number of pregnant Ongole and Bali cattle available for monitoring of fetal and placental development. However, the limited data for these two breed indicated that the pattern of fetal and placental development was very similar to that observed for the Madura.

Chapter 5 Comparison of fetal and placental development between DM, Madura, Bali and Ongole cattle

5.1 Introduction

This chapter will combine the first and second study conducted in tropically adapted beef cattle in Australia and Indonesia beef cattle to address the gap in the knowledge on fetal placental development. Moreover, this chapter will provide more detail related to the result of data analysis on DM, Bali, Madura and Ongole cattle.

The hypothesis of this study was that there is no difference in fetal and placental development during early gestation between Australia (DM cattle) and Indonesia beef breed cattle (Bali, Ongole and Madura cattle).

The main objective of this chapter was to compare fetal and placental morphometric measurements from an Australian tropically adapted beef breed with those from three Indonesian tropically adapted beef breeds .

5.2 Materials

5.2.1 Data collection and management

Data on fetal and placental development of DM from the first study were compared to data for Bali, Ongole and Madura derived from the second study.

5.3 Methods

5.3.1 Statistical analysis

To allow for a visual comparison of different fetal measurements across the four breeds of cattle, a nonparametric cubic spline regression model was used for each of the fetal morphometric variables to generate a smoothed curve as a function of gestation week. To compare fetal morphometric measurements (outcomes) between the four breeds, each outcome measure was visualised and assessed for normality. Any variable which did not appear to be normally distributed was transformed (log transformation was used). After transformation, the transformed variable was assessed to ensure that transformation was successful. For each of the outcome variables, a separate linear mixed effects model was fitted with cows as a random effect and a first order autoregressive function for the residuals. The significance of each of the explanatory variables was assessed using the Likelihood Ratio

Test with an alpha level set at 0.2 for the univariable analysis and 0.05 or less for the multivariable analysis. Wald test statistics was used to assess the significance of categorized variables in the univariable and multivariable models. Alpha was set at 0.05 or less. Data analysis was done using R[61].

5.4 Results

Bubble plots comparing the findings for each breed for each measure of fetal and placental development used are presented in Figures 21 to 26. These highlight the similarity in pattern of development across the breeds. The results from the multivariable analysis are presented in Tables 32-37. CRL of Bali, Madura and Ongole fetuses were on average shorter than that of DM fetuses by 20% (-0.20 95% CI -0.39 to -0.11, P value 0.04), 31% (-0.31; 95% CI -0.45 to -0.16, P value <0.001) and 32% (-0.32; 95% CI -0.51 to -0.14, P value <0.001), respectively (Table 32.).

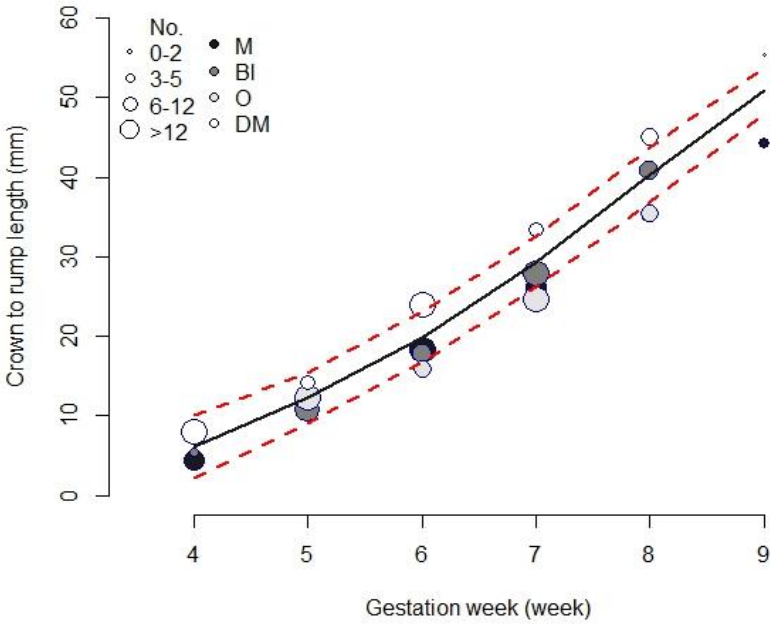


Figure 20. Bubble plot of CRL as a function of gestation week for Madura, Bali, Ongole and DM fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the CRL data. The dashed lines represent the lower and upper 95% predictions.

Table 32. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing CRL of DM, Bali, Madura and Ongole fetuses.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	2.15 (0.05)	2.06 to 2.25	<0.001
Gestation week (week)	0.43 (0.02)	0.40 to 0.47	<0.001
Breed			
DM	**Reference**		
Bali	-0.20 (0.09)	-0.39 to -0.01	0.04
Madura	-0.31 (0.07)	-0.45 to -0.16	<0.001
Ongole	-0.32 (0.09)	-0.51 to -0.14	<0.001
Gestation week × Breed			
DM	**Reference**		
Bali	-0.02 (0.04)	-0.09 to 0.06	0.68
Madura	0.05 (0.03)	-0.01 to 0.01	0.10
Ongole	0.02 (0.03)	-0.05 to 0.08	0.61

Key: SE (standard error)

Hoof diameter (Table 33) of Bali and Ongole fetuses were/tended to be on average less than that of DM fetuses by 6% (-0.06 95% CI -0.16 to 0.03, P value 0.18), and 15% (-0.15; 95% CI -0.27 to -0.04, P value 0.01), respectively, while Madura fetuses were 8% greater (0.08; 95% CI <0.001 to 0.15, P value 0.04). The rate of increase per week of gestation in hoof diameter for Madura fetuses was on average 1% less (-0.01; 95% CI -0.02 to -0.01, P value <0.001) than that of DM fetuses, while Ongole fetuses tended to be 2% greater (0.02; 95% CI -0.01 to 0.04, P value 0.16), respectively.

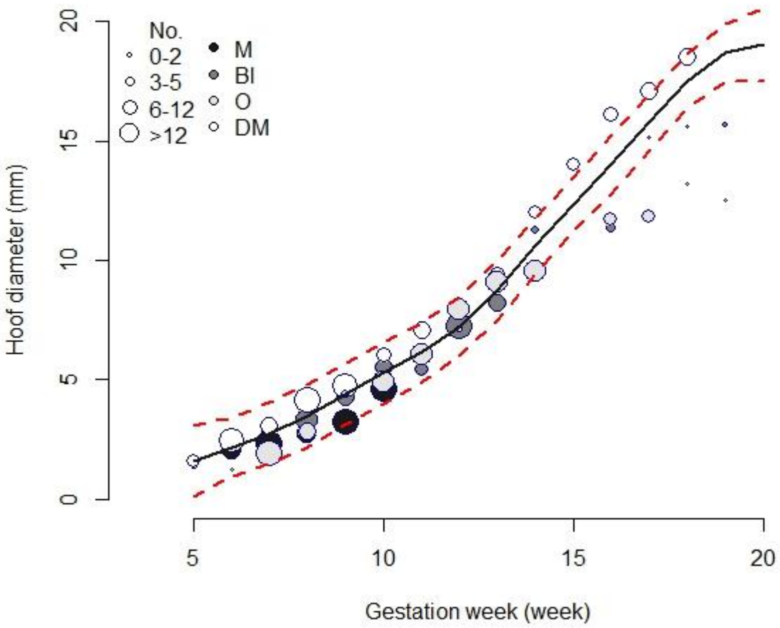


Figure 21. Bubble plot of hoof diameter as a function on gestation week for Madura, Bali, Ongole and DM fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the hoof diameter data. The dashed lines represent the lower and upper 95% predictions.

Table 33. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing hoof diameter in DM, Bali, Madura and Ongole fetuses.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.23 (0.02)	0.19 to 0.28	<0.001
Gestation week (week)	0.08 (<0.001)	0.07 to 0.08	<0.001
Breed			
DM	**Reference**		
Bali	-0.06 (0.05)	-0.16 to 0.03	0.18
Madura	0.08 (0.04)	<0.001 to 0.15	0.04
Ongole	-0.15 (0.06)	-0.27 to -0.04	0.01
Gestation week × Breed			
DM	**Reference**		
Bali	-<0.001 (0.01)	-0.01 to 0.01	0.65
Madura	-0.01 (<0.001)	-0.02 to -0.01	<0.001
Ongole	0.02 (0.01)	-0.01 to 0.04	0.16

Key: SE (standard error)

Head length (Table 34.) of Bali (-0.20 95% CI -0.30 to 0.11, P value <0.001) and Ongole fetuses (-0.03; 95% CI -0.16 to 0.10, P value 0.62) was/tended to be less than that of DM fetuses, while Madura fetuses were 9% greater (0.09; 95% CI <0.001 to 0.18, P value 0.05). The rate of increase per week of gestation in head length for Bali and Ongole fetuses were 3% (0.03; 95% CI -0.01 to 0.04, P value <0.001) and 1% (0.01; 95% CI -0.02 to 0.03, P value 0.55) greater, respectively, compared to DM fetuses, while Madura fetuses tended to be 1% less (-0.01; 95% CI -0.02 to <0.001, P value 0.10).

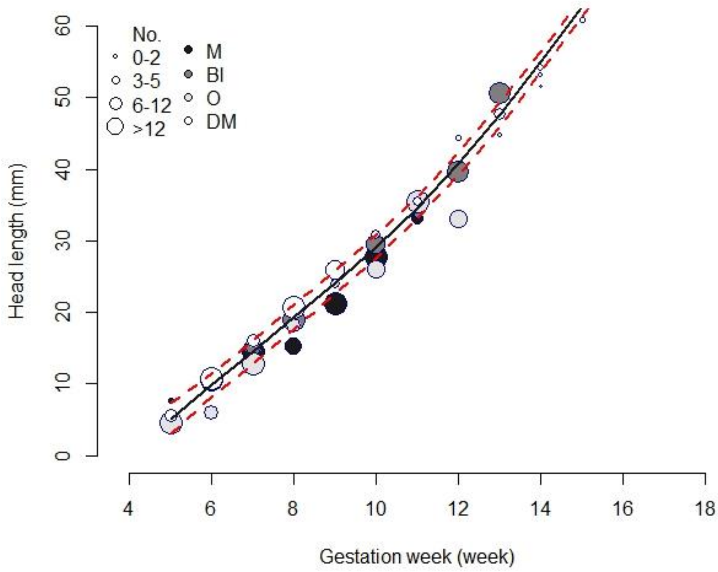


Figure 22. Bubble plot of head length as a function of gestation week for Madura, Bali, Ongole and DM fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the head length data. The dashed lines represent the lower and upper 95% predictions.

Table 34. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing head length in DM, Bali, Madura and Ongole fetuses.

Variable	**Coefficient (SE)**	**95% Confidence interval**	**P-value**
Intercept	0.83 (0.03)	0.78 to 0.88	<0.001
Gestation week (week)	0.10 (<0.001)	0.09 to 0.10	<0.001
Breed			
DM	**Reference**		
Bali	-0.20 (0.05)	-0.30 to 0.11	<0.001
Madura	0.09 (0.05)	<0.001 to 0.18	0.05
Ongole	-0.03 (0.06)	-0.16 to 0.10	0.62
Gestation week × Breed			
DM	**Reference**		
Bali	0.03 (0.01)	0.01 to 0.04	<0.001
Madura	-0.01 (0.01)	-0.02 to <0.001	0.10
Ongole	0.01 (0.01)	-0.02 to 0.03	0.55

Key: SE (standard error)

Overall trunk diameter development (Table 35.) of Bali fetuses was 12% lower (-0.12 95% CI -0.18 to -0.06, P value <0.001) than that of DM fetuses. However, the rate of increase per week of gestation in trunk diameter for Bali fetuses tended to be 1% greater (0.01; 95% CI -<0.001 to 0.02, P value 0.07) than DM fetuses. Overall placentome diameter development of Madura conceptuses were 15% greater than that of DM conceptuses (Table 36.). However, the rate of increase per week of gestation in placentome diameter of Madura conceptuses was 1% lower than that of DM conceptuses. Overall eye diameter development and rate of development (Table 37.) did not differ between breeds.

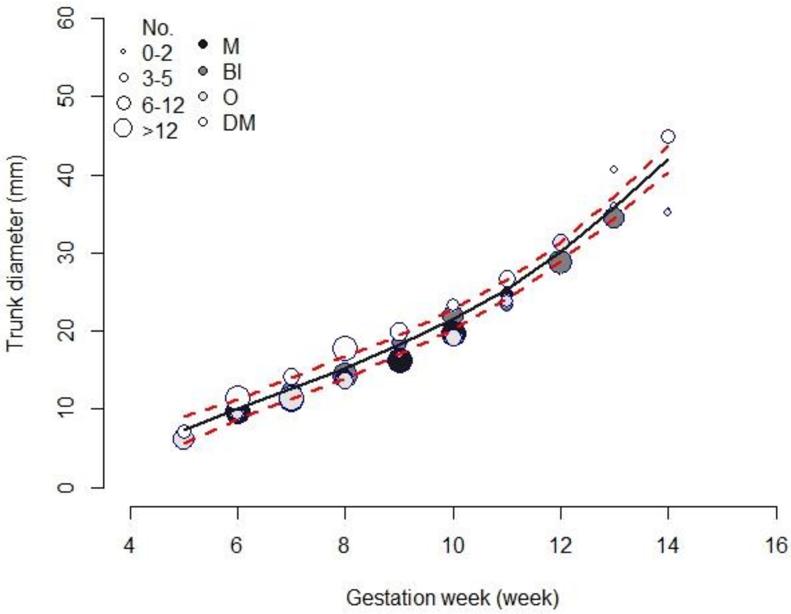


Figure 22. Bubble plot of trunk diameter as a function of gestation week in Madura, Bali, Ongole and DM fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the trunk diameter data. The dashed lines represent the lower and upper 95% predictions.

Table 35. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing trunk diameter in DM, Bali, Madura and Ongole fetuses.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.88 (0.02)	0.84 to 0.91	<0.001
Gestation week (week)	0.08 (<0.001)	0.08 to 0.09	<0.001
Breed			
DM	**Reference**		
Bali	-0.12 (0.03)	-0.18 to -0.06	<0.001
Madura	-0.01 (0.03)	-0.07 to 0.04	0.62
Ongole	-0.07 (0.04)	-0.15 to <0.001	0.06
Gestation week × Breed			
DM	**Reference**		
Bali	0.01 (<0.001)	-<0.001 to 0.02	0.07
Madura	-0.01 (<0.001)	-0.01 to <0.001	0.15
Ongole	<0.001 (0.01)	-0.01 to 0.02	0.75

Key: SE (standard error)

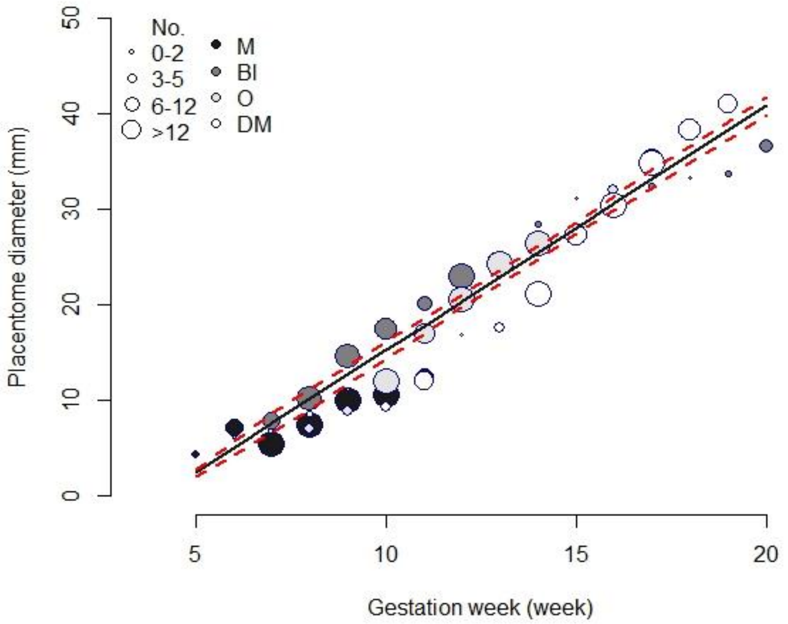


Figure 23. Bubble plot of placentome diameter as a function of gestation week for Madura, Bali, Ongole and DM cattle. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the placentome diameter data. The dashed lines represent the lower and upper 95% predictions.

Table 36. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing placentome diameter in DM, Bali, Madura and Ongole cattle.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.70 (0.05)	0.84 to 0.91	<0.001
Gestation week (week)	0.06 (<0.001)	0.09 to 0.10	<0.001
Breed			
DM	**Reference**		
Bali	-0.09 (0.08)	-0.18 to -0.06	0.25
Madura	0.15 (0.57)	-0.07 to 0.04	0.01
Ongole	-0.11 (0.08)	-0.15 to <0.001	0.19
Gestation week × Breed			
DM	**Reference**		
Bali	0.01 (0.01)	-<0.001 to 0.02	0.06
Madura	-0.01 (<0.001)	-0.01 to <0.001	0.04
Ongole	0.01 (0.01)	-0.01 to <0.001	0.62

Key: SE (standard error)

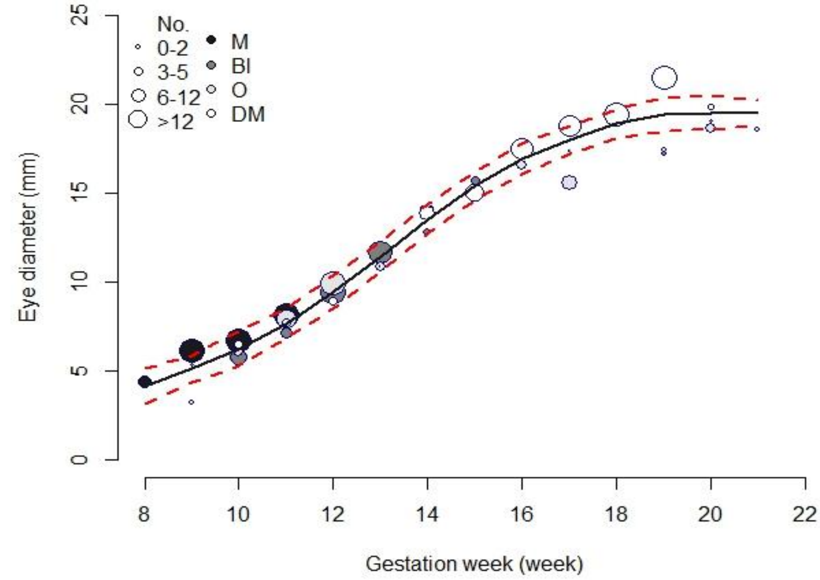


Figure 24. Bubble plot of eye diameter as a function of gestation week in Madura, Bali, Ongole and DM fetuses. Bubble size is representative of the number of observations recorded for each breed. The superimposed solid line shows a penalised cubic regression line fitted to the eye diameter data. The dashed lines represent the lower and upper 95% predictions.

Table 37. Estimated regression coefficients and their standard errors (natural log) from a mixed-effects linear regression model of factors influencing eye diameter in DM, Bali, Madura and Ongole fetuses.

Variable	Coefficient (SE)	95% Confidence interval	P-value
Intercept	0.54 (0.05)	0.43 to 0.64	<0.001
Gestation week (week)	0.05 (<0.001)	0.05 to 0.06	<0.001
Breed			
DM	**Reference**		
Bali	-0.05 (0.08)	-0.21 to 0.11	0.54
Madura	-0.06 (0.06)	-0.19 to 0.07	0.35
Ongole	-0.16 (0.17)	-0.50 to 0.18	0.34
Gestation week × Breed			
DM	**Reference**		
Bali	<0.001 (<0.001)	-0.01 to 0.01	0.94
Madura	<0.001 (0.01)	-0.01 to 0.02	0.43
Ongole	0.02 (0.03)	-0.03 to 0.08	0.42

Key: SE (standard error).

5.5 Discussion

The pattern and absolute changes in fetal and placental development among the 4 breeds of tropically adapted cattle were similar. This is despite the fact that there are marked differences between breeds in mature cow size and calf birthweight. Overall these findings support the hypothesis that there is no difference in fetal and placental development during approximately the first half of gestation among the 4 tropically adapted breeds of cattle studied. Further, given that fetal and placental development in these tropically adapted breeds of cattle appears to be similar to that of the HF then it is reasonable to suggest that across all breeds of cattle development during the first half of gestation is likely to be similar.

During the period of monitoring mean CRL, trunk and eye diameter for DM fetuses was greater than that for each of the Indonesia breeds. However, mean head length, hoof and placentome diameter for Madura fetuses was greater than that for DM fetuses and also for Ongole and Bali fetuses. It is important to note that although significant differences were detected in real terms the magnitude of these differences was small.

Because the observed pattern of fetal and placental development was generally linear for the 4 breeds of cattle studied, guidelines for estimation of stage of gestation based on ultrasonographic measurement of fetal and placental anatomy can be readily developed. Development of these will provide a more accurate and practical method of fetal aging.

Chapter 6 General Discussion and Conclusions

The aim of this work was to document fetal and placental development in Indonesian native cattle and compare it to that in DM and HF breed cattle. The novelty of this work is that it represents a comprehensive body of work that defines and directly compare fetal and placental development during approximately the first half of gestation in DM cattle (the most numerous breed of tropically adapted beef cattle in Australia), and in the three most common breeds of Indonesian beef cattle (Bali, Ongole and Madura).

In Chapter Three, pregnancy rate to FTAI for DM heifers was as expected. The observed rate of increase during the early gestation in CRL was greater than other measured parameters [19]. In Chapter Four, the trend of fetuses growth rate between each breeds were similar. In all breeds, the highest growth rate in CRL during the first weeks of gestation compared to other parameters which lower percentage in ranged week 5 up to 22 of pregnancy age. HF fetal placental development parameters tended to either be greater mean values and a higher growth rate in all measured parameters from week 4 to 13 of gestation.

In Chapter Five the growth of DM fetuses and Indonesia breeds was compared. Madura fetuses were dominating the growth in head length, hoof and placentome diameter compare during early gestational age. Madura fetuses also had the highest growth rate for CRL followed by Ongole, DM and Bali fetuses. This body of work addresses a gap in the literature about fetal and placental development in Indonesian native cattle.

The results presented in each chapter of this thesis goes someway to better defining fetal and placental development during the first half of gestation in DM cattle and in the three most common breeds of Indonesian beef cattle (Bali, Ongole and Madura).

6.1 Major findings

During approximately the first half of gestation (4th to the 22nd week), all beef cattle breeds studied had similar patterns of development in CRL, head length, trunk diameter, eye diameter, hoof diameter and placentome diameter, despite a marked difference between the mean birthweight and mature cow weight of the Indonesian breed cattle compared to the DM breed cattle. Further, the pattern of fetal development observed in these 4 tropically adapted beef breeds was similar to that reported for the HF. These findings are similar to those of Mao (2008), who observed that fetal development was not different in early gestational between week 3 and week 6 [21]. However, some differences between breeds in overall development and rate of development of both the fetus and placentome were observed. The major differences observed in head length, hoof and placentome diameter from Madura fetuses compared to DM and other Indonesian breed cattle fetuses was small magnitude.

6.2 Limitations of study

In this study a number of limitations have been identified that have influenced the outcome or the course of the study. These were as follow:

Small sample size mainly due to logistics. This is represented by a small number of cattle enrolled in this study, especially for Bali and Ongole cattle. While this issue may have influenced the precision of the results presented in this study it is unlikely to have had an effect on the point estimates provided for each measured parameters. However, this represents a form of sampling and selection bias and the effect of this on the validity of this study is difficult to predict, particularly when comparing Indonesian cattle with DM and HF cattle. Nevertheless, the internal validity of this study remains high as all observations and procedure were conducted consistently and by the same observer.

The other major limitation is the lack of concurrent data for HF fetal and placental parameters. In an attempt to overcome this limitation HF were extracted from the literature and a published formula was used to predict HF fetal and placental parameters as a function of gestation week. This may have introduced information bias as the published data were point estimates and measures of spread or uncertainty were not accessible. Therefore these results are to be interpreted with caution.

This study focused on fetal and placental development in the first half of gestation and would have been improved if the study had included monitoring of development through to parturition and comparison between gestational measures of conceptus development and measures taken at birth. A further limitation of the study is that gestational development was mainly measured in heifers and not cows preventing any comparison being investigated.

6.3 Recommendations for future research

Conduct a follow up study at a larger scale to confirm current findings. This would require setting up a multisite, multiyear longitudinal study across Indonesia and Australia. Of interest to quantify the association between fetal-placental development in the first two trimesters and third trimester of gestation. Furthermore, of equal interest is to compare how fetal-placental development in different trimesters affects neonate measurements or even survival. The current study suggest that the growth rate in different trimesters follow a similar trend but vary in magnitude. The effect of this on neonates remain unanswered.

References

1. Schlafer DH, Fisher PJ, Davies CJ. The bovine placenta before and after birth: placental development and function in health and disease. *Animal Reproduction Science* 2000;60–61:145-160.

2. Fitzgerald AM, Ryan DP, Berry DP. Factors associated with the differential in actual gestational age and gestational age predicted from transrectal ultrasonography in pregnant dairy cows. *Theriogenology* 2015;84:358-364.

3. Diskin MG, Morris DG. Embryonic and early fetal losses in cattle and other ruminants. *Reproduction in Domestic Animals* 2008;43:260-267.

4. Pierson RA, Ginther OJ. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. *Theriogenology* 1984;22:225-233.

5. Rosiles VA, Galina CS, Maquivar M, Molina R, Estrada S. Ultrasonographic screening of embryo development in cattle (Bos indicus) between days 20 and 40 of pregnancy. *Animal Reproduction Science* 2005;90:31-37.

6. Echternkamp SE. Relationship between placental development and calf birth-weight in beef-cattle. *Animal Reproduction Science* 1993;32:1-13.

7. Head H, Schick R, Wilcox C. Interrelationships of physical measures of placenta, cow and calf. *Journal of Dairy Science* 1981;64:161.

8. Prior R, Laster D. Development of the bovine fetus. *Journal of Animal Science* 1979;48:1546-1553.

9. Ginther O. *Ultrasonic imaging and animal reproduction: Fundamentals,* Book 3. 1998.

10. Mohamad K, Olsson M, Andersson G *et al*. The origin of Indonesian cattle and conservation genetics of the Bali cattle breed. *Reproduction in Domestic Animals* 2012;47:18-20.

11. Sutarno, Setyawan AD, Lymbery AJ. Genetic diversity of five indonesian native cattle breeds at microsatellite loci. *Asian Journal of Animal Sciences* 2015;9:57-64.

12. Purwantara B, Noor RR, Andersson G, Rodriguez-Martinez H. Banteng and Bali Cattle in Indonesia: Status and Forecasts. *Reproduction in Domestic Animals* 2012;47:2-6.

13. Waldron S, Nuryati Y. The Indonesian Beef Industry in “Regional Workshop on Beef markets and trade in Southeast Asian and China”, Ben Tre, Vietnam, 30th November–3rd December, 2015. 2015.

14. Sudardjat D. *National Report on Animal Genetic Resources in Indonesia: A Strategic Policy Document*. . ftp://ftp.fao.org/docrep/fao/011/a1250f/annexes/CountryReports/Indonesia.pdf. Directorate General of Livestock Services, Ministry of Agriculture Indonesia., Jakarta, 2003.

15. Hadi PU, Ilham N, Thahar A *et al*. *Improving Indonesia's Beef Industry*. ACIAR Monograph No. 95, Australian Center for International Research, Canberra, 2002.

16. Talib C, Entwistle K, Siregar A, Budiarti-Turner S, Lindsay D. *Survey of population and production dynamics of Bali cattle and existing breeding programs in Indonesia*. Australian Center for International Research, Canberra, 2003.

17. McGowan MR, McCosker K, Fordyce G, Smith D, O'Rourke PK, Perkins N, Barnes T, Marquet L, Morton J, Newsome T, Menzies D, Burns BM and Jephcott S (2014a). North Australian beef fertility project: Cash Cow. Final Report, Project B.NBP.0382, Meat and Livestock Australia, Sydney. http://www.mla.com.au/Research-and-development/Final-report-details?projectid=15462

18. Noakes D, Parkinson T, England G. Development of the conceptus. In. *Arthur's Veterinary Reproduction and Obstetrics. Eighth edition. Saunders, Edinburgh, UK*. 2001:57-68.

19. Laven RA, Peters AR. Gross morphometry of the bovine placentome during gestation. *Reproduction in Domestic Animals* 2001;36:289-296.

20. Bazer F, Kim J, Song G *et al*. Uterine environment and conceptus development in ruminants. *Animal Reproduction Science* 2012;9:297-304.

21. Curran S, Pierson R, Ginther O. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. *Journal of the American Veterinary Medical Association* 1986;189:1295-1302.

22. Buczinski SMC, Fecteau G, Lefebvre RC, Smith LC. Fetal well-being assessment in bovine near-term gestations: Current knowledge and future perspectives arising from comparative medicine. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 2007;48:178-183.

23. McKeown T, Marshall T, Record R. Influences on fetal growth. *Journal of Reproduction and Fertility* 1976;47:167-181.

24. Rattray P, Garrett W, East N, Hinman N. Growth, development and composition of the ovine conceptus and mammary gland during pregnancy. *Journal of Animal Science* 1974;38:613-626.

25. Ferrell C, Garrett W, Hinman N. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. *Journal of Animal Science* 1976;42:1477-1489.

26. Eley R, Thatcher W, Bazer FW *et al*. Development of the conceptus in the bovine. *Journal of Dairy Science* 1978;61:467-473.

27. Pace MM, Augenstein ML, Betthauser JM *et al*. Ontogeny of cloned cattle to lactation. *Biology of reproduction* 2002;67:334-339.

28. Riding GA, Lehnert SA, French AJ, Hill JR. Conceptus-related measurements during the first trimester of bovine pregnancy. *The Veterinary Journal* 2008;175:266-272.

29. Ogata Y, Nakao T, Takahashi K *et al*. Intrauterine growth retardation as a cause of perinatal mortality in Japanese black beef calves. *Journal of Veterinary Medicine Series A* 1999;46:327-334.

30. Wallace J, Bourke D, Aitken R *et al.* Relationship between nutritionally-mediated placental growth restriction and fetal growth, body composition and endocrine status during late gestation in adolescent sheep. *Placenta* 2000;21:100-108.

31. Pierson R, Kastelic J, Ginther O. Basic principles and techniques for transrectal ultrasonography in cattle and horses. *Theriogenology* 1988;29:3-20.

32. Ginther OJ. Ultrasonic imaging and reproductive events in the mare. *Ultrasonic imaging and reproductive events in the mare.* 1987.

33. Kähn W. Sonographic fetometry in the bovine. *Theriogenology* 1989;31:1105-1121.

34. Curran S, Pierson R, Ginther O. Ultrasonographic appearance of the bovine conceptus from days 10 through 20. *Journal of American Veterinary Medical Association* 1986;189:1289-1294.

35. Barnwell CV, Farin PW, Whisnant CS, Alexander JE, Farin CE. Maternal serum progesterone concentration and early conceptus development of bovine embryos produced in vivo or in vitro. *Domestic Animal Endocrinology* 2015;52:75-81.

36. Heap RB, Holdsworth RJ, Gadsby JE, Laing JA, Walters DE. Pregnancy diagnosis in the cow from milk progesterone concentration. *British Veterinary Journal* 1976;132:445-464.

37. Lonergan P, Woods A, Fair T *et al*. Effect of embryo source and recipient progesterone environment on embryo development in cattle. *Reproduction, Fertility and Development* 2007;19:861-868.

38. Spencer TE, Johnson GA, Bazer FW, Burghardt RC, Palmarini M. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. *Reproduction, Fertility and Development* 2006;19:65-78.

39. Kenyon A, Mendonça L, Lopes G et al. Minimal progesterone concentration required for embryo survival after embryo transfer in lactating Holstein cows. *Animal Reproduction Science* 2013;136:223-230.

40. Mann G, Lamming G. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001;121:175-180.

41. Robertson H, King G. Conjugated and unconjugated oestrogens in fetal and maternal fluids of the cow throughout pregnancy. *Journal of Reproduction and Fertility* 1979;55:463-470.

42. Hoffman B, Wagner W, Hixon J, Bahr J. Observations concerning the functional status of the corpus luteum and the placenta around parturition in the cow. *Animal Reproduction Science* 1979;2:253-266.

43. Zhang W, Nakao T, Moriyoshi M *et al*. The relationship between plasma oestrone sulphate concentrations in pregnant dairy cattle and calf birth weight, calf viability, placental weight and placental expulsion. *Animal Reproduction Science* 1999;54:169-178.

44. Zhang W, Nakao T, Moriyoshi M, Nakada K, Ohtaki T. Relationships among plasma concentrations of estrone sulfate during pregnancy and placental and calf weight at birth in dairy cows. *Animal Science and Technology (Japan).* 1999.

45. Zhang W-C, Nakao T, Moriyoshi M *et al.* Estrone sulfate concentrations, calf birth weight and viability, and placental weight and expulsion in dairy cattle with different gestation length. *Animal Science Journal* 1999;70:429-436.

46. Heap R, Hamon M. Oestrone sulphate in milk as an indicator of a viable conceptus in cows. *British Veterinary Journal.* 1979.

47. Isobe N, Nakao T, Uehara O, Yamashiro H, Kubota H. Plasma concentration of estrone sulfate during pregnancy in different breeds of Japanese beef cattle. *Journal of Reproduction and Development* 2003;49:369-374.

48. Takahashi T, Hirako M, Takahashi H *et al*. Maternal plasma estrone sulfate profile during pregnancy in the cow; comparison between singleton and twin pregnancies. *Journal of Veterinary Medical Science* 1997;59:287-288.

49. Szenci MAM, Beckers J, Sulon J *et al*. Evaluation of false ultrasonographic diagnoses in cows by measuring plasma levels of bovine pregnancy-associated glycoprotein. *The Veterinary Record* 1998;142:304-306.

50. Sasser RG, Alexander B, Ruder C. Pregnancy detection in postpartum cows by measurement of pregnancy specific protein B (PSPB). *Journal of Animal Science* 1991;69:466.

51. Romano JE, Larson JE. Accuracy of pregnancy specific protein-B test for early pregnancy diagnosis in dairy cattle. *Theriogenology* 2010;74:932-939.

52. Robert S. Veterinary obstetrics and genital diseases (Theriogenology)*, Woodstock, VT* 1986;14-22, 497.

53. Romano JE, Thompson JA, Kraemer DC *et al*. Early pregnancy diagnosis by palpation per rectum: influence on embryo/fetal viability in dairy cattle. *Theriogenology* 2007;67:486-493.

54. Noakes DE, Parkinson TJ, England GC. Arthur's Veterinary Reproduction and Obstetrics. In: Elsevier Health Sciences, 2001:69-118.

55. Reeves JJ, Rantanen NW, Hauser M. Transrectal real-time ultrasound scanning of the cow reproductive tract. *Theriogenology* 1984;21:485-494.

56. DesCoteaux L, Gnemmi G, Colloton J. Ultrasonography of the bovine female genital tract. *Veterinary Clinics North America Food Animal Practice* 2009;25:733-752.

57. Szenci O, Beckers JF, Humblot P *et al.* Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. *Theriogenology* 1998;50:77-88.

58. Franco O, Drost M, Thatcher M-J, Shille V, Thatcher W. Fetal survival in the cow after pregnancy diagnosis by palpation per rectum. *Theriogenology* 1987;27:631-644.

59. Sullivan R, O'Rourke P, Robertson D, Cooke D. Effects of once-yearly weaning on some aspects of herd productivity in an extensive herd in the semi-arid tropics of northern Australia. *Australian Journal of Experimental Agriculture* 1992;32:149-156.

60. White IR, Russel AJF, Wright IA, Whyte TK. Real-time ultrasonic scanning in the diagnosis of pregnancy and the estimation of gestational age in cattle. *Veterinary Record* 1985;117:5-8.

61. Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/. 2016.

62. Butler SA, Phillips NJ, Boe-Hansen GB, Bo GA, Burns BM, Dawson K, McGowan MR. Ovarian responses in Bos indicus heifers treated to synchronise ovulation with intravaginal progesterone releasing devices, oestradiol benzoate, prostaglandin F(2α) and equine chorionic gonadotrophin. Anim Reprod Sci. 2011 Dec;129(3-4):118-26. doi: 10.1016/j.anireprosci.2011.11.001. Epub 2011 Nov 15.