The early identification of twinning trait genes on Indonesian local beef cattle

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ABSTRACT: One of ways to increase local beef production is increasing beef cattle population with exploit genetic potency of twinning trait genes. This research was done as earliest identification to get basic data about twinning genes trait on PO cattle and Bali cattle as Indonesian local beef cattle. The research using five dams Bali cattle with nine her twinning calves and five dams with five her single calves that distributed on 10 farmers at Lombok island in West Nusa Tenggara province; also 10 dams PO cattle with 19 her twinning calves and five dams with five her single calves that distributed on 14 farmers at Hulu Sungai Tengah district in South Kalimantan province. DNA were isolated from the blood samples of every cattles were analyzed with PCR and sequencing to known genetic ban map and configuration specific of nucleate acids that cath up IGF-1 and IGF-2 genes as the twinning trait. The parameters were : electrophoresis fotos of genetic ban map, and configuration specific of nucleate acids that cath up IGF-1 and IGF-2 genes. Data were analyzed with descriptif. The results of this research showed that genetic ban map and configuration specific of nucleate acids that cath up IGF-1 and IGF-2 genes, at Bali cattle was not same as at PO cattle, also between cattles with twinning history and cattles with single history; but between dams and her calves were resembles, especially at cattles with twinning history. This study concluded that, the twinning trait leader on PO cattles and Bali cattles, could to be identified of their genetic with saw genetic ban map and configuration specific of nucleated acids that cath up IGF-1 and IGF-2 genes.

Key words: twinning trait genes, Indonesian local beef cattle

INTRODUCTION

At this time much discussed phenomenon of twinning birth in beef cattle. In the last few years, some districts in South Kalimantan, East Java, Bali and West Nusa Tenggara provinces has been reported, there are tendency that more and more twinning birth in PO and Bali cattles. The occurrence of twinning birth at a cow, allegedly due to abnormalities (quality or quantity) secretion of reproduction hormones (FSH, LH, estrogen) in cows, or zygot cell duplication abnormalities. Twinning birth trait is genetically, and there is a tendency to repeated on their calves.

One of the biological limitations of species *uniporous* would be one calf in each birth. But with the development of technology, greater opportunities to enhance the ability of the cow gave birth to twins (twinning). Twin births is one of the strategic technology, because it can overcome the biological limitations of cattle as a species uniporous (Komisarek and Dorynek, 2002). Twin births also increased the efficiency of biological and production, because of lower input costs for pregnant and lactating mother (Karlsen *et al*., 2000). Quoted Notter *et al*. (1979) said that total high production input costs for maintenance and pregnant and lactating dams can be reduced up to 35% through the production of twinning birth; while Guerra-Martinez *et al*. (1990) said that efficiency of beef cattle production can be increased by 24% through the birth of twins.

Through the mating program is known mating between dam and bull who has a history of twinning birth, it raises a strong suspicion that twinning births in cattle was not influenced by single genes (Morris *et al*., 1992; Gregory *et al*., 1997). By doing an intensive study, known inheritance of fertility in cattle following the pattern of quantitative traits, means controlled by many genes and interact with the environment (Gregory *et al*., 1997; Lien *et al*., 2000; Komisarek and Dorynek, 2002; Meuwissen *et al*., 2002). In harmony with the development of technology and the polymorphic markers of genetic linkage maps (Kappes *et al*., 1997) opens the opportunity to browse the twin birth control genes (quantitative trait loci/QTL) using marker characterized (marker assisted selection / MAS). Otherwise

Dekkers (2004), the development of polymorphic molecular marker mapping opportunities identification of genes with genetic variation of quantitative traits (QTL), so the cattle can be selected with the desired genes as the elders of certain properties to be included in a draft of mating, which allows the trait can be inherited (Meuwissen *et al*., 2002).

Increasing the frequency of twinning births could be done through some farm technology applications, for example : conventional breeding, molecular DNA (un-conventional breeding), biotechnology, reproduction (hormonal induction and manipulation of embryos) and giving ration that contain the active components of fertility boosters. Increasing of twinning birth via hormone induction and manipulation of embryos (embryo transfer) are temporary and require considerable expense, but the commercialization process fast done (Yamaguchi, 1992). Genetic improvement through conventional breeding (selection and mating) require a long time, but a permanent genetic progress (Van Vleck and Gregory, 1996). The use of molecular DNA technology could allow for tracing genes controlling fertility cows with early warning, so that the response properties of genetic progress were born twins will be obtained more quickly (vs. conventional breeding) (Kappes *et al*., 1997), but apliaksi technology requires a large cost (Dekkers , 2004).

Increase the frequency of twinning birth of Indonesia local cattle, although still facing many obstacles, it seems the future will be a dramatic way to increase the production of calves per occurrence, so that breeders are expected to increase revenue through the sale of more calves per year per dam. However, to achieve these goals requires extra handling technology, well, remember the birth of twins in cows is often compromised by the negative effects of such incidents distocya, retained placenta and dam reproductive disorders, also mortality and premature birth (Kirkpatrik, 2000). Gregory *et al*. (1996) reported that compared to single births, twinning birth calves have a survival rate (3 days postnatal) lower 13% with pre-weaning mortality higher than 2%. Twins (up to age 200 days) grew 15% slower, but weaning weight was 7% greater. Further reported Echternkamp and Gregory (1999) twinning birth compared to single birth distocya significantly increase the incidence of approximately 22% (42 vs. 20%), and so the number of gestation (60 or 70 days post mating) was lower by about 10% (67 vs. 77%). Based on the above matters, it seems that the positive value of the reproductive performance of twinning birth will require various improvements, especially on livestock nutrition and maintenance management.

The success of increasing the incidence of twinning birth, has been achieved from the work at large through a long selection by The Meat and Animal Research Centre, USDA) in beef cattle composite (mixed breeds). Cows with high frequency of twin built as a base population for this trait. First step is done by detecting a gene that affects ovulation rate using single marker analysis on 77 markers (43-45 informative markers per family) from three great families of grandsire (Blatman *et al*., 1996). Van Vleck and Gregory (1996) noted that after going through a series of selection activities, the frequency of twinning birth could be increased from 3.4% (1982) to 28.5% (1993). Until the year 2001 reported Meuwissen, *et al*. (2002) produced as many as 350 head of cattle parent with a frequency of twin births has reached 55%. Increased genetic potential of the cow gave birth to twins by combining conventional and molecular selection of DNA has been followed also by a number of countries such as Norway (Lien *et al*., 2000) and Poland (Komisarek and Dorynek, 2002). At least chromosomes 5, 7, 10, 12, 19 and 23 reported good potential to identify the gene positions of fertility (ovulation and twinning rate) in cattle (Kappes *et al*., 1997; Lien *et al*., 2000; Komisarek and Dorynek, 2002 ; and Kirkpatrick, 2002).

Based on the consideration of positive aspects and benefits of efforts to increase the frequency of the cow giving birth to twins on top, and based on the expectation of Agricultural Research and Development Agency that the phenomenon of twinning birth in beef cattle should be researched and studied to know whether can be used as a method to accelerate the increase in national population cattle, then forward it seems this phenomenon can be used as an alternative strategic enough to increase the biological efficiency of beef production process.

MATERIALS AND METHODS

This research is a combination of field activities in the form of cow's blood sample for DNA isolation and sample making interviews to farmers/officers concerned to know the incidence of

twinning birth beef cattle, and values of local wisdom, whereas the laboratory activities such as making isolation of the DNA sample, and analysis PCR and DNA Sequencing.

Materials

To obtain a DNA sample suspected of controlling the nature of twinning birth :

1. blood samples from :
   a. 5 Bali cattle cows and 9 their twinning calves in Pringgesela and Pringgerata village, Pringgasela sub-district, East Lombok district; Lendangnangka village, Masbagik sub-district and North Jenggik villages, Terara sub-district, Central Lombok district; and in Narmada village, Narmada sub-district, West Lombok district West Nusa Tenggara province
   b. 10 PO cattle cows and 19 their twinning calves in Sei, Rengas, Ilung, Murung and Pau villages, Pandawan sub-district, Hulu Sungai Tengah district, South Kalimantan province. As control were 5 Bali cattle cows and 5 PO cattle cows that have single calf at each locations
2. a set of tools and materials of blood sampling, DNA isolation, PCR and Sequencing analysis
3. genes controlling twinning birth, are identificated as the IGF-1 and IGF-2
4. primer pairs (mix) nucleotide sequences (forward and reverse) as templates (template) DNA genome in the DNA amplification reaction (PCR reactions) :
   a. IGF-1 gene : forward 5-3-CCTCTGCGGGGCTGAGTTGGT
      : reverse 5-3-CGACTTGGCGGGCTTGAGAGGC
   b. IGF-2 gene : forward 5-3-TCTGTGCGGCGG GGAGCTGGT
      : reverse 5-3-AGTCTCCAGCAGGGCCAGGTCG

Methods

Preparation of DNA Isolated Samples

Blood samples taken at the jugular vein of dams and her twinning calves or her single calf (control), stored in blood tubes containing an anti-coagulant heparin. Preparation of sample DNA isolation, performed using modified method of Sambrook *et al*. (1989). DNA purified by phenol-chloroform method.

Analysis of DNA Samples Isolated

Identification of fertility IGF1 and IGF2 genes, using the reaction Polymerase Chain Reaction (PCR) and Sequencing (Sambrook and Russell, 2001), at the Laboratory of Biochemistry, Faculty of Veterinary Medicine - Gadjah Mada University

Sequencing

Preview arrangement nucleotides IGF1 and IGF2 genes, obtained by mapping the genes through sequencing

Parameters Observed:

a. banding pattern of gene IGF-1 and IGF-2
b. composition and type nucleotides of IGF-1 and IGF-2 genes
c. the local value wisdom of maintaining cattle with twinning history

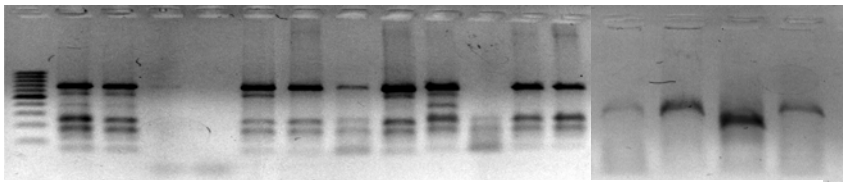
Data obtained are processed and presented by descriptive.

RESULTS AND DISCUSSION

Banding Pattern of Gene IGF-1 and IGF-2

Bali Cattle

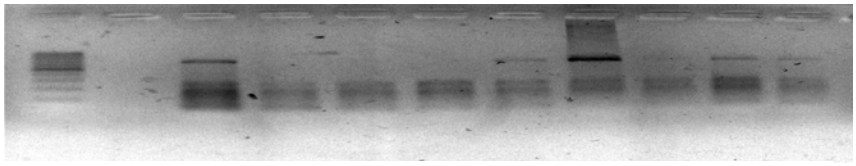
PCR results for IGF-1 and IGF-2 gene from Bali cow with her twinning calves, its electro-foresis listed in Figure 1 and 3, while as control (Bali cow and her single calf) in Figure 2 and 4.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Description:
1 = DNA Marker
2 and 3 = cow I and her twinning calf ; 4 and 5 = cow II and her twinning calf
6, 7, 8 and 9 = cow III and her three twinning calves ; 10, 11, 12 and 13 = cow IV and her three twinning calves
14 and 15 = cow V and her twinning calf ; 16 and 17 = cow VI and her twinning calf

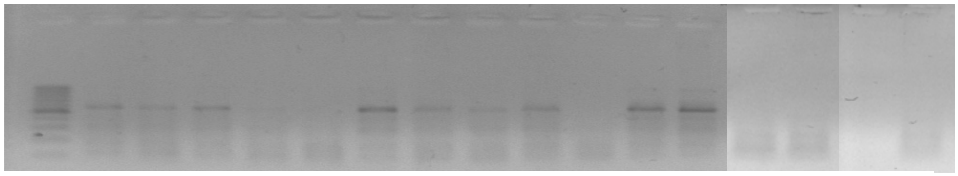
Figure 1. Electroforesis IGF-1 gene of Bali cow and her twinning calves



1 2 3 4 5 6 7 8 9 10 11

Description:
1 = DNA Marker
2 and 3 = cow I and her single calf ; 4 and 5 = cow II and her single calf
6 and 7 = cow III and her single calf ; 8 and 9 = cow IV and her single calf
10 and 11 = cow V and her single calf

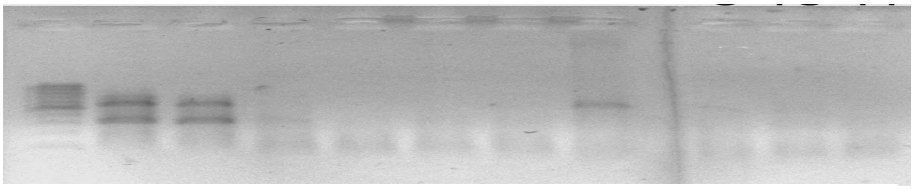
Figure 2. Electroforesis IGF-1 gene of Bali cow and her single calf



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Description:
1 = DNA Marker
2 and 3 = cow I and her twinning calf ; 4 and 5 = cow II and her twinning calf
6, 7, 8 and 9 = cow III and her three twinning calves ; 10, 11, 12 and 13 = cow IV and her three twinning calves
14 and 15 = cow V and her twinning calf ; 16 and 17 = cow VI and her twinning calf

Figure 3. Electroforesis IGF-2 gene of Bali cow and her twinning calves



1 2 3 4 5 6 7 8 9 10 11

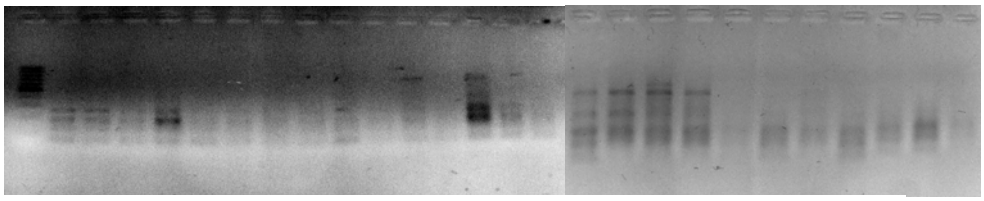
Description:
1 = DNA Marker
2 and 3 = cow I and her single calf ; 4 and 5 = cow II and her single calf
6 and 7 = cow III and her single calf ; 8 and 9 = cow IV and her single calf
10 and 11 = cow V and her single calf

Figure 4. Electroforesis IGF-2 gene of Bali cow and her single calf

PO Cattle

PCR results for IGF-1 and IGF-2 gene from PO cow with her twinning calves, its electroforesis listed in Figure 5 and 7, while as control (PO cow and her single calf) parent of Bali cattle and their only child) in Figure 6 and 8.

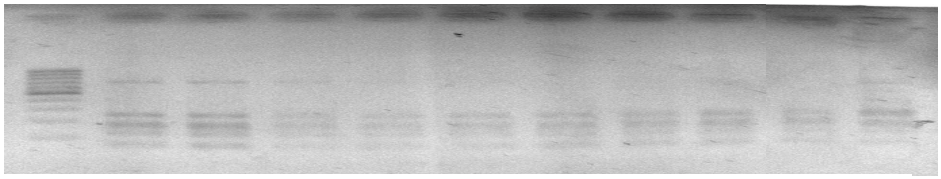
1 3 5 7 9 11 13 15 17 19 21 23 25 27



2 4 6 8 10 12 14 16 18 20 22 24 26

Description:
1 = DNA Marker
2, 3 and 4 = cow I and her twinning calves ; 5, 6 and 7 =cow II and her twinning calves ;
8, 9 and 10 = cow III and her twinning calves ; 11, 12 and 13 = cow IV and her twinning calves
14, 15 and 16 = cow V and her twinning calves ; 17, 18, 19 and 20 = cow VI and her three twinning calves
21, 22 and 23 = cow VII and her twinning calves ; 24 and 25 = cow VIII and her twinning calf
26 and 27 = cow IX and her twinning calf

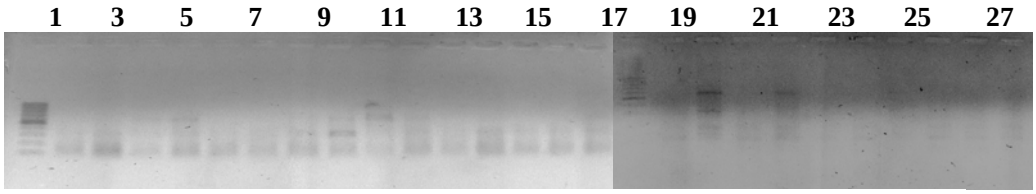
Figure 5. Electroforesis IGF-1 gene of PO cow and her twinning calves



1 2 3 4 5 6 7 8 9 10 11

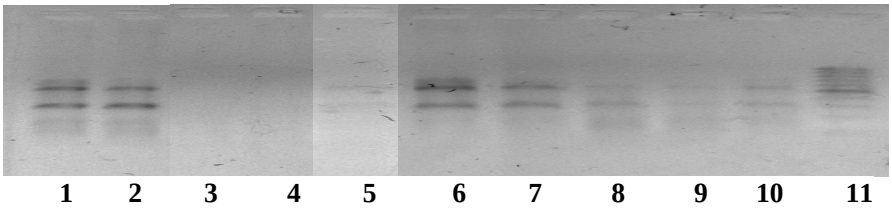
Description:
1 = DNA Marker
2 and 3 = cow I and her single calf ; 4 and 5 = cow II and her single calf
6 and 7 = cow III and her single calf ; 8 and 9 = cow IV and her single calf
10 and 11 = cow V and her single calf

Figure 6. Electroforesis IGF-1 gene of PO cow and her single calf



Description:
1 = DNA Marker
2, 3 and 4 = cow I and her twinning calves ; 5, 6 and 7 =cow II and her twinning calves ;
8, 9 and 10 = cow III and her twinning calves ; 11, 12 and 13 = cow IV and her twinning calves
14, 15 and 16 = cow V and her twinning calves ; 17, 18, 19 and 20 = cow VI and her three twinning calves
21, 22 and 23 = cow VII and her twinning calves ; 24 and 25 = cow VIII and her twinning calf
26 and 27 = cow IX and her twinning calf

Figure 7. Electroforesis IGF-2 gene of PO cow and her twinning calves



Description:
1 and 2 = cow I and her single calf ; 3 and 4 = cow II and her single calf ;
5 and 6 = cow III and her single calf ; 7 and 8 = cow IV and her single calf
9 and 10 = cow V and her single calf ; 11 = DNA Marker

Figure 8. Electroforesis IGF-2 gene of PO cow and her single calf

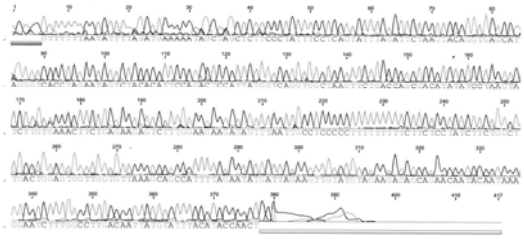
From Figure 9, it appears that :

1. there are similarities banding pattern of IGF 1 and IGF-2 genes (black line) between the dams/cows with her calf/calves. Equation banding pattern of these genes appear highly/more obvious at the dams/cows with her twinning calves
2. banding pattern of IGF-1 and IGF-2 genes of cows that have twinning history, are different with cows that have single history
3. IGF-1 and IGF-2 genes of cows with twinning history, appear to have more genes bands, longer distant and pattern distribution are more uniform than cows with single history.

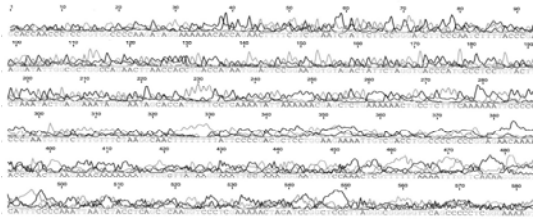
Differences in banding pattern of IGF-1 and IGF-2 genes among cows having twins with no history, and similarities banding pattern IGF-1 and IGF genes between cows and her calf/calves, it appears increasingly clear on the sequencing results (Figure 9) which shows the similarity of type and arrangement of nucleotides

The Local Values Wisdom of Maintaining Cattle with Twinning History

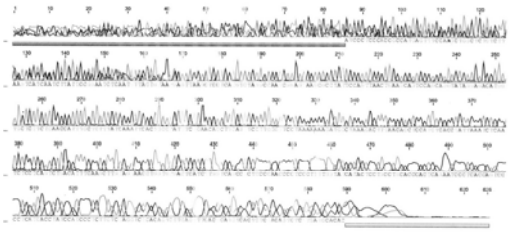
In Table 1 listed some of the local values wisdom of maintaining cattle with twinning history, the results of interviews using a semi-closed question to 10 farmers who their cows had been twinning birth.



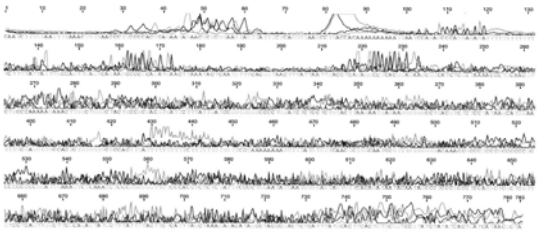
The PO calf is borned single



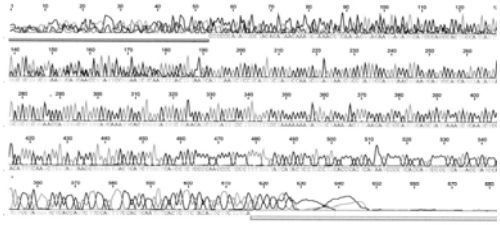
The Bali calf is borned single



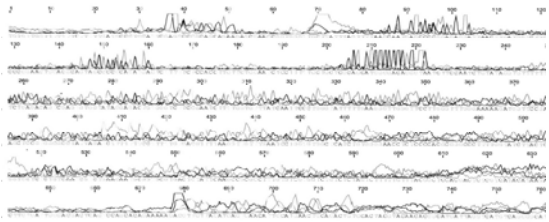
The PO cow borned single



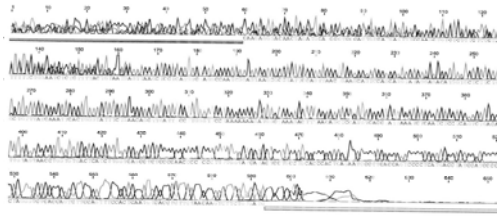
The Bali cow borned single



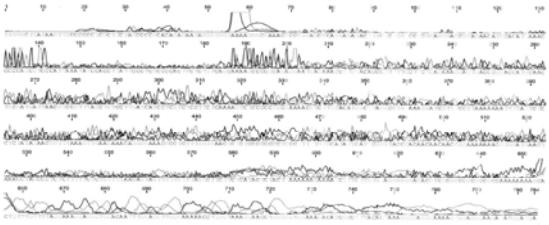
The PO cow borned twin



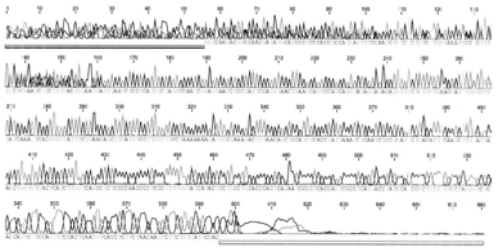
The Bali cow borned twin



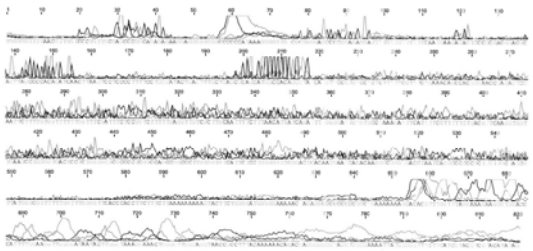
The PO calf is borned twin (a)



The Bali calf is borned twin (a)



The PO calf is borned twin (b)



The Bali calf is borned twin (b)

Figure 9. Differences configuration of IGF genes nucleate acids between twinning and single birth at PO and Bali beef cattle.

Table 1. The local value wisdom of maintaining cattle with twinning history

a.	Questions	Answers
b.	special treatment before mating	: 100% no
c.	mating cows	: 80% nature ; 20% IB
d.	bull/straw	: 40% PO
		: 30% Bali
		: 20% Limousin
		: 10% Simmental
c.	its dam/cow have twinning history	: 80% no, 20% yes
d.	S/C at twinning birth	: 70% 1x, 30% 2x
e.	there are special food for the twinning birth	: 30% no, 70% didn't know
f.	the number of twin calves	: 80% 2 heads, 20% 3 heads
g.	sex twin calves	: 70% all females, 30% female&male
h.	dam/cow had birth	: 80% the first, 20% second
i.	feeling happy have twinning calves	: 80% yes, 10% no, 10% alike
j.	raise the price of cattle	: 50% yes, 50% no
k.	next, their calves want twinning birth too	: 80% yes, 20% didn't know
l.	there are special treatment to dam/caw	: 80% yes, 20% no
m.	there are special treatments to calves	: 100% no
n.	body condition of cow/dam during lactation	: 60% bad, 40% enought
o.	body condition of calf/calves during growth	: 70% bad, 30% enought
p.	mortality of calves	: 40% one dead
		: 20% all dead
		: 40% all lived

CONCLUSIONS

Twinning trait on PO and Bali cattles, can be genetically identification through pattern band configuration of genes and specific composition of its nucleotides in its IGF-1 and IGF-2 genes. The phenomenon of twinning birth on PO and Bali cattles in farmers, occurred inadvertently and it are good responsed by farmers

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