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Twinning and Ovulation Rate for Sustainable Production in Cattle

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ABSTRACT: Profits is very important in most industries and determined by the cost and amount of products generated. In the livestock industries, higher number of viable animals produced will improve the economies of scale, hence, their profit. In general, twinning can be a good approach to increase reproductive capacity, and consequently the production volume. Cattle are monovular animal, typically give birth to one offspring and multiple birth is rare. Multiple gestation is mostly unfavorable in the dairy industry due to their negative impact on reproductive and production performance on cow and calf. Twinning is highly correlated to ovulation rate and various genes that are affecting hyperprolificacy in sheep have been identified. Many studies have reported quantitative trait loci (QTL) that are associated with high ovulation rate and twinning in cattle. Besides the genetic factor, twinning can also be induced via hormonal methods. Knowledge on the factors that causing hyperprolificacy can assist the breeders or farmers for their selection, based on their objectives and strategy.

Keywords: Hyperprolificacy; Livestock industry; Reproductive performance; Qantitative trait loci; Twinning technology

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INTRODUCTION

Profits in most industries are determined by the amount of product and the cost of production. It is important in the beef or dairy cattle production, where the farmer can produce more viable animals that will improve the economies of scale, together with the production cost per animal. In general, twinning can be a good approach to increase reproductive capacity, and consequently the production volume. Twinning can increase reproductive efficiency by 20-30% in beef cattle (Echternkamp et al., 2007b) and 79 % greater sale for twins weaned calf compared for singles (Guerra-Martinez et al., 1990). It causes a lower cost per unit output by spreading the cost of production over more animals. In addition, it will increase the number of candidates in selection program for superior females.

Cattle are monovular animal and typically give birth to one offspring. Twinning is rare in beef cattle (< 1%) while higher occurrence recorded in dairy cattle with 4-5 % (Komisarek and Dorynek, 2002). Holstein in the USA shown consistent increment of twinning from 1983 to 2003 and expected with the same pattern due to selection for higher milk production traits continues (Cabrera and Fricke, 2021). Factors that influencing twinning include ovulation rate, milk production, genetics, parity and season (Fricke, 2001). Besides that, incidence of twinning were associated with low progesterone level during the selection of dominant follicle in dairy cows (Carvalho et al., 2019). Pearl wrote: "From the standpoint of the practical breeder it is highly important that the phenomenon of multiple gestation in normally uniparous animals be carefully studied. Any definite and heritable increase in the fecundity and fertility of the domestic animals, if it can be gained without loss of other desirable qualities, is greatly to be desired. Cases of multiple gestation are the ‘favorable variations’ which must serve as the foundations for the creation of more fertile breeds and races" (quoted by Rutledge (Rutledge, 1975)). In dairy production, twinning is undesirable mostly due to negative impact on cow and calf related to reproductive difficulties (Kirkpatrick, 2002; Komisarek and Dorynek, 2002), decreased pregnancy rate, longer pregnancy interval (Echternkamp et al., 2007a), and metabolic disorders (Fricke, 2001) despite few were reported with superior milk yield (Sawa et al., 2015). Besides the postpartum reproductive difficulties due to twinning, increase of ovulation rate can increase prenatal and neonatal mortality. All these negative effects of twinning will cause economic loss due to interference with dairy cattle management, veterinary intervention, and maintenance (Cabrera and Fricke, 2021). However, freemartins have higher carcass quality compared to fertile females (Gregory et al., 1997), a desirable characteristic in the beef cattle sector.

There is a high correlation (0.8-0.9) between ovulation rate and twinning rate (Van Vleck et al., 1991), with low heritability (h^2 = 0.1), increased to intermediate heritability (h^2 = 0.35) of ovulation rate for cattle selected for twinning (Gregory et al., 1997). A lower heritability of multiple ovulation (0.028 ± 0.003) and twin birth rate (0.017 ± 0.004) was recently reported, for herds of cattle in Ireland from year 2002 to 2012 (Fitzgerald et al., 2014). More recently, heritability estimates for twinning rate were 0.0192 ± 0.0009 and 0.1420 ± 0.0069 for linear and threshold models, respectively for the US Holstein cattle (Lett and Kirkpatrick, 2018). Most breeds in sheep have heritability of ovulation rate range about 0.15 – 0.25 (Vinet et al., 2012). Heritability described as the proportion of genetic factor over the phenotypic variation observed. Low heritability and postpartum reproduction difficulties can cause slow response to selection. It appears to be challenging and time consuming due to the composite factor (genetic and non-genetic) effect, the development of a population with high twinning frequency would likely be required for consideration of a twinning technology by the beef cattle industry (Gregory et al., 1997).

MATERIALS AND METHODS

Mechanism of Twinning

Twinning can be categorized as monozygous and dizygous, where embryos are produced from one oocyte for the former, whereas two oocytes were fertilized to produce dizygotic twins. The monozygous twins were formed by a single, fertilized egg, which then cleaves, producing two separate embryos. Monozygous twins are considered very uncommon. By using microsatellite markers, Silva del Río et al. (Silva del Río et al., 2006) calculated only 0.33% monozygous twins birth in Holstein cattle, similar to 4 per 1000 maternities in human. As the monozygous twins originate from a cleaved single zygote, they are of the same sex, genotype, and phenotype. Most of the twins in dairy cattle are dizygous or due to multiple ovulations from a single follicular wave (Wiltbank et al., 2000).

Twinning in cattle is affected by multiple factors including genetics, breed, parity, ovulation rate and milk production (Fricke, 2001). Multiparous cows showed higher occurrences of multiple pregnancies compared to heifers (Sawa et al., 2015), with increasing incidence of twin pregnancy in successive lactations with the highest increase between first and second lactation (Fricke, 2001). Increase in peak milk production has been suggested to be a key contributor for increases in twinning rate possibly associated with higher energy diets supplement (Kinsel et al., 1998), or the "flushing" effect observed during the breeding season with high energy concentrate supplementation (Echternkamp et al., 2007a). This association may be due to increased energy intake increasing blood flow to the liver causing increased steroid metabolism (Wiltbank et al., 2000).

Scaramuzzi (Scaramuzzi et al., 2011) proposed a "window" theory, involving two different mechanisms that increase ovulatory follicle number. Lower threshold levels of FSH widen the window or time span above the threshold level allowing gonadotropin-dependent follicles to continue to grow and escape atresia. However, threshold concentrations of FSH were never confirmed and a universal value is unlikely to exist. In a normal regulatory process, the follicle sends negative feedback to the pituitary gland and lowers FSH production. As a part of the feedback mechanism, activin and inhibin that are mainly produced by the granulosa cells, antagonistically increase and decrease, respectively, FSH synthesis and release by the anterior pituitary (Knight et al., 2012). At the beginning of follicle deviation at a later stage of development, estradiol secretion is increased by the granulosa cells, continuing the depression of FSH resulting in an FSH deprived environment that suppresses small follicle development, while the largest follicle establishes dominance (Ginther et al., 2000). Ewes that carry the Boorola mutation have a larger cohort of growing follicles responsive to the FSH threshold level required for further development (Scaramuzzi et al., 2011). Multiple ovulations can be achieved with unaltered feedback context; smaller size follicles in total produce similar amounts of estradiol relative to a single large follicle (Vinet et al., 2012).

RESULT AND DISCUSSION

Exogenous Induction

Besides the genetic factors, twinning can also be induced via hormonal methods, which was previously studied in cattle and other ruminants (Johnson et al., 1975; Najafi et al., 2014). Most of the studies used pregnant mare serum gonadotropin (PMSG), currently termed equine chorionic gonadotropin (eCG). The eCG possesses LH- and FSH-like activity, with high affinity for receptors of both hormones in the ovary (De Rensis and López-Gatius, 2014). eCG belongs to the glycoprotein family along with LH and FSH, and has beneficial effects on follicular development and reproductive activity including embryo development and survival (see review by De Rensis and López-Gatius, 2014) (De Rensis and López-Gatius, 2014). Recently, Martinez *et al.* (Martinez et al., 2014), reported that strategic administration of eCG increased double ovulation by 30% and twinning by 20% in treated beef heifers. The protocol was optimized by intra-muscular administration of eCG on day 3 of a synchronization procedure (total of 6 days), with dose of eCG and timing of treatment affecting the follicular dynamics and the increase in double ovulation.

Double ovulation is desirable for twinning, while incidence of more than two ovulations can lead to unfavorable multiple pregnancy (i.e., triplets and quadruplets). Nevertheless, response to hormonal treatments is highly variable, and it is difficult to predict the production of only two follicles. A formulation for hormonal induced twinning has yet to produce an optimal and uniform of result. However, there are several strategies that can be applied that may produce only single or twin pregnancies. Negative effects of multiple pregnancy can be prevented by removal partially or total sets of embryo, termed 'embryo reduction' (Andreu Vázquez, 2012).

It can be carried out by manual rupture (MR) of amniotic vesicle, or transvaginal ultrasound guided aspiration (TUGA) to remove the allanto-amniotic fluid using a guided needle. Nonetheless, this treatment is not a risk-free procedure; pregnancy losses were reported to be 13.7% higher compared to non-treated twin-pregnancy cows in the same dairy herd. Besides that, for a more preventive approach, embryo transfer can be applied to increase twinning rate with more control on number of fetuses desired. However, success in producing twin sets varies.

Intuitively, due to their uncertainty and variability, it is possible that none of these approaches is superior to another; not to mention the man-hour cost. Knowledge regarding the interaction of environmental factors and reproduction can be useful to control ovulation rate and twinning. For example, the 'flushing effect', where dietary supplementation is used to increase ovulation rate, or alternatively, ovulation rate could be reduced by decreasing the quantity or quality of feed. In addition, the inhibitory effect of estradiol, can be manipulated to reduce or increase the production of FSH, the key hormone in folliculogenesis. In the same fashion, theoretically, inhibition of estradiol metabolism by reducing feed intake, could decrease liver blood flow potentially allowing estradiol to be available longer, inhibiting FSH secretion (Wiltbank et al., 2000). Apart from the genetic and environmental factors, increased twinning rate can also be influenced by ability of the mother to maintain multiple pregnancy, termed uterine capacity, and maternal and embryonic interaction of the development and survival of the two embryos.

Gene Mutations Affecting Prolificacy

Mutations have been identified at several loci on the chromosomes that affect fertility for heterozygous and homozygous ewes in some sheep breeds (**Table 1**). Except for the Santa Ines breed (Silva et al., 2011), ewes heterozygous for the mutations of *BMP15* and *GDF9* were reported to have an increase of 32-100% in the ovulation rate, while homozygotes were sterile, with primary ovarian failure (Juengel et al., 2013).

The mutation in *GDF9* gene inhibiting the conversion of Gdf9 protein into mature protein, thus, probably causing ovarian and uterine hypoplasia in homozygous ewe (Souza et al., 2014). The heterozygous genotype promotes early termination of production of biologically active mature Bmp15, causing lower *BMP15* system activity, resulting in an increase in the ovulation rate. However, Demars et al. (Demars et al., 2013) have identified two novel mutations of *BMP15* that do not cause sterility in homozygous ewes and that have additive effects on the ovulation rate, similar to effects observed for *BMPR1B* (Davis et al., 1982; Mulsant et al., 2001). The alleles were named $FecX^{Gr}$ (in French Grivette) and $FecX^{O}$ (in Polish Olkuska) variants, where homozygous ewes showed greater litter size and a higher ovulation rate, respectively. Partial inhibition of *BMP15* signalling pathway observed *in vitro* activity for $BMP15^{N337H}$ ($FecX^{O}$) well explaining the hyperprolific phenotype of homozygous Olkuska sheep (Demars et al., 2013). Concurrently, $FecX^{GR}$ mutation induced complete inhibition of *BMP15* signalling. The same authors hypothesized that heterodimerization of Bmp15 with Gdf9 had sustained the biological activity.

Table 1. List of gene mutations affecting the ovulation rate in sheep.

Gene / symbol	Mutation	CHR1	Founder breed	REF2
BMP15 / FecXI	Missense single nucleotide (Valine -> Aspartic acid)	X	Romney	(Bodin et al., 2007; G H Davis et al., 2001)
BMP15 / FecXH	Premature stop codon	X	Romney	(Bodin et al., 2007; G H Davis et al., 2001)
BMP15 / FecXB	Missense single nucleotide (Serine -> Isoleucine)	X	Belclare	(Hanrahan et al., 2004)
BMP15 / FecXG	Premature stop codon	X	Belclare, Cambridge	(Hanrahan et al., 2004)
BMP15 / FecXL	Missense single nucleotide (Cysteine -> Tyrosine)	X	Lacaune	(Bodin et al., 2007; Vage et al., 2013)
BMP15 / FecXR	Premature stop codon	X	Rasa Aragonesa	(Martinez-Royo et al., 2008; Monteagudo et al., 2009)
BMP15 / FecXGr	Missense single nucleotide (Threonine -> Isoleucine)	X	Grivette	(Demars et al., 2013)
BMP15 / FecXO	Missense single nucleotide (Asparagine -> Histidine)	X	Olkuska	(Demars et al., 2013)
FecX2W	-	X	Coopworth	(George H Davis et al., 2001)
GDF9 / FecGH	Missense single nucleotide (Serine -> Phenylalanine)	5	Belclare, Cambridge	(Hanrahan et al., 2004)
GDF9 / FecGT	Missense single nucleotide (Serine -> Arginine)	5	Icelandic	(Nicol et al., 2009)
GDF9 / FecGE	Missense single nucleotide (Phenylalanine -> Cysteine)	5	Santa Ines	(Silva et al., 2011)
GDF9 / FecG	Missense single nucleotide (Valine -> Methionine)	5	Norwegian white sheep	(Vage et al., 2013)
BMPR-1B / FecBB	Missense single nucleotide (Glutamine -> Arginine)	6	Booroola, Merino	(Mulsant et al., 2001; Souza et al., 2001)
B4GALNT2 / FecLL	2 SNPs (single nucleotide polymorphism) close / within the gene	11	Lacaune	(Drouilhet et al., 2013)
Davisdale/FecD	-	-	Leicester x Romney	(Juengel et al., 2011)

[1]Chromosome; [2]References

The hyperprolificacy of Booroola ewes was suggested by decreased in receptor activity with polymorphism in *BMPR1B*, causing reduced sensitivity of *BMP4* action towards granulosa cells proliferation and inhibition of progesterone production (Demars et al., 2013; Fabre et al., 2006; Mulsant et al., 2001). Besides that, the reduced BMP inhibiting action in the granulosa cells were also increase the FSH sensitivity (Fabre et al., 2006). The mutant allele of this gene showed an additive effect, where the highest ovulation rate and litter size were in the homozygous ewes. Meanwhile, Juengel *et al*. (Juengel et al., 2011) have proposed a novel major gene of the same phenotype without interaction with the *TGF-β* pathway, namely, Fecundity Davisdale (FecD). Similarly, the $FecL^L$ gene in Lacaune sheep showed an additive effect and is controlled by an autosomal gene (Martin et al., 2014). Beta-1,4-N-acetyl-galactosaminyl transferase 2 (*B4GALNT2*) was proposed as the causative gene (Drouilhet et al., 2013) and does not belong to the *TGF*- β signalling pathway.

Quantitative Trait Loci for Twinning and Ovulation Rate

Conventionally, genes for the trait of interest were mapped via correlation of genomic region to the phenotype with the resulting location referred to as a quantitative trait locus (QTL). In several populations of cattle, QTL affecting twinning and ovulation rate have been reported on chromosomes 5, 7, 8, 10, 12, 14, 19, and 23 (Allan et al., 2009; Arias and Kirkpatrick, 2004; Blattman et al., 1996; Cobanoglu et al., 2005; Cruickshank et al., 2004; Gonda et al., 2004; Kappes et al., 2000; Kim et al., 2009; Kirkpatrick et al., 2000; Lien et al., 2000). Among those reported QTLs, a QTL associated for twinning rate was on BTA10 (Cobanoglu et al., 2005), the same chromosome of recently reported candidate region for ovulation rate in cattle (Kirkpatrick and Morris, 2015). However, it is a distal region of the chromosome, which is unlikely to represent the candidate region. In the most recent finding, a major QTL was identified on chromosome 11 of Holstein cattle where Luteinizing Hormone / Choriogonadotropin Receptor (*LHCGR*) and Follicle Stimulating Hormone Receptor (*FSHR*) are located (Widmer et al., 2021). Garcia-Guerra et al. (Alvaro Garcia-Guerra et al., 2018) listed chromosomal locations of quantitative trait loci and single nucleotide polymorphisms associated with twinning rate and ovulation rate in cattle.

The Story of "Treble", a Highly Prolific Cow

A cow named "Treble" had calved three sets of triplets in her lifetime (Morris et al., 2010). Triplet births in cattle are a rare occurrence; estimates range between 1 in 3500 births to 1 in 105,000 births, depending on breed (Jones and Rouse, 1920). Treble's son, "Trio", which was born in 1996 as part of the second set of triplets, sired 131 daughters from 2008-2011 at University of Wisconsin-Madison research farms using artificial insemination (Kirkpatrick and Morris, 2015). The high prolificacy trait of the founder cow was observed in her descendants sired by Trio, producing twins and triplets. This is evidence of transmission of a genetic factor across generations for this exceptional record of prolificacy. Preliminary work has confirmed that the ovulation rate is caused by a single gene, which was mapped to a narrow genomic location on chromosome 10 to an area of 1.2 Mb (Kirkpatrick and Morris, 2015).

There are seven of each protein coding and non-coding genes that reside in this candidate gene region (http://www.ncbi.nlm.nih.gov/). The protein coding genes are: *SMAD6, SMAD3, AAGAB, IQCH, C10H15orf61, MAP2K5* and *SKOR1*. Three of those genes are considered as strong positional candidate genes; *SMAD3* and *SMAD6* that are involved in *TGF-ß* signalling system (Shi and Massagué, 2003), while *IQCH* has been associated with menarche in human females (Elks et al., 2010). In the recent study, *SMAD6* were found overexpressed in the granulosa cells of the carrier females compared to the non-carrier of the allele (Kamalludin et al., 2018). The *SMAD6* has inhibitory role in the BMP signalling pathway--high expression of this gene might had interfere the pathway and causing higher ovulation rate of the carrier. The carrier group shows greater circulating FSH, smaller individual follicles and higher ovulation rate compared to the non-carrier (A. Garcia-Guerra et al., 2018).

CONCLUSIONS

Production cost has always been one of the major factors in shaping the well doing and profits in many industries. Twinning can increase reproduction ability, production volume via positive effects that can contribute towards of the same focus above. However, depending on which industry, it also carries some negative impacts. That has made twinning an unfavorable phenotype in the dairy cattle industry, albeit higher occurrences have been recorded in the dairy cattle compared to the beef cattle. Twinning is highly correlated with ovulation rate in cattle. There are various genes that are affecting hyperprolificacy in sheep have been reported. None of those genes is corresponding to the candidate genes that effecting ovulation rate in cattle, as reported by Kirkpatrick and Morris (Kirkpatrick and Morris, 2015), the only major gene affecting ovulation rate that was reported for cattle (Kamalludin et al., 2018).

Combination of approaches including molecular markers selection for breeding, embryo transfer and hormonal manipulation is promising to rise the twinning rate despite their low heritability. Having said that, identification of the causative gene for this phenotype is a decent approach to apply in selecting (for or against) and breeding the herd while increasing or decreasing the gene frequency in the population of interest. Discovery of molecular markers that affecting the ovulation rate can be used not only for selection in cattle production, but also a valuable resource for further investigation of their effect on other species.

The negative impacts of twinning can be minimized by prior measures such as appropriate nutrition management, suitable calving facilities and expertise, and early detection of twin pregnancies for better managements. In some region, the domestic ruminant played an important role to convert low quality forage to high quality protein due to their high adaptability to the environment, besides their lower cost of production. They often reported inferior in production traits compared to the exotic breeds ie. Kedah-Kelantan cattle in Malaysia. With proper knowledge and management, twinning technologies is likely to be a good choice to increase their production by increasing their numbers. Along with ability to attend and handle multiple pregnancy complication by the small holders, twinning technology can also be beneficial in various level and interest.

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