Chapter

The Incidence of Ovulation and Detection of Genes Associated with Ovulation and Twinning Rates in Livestock

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Abstract

Cattle is a monotocous species that generally produce only one offspring per conception. However, multiple ovulations are a naturally emerging reproductive phenomenon typically controlled by genetic structure and environmental factors. On the other hand, few genes or causative mutations might explain significant genetic variations between animals for the reproductive traits. Studies report different methods, including QTL analysis, fine mapping, GWAS, and MAS selection, to improve such traits due to their economic importance. The recent fine-mapping study, which narrows the genomic region, indeed, influencing multiple ovulation, gives positive signals that causative mutation controlling high ovulation rate may be identified shortly. In conclusion, identifying the major genes that considerably affect ovulation and twinning rates provides the opportunity to increase reproduction efficiency by improving genetic gain in livestock species.

Keywords: ovulation rate, twinning rate, polymorphism, QTL, MAS, livestock

1. Introduction

Complex traits are typically influenced by multiple genes by their combined contributions and modifications of environmental factors. However, a few genes or loci account for most variation between individuals for any given domesticated species. Researchers develop various methods, such as marker-assisted selection (MAS), to improve production and reproduction, and performance traits because of their economic significance in dairy and beef cattle over the last 50 years. This chapter presents issues about the major traits with economic values for the genetic improvement of livestock reproduction. It also covers aspects from basic information about physiological mechanisms of ovarian follicular development in ruminants to incidence of multiple ovulations to the fundamental studies of ovulation rate in model species to all aspects of ovulation rates and genetic studies to identify quantitative trait loci or causative mutations affecting ovulation rates and more explicitly twinning rates in bovine species.

2. The overview of ovulation induction

Ovulation is the release of a fertilization-competent oocyte (mature female germ cells) from the ovary into the fallopian tubes in the female abdominal cavity where male sperm cells fertilize; thus, it is an essential and sophisticated biological process for sexual reproduction. Ovulation is an ovarian response that is initiated due to the surge of luteinizing hormone (LH) through the anterior pituitary gland. Thus LH surge triggers ovulation and estrus [1, 2] and the development of the corpus luteum, which initiates a series of ovarian activities in females. It works with the secretion of follicle-stimulating hormone (FSH), which plays a fundamental role in regulating for development of ovarian follicles as well as selection. It also stimulates granulosa cell differentiation, regulating gonadal functions, including steroidogenesis [3, 4]. LH, along with FSH, are considered gonadotrophic hormones because of their role in controlling the function of the ovaries in the development of preovulatory follicles to stimulate certain molecular events. This complex spectrum of events includes various types of ovarian cells, the activation of various signaling pathways, and the controlled expression of specific genes affecting the overall mechanism. LH and FSH levels are regulated and highly dependent on the pattern of release of gonadotropin-releasing hormone (GnRH) from the hypothalamus [5, 6].

Up to the last decade, a significant focus on ovulation and related features has been the association analysis of known candidate genes. The entire process of ovulation varies in mammals, following where they can be spontaneous or induced manners [7]. Spontaneous ovulation is the ovulation process in which females exhibit a constant cycle of reproductive hormones and does not require to be aroused in any way through a male to generate a preovulatory LH surge associated with reproduction. Species that are naturally ovulating through estrous produce mature ova through a process necessary for fertilization to occur. The females are ovulating spontaneously include mice, rats, domestic dogs, sheep, goats, horses, pigs, monkeys, and humans [8, 9]. The entire cycling process of ovulation varies among species. For example, while humans and primates experience monthly menstruation processes, all other animal species ovulate through various other ovulatory mechanisms [10].

Whereas female who displays mating-induced ovulation will have spontaneous development of follicles to maturation due to some component of coitus that is an externally-derived and receipt of genital stimulation during, or just before mating. Therefore, despite exhibiting high estradiol levels resulting from follicular maturation, they do not ovulate because they entail priming of males resulting in a long mating cycle to ensure successful fertilization [11].

Often, steroid-induced LH surges are not seen in ovulation types induced during reproductive periods, indicating insufficient or reduced secretion level of GnRH due to lack of positive feedback from estrogen and progestin hormones upon gonadotropin secretion. However, paradoxically, some natural ovulating species may undergo an occasionally induced preovulatory LH surge due to mating. Species in which females are triggered in induced ovulation include rabbits, domestic cats, ferrets, and camels [8].

Reproduction is a highly dynamic process and has significant consequences on livestock profitability. Reproductive success is conditioned by fertility, productivity, and fecundity. In particular, there are several minor genes and some major genes, which are fecundity related genes (Fec) that significantly affect reproductive traits, like ovulation rate, prolificacy, and litter size genetically [12].

Livestock species are mainly classified either as monotocous species, like cattle, water buffaloes, and horses, or multiparous species, like goat, sheep, and pig based on ovulation rate depending on the characteristic of a species [13].

Biological factors for the consistent multiple ovulations and how to improve or control the ovulation rate in other single-ovulating species in livestock have been of interest to some researchers to understand and intervene in the process of follicular development by applying assisted reproductive technologies. Therefore, identifying various experimental animal models with multiple ovulation rates could efficiently enhance the selection response in farm animals.

Specifically, the reproduction process is primarily influenced by genetic and environmental factors for a transformation from primordial follicle to mature ovulatory stage and typically has low to medium inheritance; thus, traditional phenotype-based selection methods are often time-consuming processes due to a lack of efficiency.

It is more effective to select breeding animals based on their genotypic structure to increase ovulation rate, prolificacy, and litter size as reproductive abilities in livestock species. Eventually, selecting animals based upon highly polymorphic marker information for reproduction efficiency (MAS) will be of great importance for future breeding programs in the livestock production system.

3. The use of molecular genetic markers and techniques to improve reproductive performance in livestock

Genetic improvement of reproductive efficiency is one of the most effective strategies available to improve the performance of farm animals. Especially in the last 50 years, selection program based on classical or molecular genetic principles has led to significant positive changes in dairy and beef cattle [14]. Reproductive efficiency is influenced mainly by environmental factors such as dietary regimen, animal health and management, and their interactions, as well as by many genes in dairy animals. Reproductive traits generally have low-to-moderate heritability and do not show excellent progression to phenotype-dependent selection by classic selection methods. Therefore, determining the genes that affect the reproductive ability and including them in the selection program is one of the crucial arguments in increasing the efficiency and success of the selection process.

Genetic markers of follicle number in cattle ovaries can identify heifers that will become highly fertile cows because genes play an active role not only in the physical structure of an organism but also in its functioning. Therefore, analysis of the farm animal genomes will enable us to identify putative genes that are supposed to affect fertility and cow productivity, which are economically important traits in livestock, as the ultimate goal. Specific chromosomal regions, which contribute to complex traits, are called quantitative trait loci (QTL). Several studies were conducted to identify genetic variation in quantitative traits in livestock and laboratory species since the genetic variation is an essential part of breeding programs. A possibility of detecting loci that affect quantitative traits using genetic markers has been realized since Sax's study with beans, which utilized seed-coat characters as markers due to the relationship with seed size in 1923 [15].

Selecting desirable alleles at particular loci based on marker information will increase the selection response for the next generation. Short sequences of DNA, called genetic markers, are specific DNA regions in the animal genome that indicate variation within the population. These polymorphic regions can be positively or

negatively associated with particular reproductive traits of interest. One of the main tools for genetic improvement is the wide usage of molecular markers such as microsatellites, minisatellites, and single nucleotide polymorphisms (SNPs) using different methods such as PCR-RFLP, SSCP, SSR, qRT-PCR, and whole-genome analysis or the next-generation sequencing [16].

Especially microsatellite markers are not only highly polymorphic but also reasonably abundant throughout the genome [17]. The relationship between marker alleles and phenotypic observations on the trait is used widely in linkage analysis to map a segregating QTL in a population. The presence of highly polymorphic DNA markers in genetic maps in various farm animals and their relationship to phenotypes provide an effective tool for QTL affecting traits. However, identifying markers closely linked to the target region and determining the association between marker allele and QTL allele, which control the quantitative traits, are rather complex processes. A high-resolution marker map and precisely recorded phenotypic values are needed to determine the linkage between marker loci and QTL with low to moderate effect controlling the traits like reproductive performance [18]. Therefore, the QTL region affecting mainly low-moderate heritable traits is detected to find molecular markers that can be applied in the MAS system, enhancing the genetic gain for the reproductive trait of interest.

Several reproductive traits have been associated with fertility in dairy cattle, including age at puberty, early ovulation, size of ovulatory follicles, multiple ovulation, ovarian cystic structure, embryo survival, and heat detection [19, 20], which heritability rates are around 1–5% [21]. The prediction of these heritability ratios still notifies that there is a potential to make genetic progress selecting against reproductive traits in bovine. Genome-wide association studies (GWAS) are widely used powerful techniques to discover genetic variants strongly associated with various complex traits concerning any disease resistance, productive and reproductive abilities in different organisms over the last twenty years. For this purpose, chip-based microarray technology has been developed as a high processing platform to support GWAS analysis. GWAS is a technique that assays high-density SNP markers located throughout the genome to identify putative locations, either causative or in linkage with continuous phenotypic variation. The availability of millions of SNPs markers makes the system easily genotyping on throughput platforms by covering the whole genome [22]. Various GWAS studies have been carried out on livestock, especially in dairy cattle [23], beef cattle [24], water buffalo [25], pigs [26], sheep [27], and goat breeds [28]. However, the large number of potential genes identified by GWAS have not been fully validated yet. As the best-powered studies, they are combining researches of GWAS data and genomic selection (GS) with MAS in livestock species will precisely accelerate the accomplishment of analyzing massive genotypic data through millions of genetic markers which are collected from up to hundreds of thousands of phenotyped animals with diseases and traits of interest soon [29, 30]. In addition, other new technologies, including RNA-sequencing technology, to be implemented through the genome-wide sequencing of mRNAs in animal species can be widely applied in such studies over time [31]. In conclusion, it is expected that many more major genes, causative mutations, and even several genes with minor effects will be definitively identified shortly due to the drastic decrease in prices for SNP genotyping and DNA and mRNA sequencing with the substantial increase in livestock genomic studies.

4. Developmental stages of ovarian follicles

Folliculogenesis, the complex biological process of forming ovulatory follicles among the cohort of growing primordial follicles on the ovaries produced by

female animals throughout their lifetimes, causes changes in ovarian morphological characteristics during the typical estrus cycle, an essential aspect of female reproduction [32]. Cattle are a monovular species that can produce several hundred thousand primordial follicles at the onset of puberty, depending on their physiological mechanism. However, practically less than 1% of these follicles will grow and be ovulatory in the late stages of development due to atresia. Selecting a single dominant follicle among many growing primordial follicles is an essential step in livestock reproductive technology. Therefore, any intervention or malfunction in this process can lead to infertility or multiple ovulation in females. The current follicle selection process focuses on the role of follicle growth and selection of the dominant follicle regulated by LH, FSH, and insulin-like growth factor family (IGF) hormone mechanisms [33].

Many studies on the growth stages and developmental processes of follicles in animals have also presented different models. As one of the most notable models, Rajakoski proposed the developmental stages of antral ovarian follicular growth in cattle occur in a wave-like pattern [34]. Many researchers reported that each cycle usually involves two or three waves. As a result of applying transrectal ultrasonography technology, the concept of follicular waves known to that day has been re-investigated and facilitated the understanding of the pattern of follicular waves during the estrus cycle [35, 36]. Therefore, ultrasonography technology has provided more detailed information about the follicular developmental stage and the follicular wave dynamics. In addition, monitoring the growth and development patterns of follicles has enabled us to make more detailed observations about follicle selection and understand how it relates to the endocrine secretion mechanism during the maturation of follicles [36, 37].

First, Pierson and Ginther observed individual follicular development during the cycle using this technology [37]. Later, various intensive studies were carried out to investigate these developmental stages of animal husbandry, especially in sheep and cattle breeds [38, 39]. The follicular wave pattern in ruminants is typically two or three follicular waves per cycle in cattle; incredibly primitive and very fertile dairy cows usually have two wave cycles, while nulliparous dairy heifers aged 2–2.5 years have three-wave cycles [40]. However, it is three to six waves per cycle in sheep [41]. Studies were proving that the developmental processes of follicles within a follicular wave are highly variable among waves. After puberty, all primordial follicles have an equal chance of becoming mature follicles. Primary follicular wave is characterized as the synchronous growth of a group of small antral follicles. One of them is eventually selected to be dominant and thereby becoming ovulatory among the group of follicles within each follicular wave. But all other remaining "subordinate" follicles of the ovulation wave will regress and degenerate during the typical estrus cycle [42, 43]. The dominant or mature follicle of the wave is typically the largest in diameter. Still, the subordinate follicles belong to the same group of follicles which the dominant follicle comes from [40].

Traditionally, in cattle, the day when a follicular wave can be first detected determines the day when the first observation of the dominant follicle can be made retrospectively [44]. A first dominant follicular wave emerges when the follicles are 4–5 mm in diameter at approximately the day of ovulation [42]. Subsequently, a second ovulatory wave can be detected about 9 or 10 days later [45]. The main event that causes single ovulation to occur in cattle is called follicle selection. Diameter deviation occurs approximately 2 to 3 days after the emergence of the follicular wave in the selection of follicles in the morphological process. Thus, while the future dominant follicle grows continuously, the growth rate of the lower follicles slows down, and then their growth is stopped entirely, and they undergo degeneration. Although this deviation varies among individual animals, it is widely accepted

as it has been observed in this range in many studies using both Bos taurus and Bos indicus breeds [46–48]. However, the high progesterone concentration prevents the first dominant follicle from maturing, as the corpus luteum has not regressed yet. Thus, the first dominant follicle cannot be functional and ovulatory. Subsequently, a second ovulatory wave can be observed. The dominant follicle from this wave can keep on growing and ovulating during the corpora lutea (CL) regression. In addition, a third source of the ovulatory follicle becomes apparent on day 16 after ovulation in some cattle breeds due to the regression of the second dominant follicle during luteolysis. Even if each wave involves simultaneous emergence of a cohort of follicles, usually one of them, sometimes two, become dominant follicle(s), and all of the others eventually become subordinates. A single oocyte is released from the dominant follicle due to either naturally occurring or artificially induced ovulation. On the other hand, subordinate follicles begin to regress right after a short growing phase [44, 45].

It was noticed from individual to individual that the follicle size at ovulation was quite different. Dairy heifers showing two-wave cycles have a follicle at a diameter of 16.5 mm in ovulation. However, follicle size is smaller in heifers (13.9 mm) with three-wave cycles [44]. Similarly, the size of ovulation follicles has been reported as 14.8 mm in Holstein heifers. However, the follicle size observed for lactating dairy cows was slightly larger and was found to be 17.4 mm [49]. In many studies of follicular diameter deviation, both the future dominant follicle and the most significant lower follicle were more prominent in Bos taurus. However, diameter deviation occurred at similar times after wave emergence in Bos taurus and Bos indicus. Bos indicus has a smaller follicle size when the deviation in the follicle diameter cannot be fully revealed. Nevertheless, the results of the studies support the idea that the future dominant follicle generally has a size advantage over the largest subordinate one [46, 48, 50].

Reproductive biotechnology has recently emerged as a powerful tool to improve livestock productivity and reproductive performance. Therefore, these modern reproductive technologies have started to be used instead of conventional classical techniques in many reproductive-based studies recently. Progress in our understanding of follicle development and selection has sparked the development of synchronization protocols for fixed-time artificial insemination (AI), in addition to the applications of other cutting-edge reproductive technologies such as in-vitro fertilization (IVF), embryo culturing and transfer (ET), cloning, estrus synchronization, transgenesis, and much more new emerging reproductive biotechnologies [51, 52]. As a result, these developments in terms of sustainable livestock productivity are important for optimal follicle growth and making the right choices to increase reproductive efficiency in livestock species.

5. The incidence of multiple ovulations

Cattle are a uniparous species that means females usually produce only one progeny per conception due to the single dominant follicle in each ovulatory cycle. Alterations in follicle selection can lead to codominant follicles and multiple ovulations, which are the basis for multiple births in cattle and sheep [53]. In rare cases, the synchronous emergence of two follicles as a physiological pattern, albeit in a monovular type, is altered so that the follicle selection mechanism allows both to be selected as the dominant follicle among several follicles in the follicular wave. The ease of evaluating follicular events by trans-rectal ultrasonography and the accuracy of the data obtained from these studies have allowed cows to be widely used as an ideal research model in follicular studies in ruminants and humans [54, 55].

Ultimately two oocytes are released from codominant follicles at the end of ovulation due to either natural stimulation or artificial inducements. In the development of codominant follicles, deviations occur in the diameter of the follicles when the largest follicle and the second-largest follicle are close to 8.5 mm. The third-largest follicle has a low growth performance, and the deviation in 2nd largest follicle may occur 36–50 h after the deviation of the first follicle [56]. Ovulation of two future dominant follicles occurs either from the same ovary simultaneously, or each follicle consists of a separate ovary [57]. In a study, synchronous production of two oocytes from different follicles was observed due to the evidence of two corpus luteum (CL) on the ovaries of cattle [58]. Also, research about follicular development during the estrous cycle in twin-calving cows indicated that double and triple ovulations coincide from different ovulatory follicles of the same follicular wave rather than ovulation of single mature follicles from two consecutive waves [59]. In addition, the authors noted that the cysts in the ovary and lack of CL possibly increased the incidence of double ovulation during pregnancy [60]. Therefore, as more than one follicle deviates and becomes dominant, the chance will be increased for ovulation of more follicles simultaneously. After all, twins, triplets, or overall multiple births in rare circumstances will become a reality if all subsequent events commonly occur for both oocytes from fertilization to parturition.

The natural incidence of twin or triplets birth in cattle is mainly due to multiple ovulations that have been summarized in many studies, even if the results are inconsistent [61]. While the multiple births five decades ago is around 1–5% depending on breed, genetics, parity, and other environmental conditions [62], this rate has increased up to 10–22% in lactating dairy cows today. There have been many studies conducted about regulating multiple birth rates in cattle by selecting genetically favorable animals [63], utilizing hormonal treatments [64], or utilizing embryo transfer techniques [65]. One of the reasons affecting ovulation rates is low progesterone secretion in older cows, which might be the main reason for the increase in circulating LH level and eventually causes enhance ovulation numbers as progesterone has a suppressive effect on LH release. In addition, growth hormone and nutritional treatments greatly influence a multi-ovulation response of an individual [66]. Also, the ovulations of two follicles simultaneously caused to increase in days of milk among pregnant animals [60]. In a recent study, the incidence of multiple ovulation rates in early lactating animals was 14.1%, but they did not significantly affect various reproductive outcomes of cattle [67]. Although the underlying mechanisms of multiple ovulation have been studied extensively, the dynamics of the entire mechanism are still not fully explained.

Monozygotic twins are genetically and physically identical since they are formed from one fertilized egg, splitting into two identical halves during early embryonic developmental stages. Thereby both individuals are always the same sex. In the case of dizygotic or fraternal twins, two different sperm fertilize two completely different ova simultaneously. Thus the successful result of ovulation and fertilization of two oocytes will be dizygotic twins. Dizygotic twins are not identical genetically or phenotypically as monozygotic twins are. They are not necessarily the same sex as opposed to monozygotic twins. They can also be similar or different from siblings born from the same parents during different gestations [68].

Twin or triplets birth is an unavoidable issue in dairy and beef cattle production systems which negatively affects the health, production, reproduction and overall decreases the productive life span of animals [69]. The study reports that the calf survival incidence from twin- and triplet-producing animals were relatively low, around 44% depending on the breed composition [70]. In a recent study of the economic analysis of multiple births, the economic loss to the livestock breeder from each twin calving was estimated at between $59 and $161 in cow-calf

production systems [71], even if twin calving could reduce substantially beef meat production costs owing to more calf growth at weaning [69]. Thus twin or triplet calving causes to lessen overall cow reproductive efficiency, productivity, and thus the profitability of enterprises. In conclusion, a complete understanding of the complex process of follicular growth during the estrus cycle and the development of oocytes will undoubtedly improve the knowledge to maximize and control the efficiency of reproduction in livestock species, especially the existence of dizygotic twinning since the fertilization of more than one oocyte after ovulation will be the main reason of multiple births.

6. Studies on ovulation rate in small ruminants as a model organism

Detection of the specific major genes that control reproduction traits provides the opportunity to improve genetic gain in livestock species. However, fertility traits generally have low heritability, and reproductive improvements in a phenotypic selection based on observable data are pretty low and limited. However, ovulation rate and litter size in sheep are important fertility traits, and they have high economic values for breeders [72]. The ovulation rate mainly determines the productivity of sheep. Ovulation rate and litter size are expressed in only one gender and can only be recorded relatively late in the animal's lifespan. Focusing on improving fertility traits will have a long-term impact on the profitability of the sheep production system [73]. For more than a decade, sheep have been used by many researchers as an essential model organism to identify genes that control reproductive functions such as high fertility rate and ovarian follicle selection and also to investigate the physiological mechanisms involved in this reproductive system. Strong evidence was detected for major genes controlling prolificacy in sheep [74]. Specifically, the genetic influence on prolificacy variability in sheep has demonstrated that many genetic mutations have essential roles in controlling the ovulation rate. Those genes were tested in several populations based on the patterns of phenotypic segregation. Therefore, the selection of breeding animals will be more effective based on genotyping for relevant candidate genes to improve fertility and fecundity traits such as ovulation rate and litter size in small ruminants.

In a study conducted in this context, it was observed that sheep developed from the Booroola Merino strain had an autosomal mutation that increased the ovulation rate by approximately one and a half ova [75]. Therefore, it was an excellent candidate to investigate the mechanisms controlling ovulation rate in mammals [74, 76]. It was reported that the Booroola fecundity gene (FecB) has a partial dominant effect on litter size due to embryonic loss in homozygous carrier animals with high ovulation rates [77]. Subsequently, it was observed that, on average, one copy of the FecB gene enhanced the number of offspring by about 1.5, with increasing ovulation rates of about 1.65 ova per copy of the gene. The gene was accurately mapped to chromosome (Chr) 6 in a region where the bone morphogenetic protein receptor 1B (BMPR1B) was located in sheep [78]. This region is syntenic to Chr 3 or 5 in mice and Chr 4 in humans [79].

Moreover, the other major gene was detected, increasing ovulation rate and litter size in Inverdale sheep. The Inverdale fecundity gene (FecXI) has been located on the X chromosome and increases ovulation rates in the heterozygous ewes [80]. But, homozygous ewes are observed to be infertile due to lack of follicle development [81]. Another fecundity gene (FecXH) was also identified successfully on the X chromosome in the Hanna sheep population [82]. Both FecXI and FecXH were mapped in the bone morphogenetic protein 15 (BMP15) site. However, different point mutations were identified in the BMP15 gene in Inverdale and Hanna sheep

populations. If ewes are heterozygotes for any of them, it causes to increase ovulation rate to two-three ova. However, if sheep is homozygous for Booroola mutation, it dramatically raises ovulation rate from 5 to 14 [83]. Another study investigating the ovulation rate records obtained from daughters of ewes inseminated by Coopworth rams to understand the inheritance pattern of ovulation rate also proved that there was another maternally inherited gene affecting productivity traits located on the X chromosome (FecX2w). But the location of this gene is entirely different from the gene on Inverdale FecX locus [80]. The findings of studies conducted about four decades ago have guided many subsequent types of research on this subject, in which sheep are extensively used as model organisms in this subject.

Currently, the segregation of five major genes that affect ovulation rate and prolificacy has been characterized at the molecular level in various sheep and goat breeds that cause significant phenotypic variations. Overall the detected genes are bone morphogenetic protein receptor, type 1B (BMPR1B; in Booroola Merino, Javanese, Small Tail Han, Hu, Garole, and Kendrapada breeds) [78, 84, 85], bone morphogenetic protein 15 (BMP15; in Inverdale, Hanna, Romney, Belclare, Cambridge, Galway, Lacaune, Raza Aragonesa, Olkuska, and Grivette breeds) [82], growth differentiation factor 9 (GDF9; in Belclare, Cambridge, Icelandic Thoka, Santa Ines, Embrapa, Finnish Landrace, Norwegian White Sheep, Ile de France, and Baluchi breeds) [86], beta-1,4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2 in Lacaune) [87], and leptin receptor (LEPR in Davisdale sheep) [88]. Causative polymorphism studies in different prolific sheep breeds showed at least 12 identified allelic variants for the BMP15, BMPR1B, and GDF9 genes encompassed in the transforming growth factor-beta (TGF-β) signaling pathway secreted from the oocyte. TGF-β is significantly associated with ovulation rate, litter size, and prolificacy and thus plays a critical role in the folliculogenesis of small ruminants. Many studies reported that the mutations in TGF-β pathway-related genes enhanced ovulation rate (35–100%) in heterozygous animals [89]. Moreover, even if causative mutations for fecundity are not fully discovered, two other genetic variants were identified as FecX2W [90] and FecD [91], which are segregated in prolific sheep breeds in recent studies. Similarly, about 20 different candidate genes, including TGF-β related genes, were also detected to play a crucial role in regulating folliculogenesis and prolificacy-related traits in several goat breeds [28]. To improve the genetic makeup of animals affecting high productivity in livestock, over 30 small ruminants, mostly high-yielding sheep and few goat breeds, have been actively used in candidate gene studies that focused on detecting variation related to reproductive performance-related traits.

7. Ovulation rate studies in bovine

7.1 Cattle as a model animal for multiple ovulation

As a uniparous species, cattle produce only one progeny in most cases, resulting from ovulation of a single follicle during the pregnancy. Nevertheless, the natural incidence of twin or triplet calving in cattle is mainly due to multiple follicular ovulations concerning breed differences, age of dam, parity, season, the effects of feeding and management systems, geographic location of raised animals, and other environmental effects [62]. Specifically, the incidence of double birth was observed as approximately 1% in beef cattle [92]. In comparison, this rate was determined as 4–5% on average, ranging from about 1% for heifers to nearly 10% for older cows in dairy cattle [93, 94]. Several studies were conducted concerning underlying causes of multiple ovulation rates, particularly twinning rates in cattle by selecting

genetically highly polymorphic animals [63], using trans-rectal ultrasonography or quantifying by circulating AMH concentrations, utilizing embryo transfer techniques [65], or utilizing hormonal treatments [64].

The ovulation rate is closely related to the twinning rate in cattle due to the high genetic correlation between ovulation and twinning rates, ranging from 0.75 to 1.0 [95]. Although the genetic control of multiple ovulation in cattle by major genes has long been the subject of research, and there has been significant interest in the mechanism underlying multiple ovulation in bovine species [70], genes with significant effects on ovulation rate have unfortunately not been identified until recently [96].

7.2 QTL studies about twinning and ovulation rates

The selection of genetically superior animals in terms of twinning frequency has been practiced in long-term experimental herds in different countries. For this purpose, various research herds for multiple ovulation studies have been implemented to be established in various countries for four decades. These herds were begun to set up in the early eighties to select for increased twinning rates in France [97], Australia [76], New Zealand [98], and Meat Animal Research Center (MARC) of the USDA-ARS in the USA [99] to develop effective genetic strategies to improve production efficiency including twinning and ovulation rates, meat quality, and animal health in dairy and beef cattle production. MARC twinning population initiated with a total of 307 well-suited cows from twelve different experimental beef, dairy, and dual purposes breeds to study involved in follicular development and recruitments and identify genes affecting primarily twinning rate; later taken into account of ovulation rate in 1981 [63, 100]. These cows were selected based on their high twinning frequencies. The twinning rate can be defined as sequential events due to ovulation, conception, and embryonic survival [101]. Sires whose dams were founders of the herd and sires whose daughters had high twinning rates were used for breeding the founder cows. In addition, semen collected from sires that mainly originated from Swedish and Norwegian breeds was used in the project. The founder breeds in the herd were mainly Holstein (18%), Swedish Red and White and Norwegian Red (12.8%), Swedish Friesian (16.1%), Pinzgaurer (18.4%), Simmental (15.8%), Charolais (5.3%), Angus and Hereford (8.3%), and other breed crosses (5.3%) [102]. The primary objective of the research was to increase the twinning rate in the herd. Therefore, they selected animals based on twinning performance. However, later on, they also evaluated animals' ovulation rate records for 8 to 10 estrous cycles since ovulation rate is highly genetically correlated with the twinning rate (0.90) [58]. Thus, they used an animal model with multi-trait repeated records to predict breeding values for twinning rates in 1990. By applying this methodology, they were able to use information not only from the individual but also from all available relatives for twinning and ovulation rates. The most significant advantage of using ovulation rate records as an estimator of twinning rate is to reduce generation interval and reduce the number of cows retained for several generations. The estimated twinning rate was about 4% in 1984. But this prediction rose linearly to 35% in 1996 [100]. In the latest report, all the cows with lower estimated breeding values (EBV) were culled from the herd. Thus herd size was reduced from 750 to 250 cows giving birth annually. The twinning rate then was enhanced from 35% to over 50% annually since 1997 [103, 104].

Many studies have been conducted to identify ovulation rate and twinning rate QTL in different cattle populations. Several genomic regions for putative ovulation rate were detected on BTA7 and 23 [105], on BTA5, 7, and 19 [106], on BTA5 [107, 108], on BTA7, 10, and 19 [109], on BTA14 [101] for ovulation rate using the USDA

Meat Animal Research Center (MARC) twinning herd, a herd with a substantial contribution from Holstein–Friesian and Norwegian Red breeds [110]. A suggestive twinning rate QTL on BTA5, 7, 12, and 23 have been identified in the Norwegian dairy cattle population [111, 112]. Twinning rate QTL based on genome-wide searches have also been observed on BTA5, 7, 19, and 23 [113], on BTA8, 10, 14, 21, and 29 [114], on BTA2, 5, and 14 [115] in North American commercial dairy cattle populations; on BTA6, 7, and 23 in the Israeli-Holstein cattle using daughter design [116], on BTA20 and 28 in the INRA experimental herd selected for twinning [117]. In studies using composite MARC herds, it was determined that cows producing twins based on genetic selection for high twinning and ovulation rates over multiple generations produced about two-fold more secondary follicles than animals in the control groups. The probable reason for the higher twinning and ovulation rates in this herd may be the combined effects of multiple genes associated with these quantitative traits [63, 100]. Multiple positional candidate gene regions associated with ovulation rate, twinning rate, and multiple birth rates in various cattle breeds have been identified by linkage analysis, interval mapping, linkage disequilibrium (LD) analysis, the combined linkage-linkage disequilibrium analysis (LDLA), and GWAS analyses even if only a few have been replicated. Depending on the statistically significant level, the QTL or single nucleotide polymorphisms (SNPs) determined in the studies so far were diverse throughout the bovine genome. They spanned about 18 of the 30 bovine chromosomes given in **Table 1** [89].

It is noteworthy that a crucial QTL region was detected on BTA5 in the MARC experimental herd, commercial dairy cattle populations raised in North American and Norway. Some of the founder sires in the composite MARC population were originated from Scandinavian countries whose progenies gave multiple births. Therefore, the probability of detecting the same QTLs in future studies is quite high due to sharing a significant portion of the founder genes in two different populations [63]. Furthermore, different studies have reported that IGF-1 as a candidate gene (especially the 2nd intronic region) in BTA5 is substantially associated with the twinning rate in US Holstein cattle [118, 119]. The presence of several QTLs for twinning rate and ovulation rate was detected, which were spanned 24 out of the 30 bovine chromosomes as a result of studies using high-throughput single nucleotide polymorphism (SNP) genotyping throughout the genome based on linkage (LE) and linkage disequilibrium (LD) analyses.

7.3 Novel candidate genes affecting multiple births in cattle

In cattle, twin or triplet births are naturally occurring reproductive processes, although not a joint physiological event in bovine. Models derived from the study of high prolific sheep breeds provide a framework for searching the regulation of follicular development in monotocous species, such as in cattle or humans.

A highly fertile cow named 'Treble' was born in 1993 at one of the cattle herds in New Zealand. Although the breed's origin is unknown, it has been assumed to likely include a hybrid of Hereford, Holstein, Angus, and Jersey breeds based on the coat color pattern. Treble calved three sets of triplets her life span as one heifer and two stillborn calves at the first time in 1995, two heifers and one bull, named as Trio at the second time in 1996, all stillborn calves due to considerable difficulty during the delivery period at the third time in 1999. Treble was cloned later, and two clone progenies were born in AgResearch Centre, NZ, in 2000. On the other hand, a son of a highly prolific cow named the 'Triple' was bred with a group of cows with high calving rates that had several progenies by 2008. Thirteen daughters out of his total of forty-four daughters produced a total of fifteen twin and six triplet sets, where triplet calving were 29% of all multiple calving, supporting the idea of a naturally-occurring

Trait	Chr (Appx. location as cM)	Population	Positional candidate genes (Chr)	Method	References
Ovulation rate	7 (40), 23 (27)	MARC Twinner	*CYP21* (23)	Interval Map.	[105]
Ovulation rate	5 (107), 7(5, 57) 19 (65)	MARC Twinner		Interval Map.	[106]
Ovulation rate	5 (40)	MARC Twinner		Interval Map, Assoc./LA	[107, 108]
Ovulation rate	7 (30), 10 (75), 19 (65)	MARC Twinner	*AMH* (7), *ESR2* (10), *IGFBP4* (19)	Interval Map.	[109]
Ovulation rate	14 (61)	MARC Twinner		Interval Map.	[101]
Twinning rate	5 (68), 7 (108), 12 (9), 23 (30)	Norwegian Cattle	*IGF1* (5), *CYP21* (23)	Interval Map.	[111]
Twinning rate	5 (64)	Norwegian Cattle	*MGF*	LDLA	[112]
Twinning rate	5 (68)	US Holstein	*IGF1* (5)	Interval Map./ LDLA	[113, 115]
Twinning rate	8 (117), 10 (41), 14 (68)	US Holstein		Interval Map.	[114]
Twinning rate	6 (55), 7 (25), 23 (67)	Israel Holstein	*AMH* (7), *CYP21* (23)	GWAS	[116]
Twinning rate	10 (49), 20 (27), 28 (8)	INRA Twin		LDLA	[117]
Twinning rate	4 (44), 5 (67), 6 (8,44), 7 (68,76), 8 (58), 9 (34), 11 (47), 14 (21, 38), 15 (23), 23 (51), 28 (9)	US Holstein	*IGF1* (5)	LDLA/GWAS	[118, 119]
Twinning rate	10 (14)	MARC Twinner	*SMAD3*, *SMAD6*, *IQCH* (10)	Linkage/Fine Map.	[120]
Twinning rate	24 (40)	Italian Maremmana	*ARHGAP8*, *TMEM200C* (24)	GWAS	[121]
Multiple birth rate	11 (31)	Swiss Holstein and Simmental Cattle	*LHCGR*, *FSHR* (11)	GWAS	[122]

Table 1.
Chromosomal locations of quantitative trait loci (QTL) and single nucleotide polymorphisms (SNP) associated with ovulation rate, twinning rate, and multiple birth rate in various cattle breeds [89].

major bovine allele contributing to a high fecundity rate in a family of cattle with triplet calving ability throughout the generations in New Zealand. The possible scenario for this situation might be that a gene or set of genes should be segregated as a single copy from a dam (Treble) to some descendants through its son (Trio) for single gene inheritance. Moreover, such a unique gene allele is expected to be segregated as dominant or partially dominant in female animals [70].

Several daughters (131) of Trio were born by AI in the USA by following the importation of his sperms at a University of Wisconsin (UW)-Madison research farm from 2008 to 2011. The research reports that a significant bovine allele for high ovulation was identified and mapped on a 2-Mb window on BTA10 (+1.02 CL per cycle for carriers vs. noncarriers for the marker allele of the high ovulation rate) by using fine mapping techniques employed the animals raised at UW-Madison research farm [120]. Thus, the daughters of Trio proved that there was evidence of a high-fecundity allele transmitting on BTA10 that had a major influence on multiple ovulations in cattle [96]. The detected location was not overlapped with any major genes previously reported for the high ovulation rate and litter size in prolific sheep breeds. Eventually, in addition to the noteworthy reproductive performance of Treble, all of her descendants, including Trio, also displayed extraordinary reproductive performance. Therefore, the members of the Treble family with highly reproductive ability should be heavily employed in gene mapping studies to discover major genes with high fecundity rates. It can provide a significant resource for the subsequent investigation of genetic diversity in bovine productivity [70]. In the follow-up study, the location of a major gene for high ovulation rate was strongly detected at 1.2 Mb region of BTA10 using half-sib daughters sired by a bull that assumed to be carriers of the Trio allele due to a single mutation. It is noteworthy that the novel region obtained does not overlap with any major gene previously reported, which significantly affecting ovulation rates in ruminants. Thus, the study reports that the newly identified regions could be employed to track inheritance patterns for multiple ovulation rates using from the carrier father's lineage since the screening of the aforementioned candidate gene consist of any functionally putative causative mutations in the coding region and 5′ or 3′ flanking regions, reminding that the polymorphic SNP region might affect the expression level of any candidate gene controlling the high reproductive performance of animals [96]. When the follicular and hormonal dynamics of animals carrying the high prolific Trio alleles were examined in animals raised at UW, the Trio carrier animals displayed multiple ovulation. The carriers produced more dominant and ovulating follicles with smaller diameters and volumes in this process due to the slower follicle growth rate close to the beginning of deviation during the entire follicular wave. In the study, even if the deviation times were similar between heterozygous bearing allele from Trio and half-sibling noncarriers, a significant increase in the selected number of multiple dominant ovulatory follicles in cow having Trio allele was reported to be associated with the enhanced concentration of FSH secretion close to the deviation time in the follicle. There was also evidence that smaller-sized follicles had more LH receptors in animals carrying the Trio allele than noncarriers, supporting the potential novel physiological mechanisms causing the production of multiple ovulatory follicles in the Trio allele carriers [89].

This newly identified candidate region covering 1.2 Mb in BTA10 contains seven protein-coding genes, of which three of them might be taken into account as putative candidate genes. These genes are the small-mothers against decapentaplegic (SMAD) family member 3 (SMAD3), SMAD family member 6 (SMAD6), those of which are the primary signal transducers for the receptors of the transforming growth factor-β (TGFβ)/Bone Morphogenic Protein (BMP) superfamily ligands [123], and IQ motif containing H (IQCH), which is strongly related with the first menstrual cycle in human females [124]. The other follow study stated well-conserved SMAD6 gene, which plays a crucial role in preventing the BMP/SMAD-dependent signaling pathway, was 9.3 times more expressed in carrier animals for the high fecundity Trio allele versus noncarriers using animals in UW-Madison research farm by applying quantitative real-time PCR technique.

Ultimately, the effect of over-expression of the SMAD6 gene displayed a similar impact of causative mutations on the functions of BMP15, BMPR1B, and GDF9 genes as part of a signaling pathway that may alter the incidence of ovulation rate upward in prolific sheep breeds [125].

In another study to determine the genetic basis of the observed increases in twinning and calf mortality in Italian indigenous Maremmana cattle breed, the most significant SNP markers (Hapmap22923-BTA-129564) were located near two genes, ARHGAP8 and TMEM200C on BTA24, which could be putative functional candidates for cattle twinning rates [121]. Furthermore, in a very recent study, the researchers detected a major QTL mapped to a 70 kb window between 31.00 and 31.07 Mb on BTA11 for multiple maternal births, explaining approximately 16% of the total genetic variation based upon linkage-disequilibrium analysis (LD) using the whole-genome sequence information of the Swiss cattle population. The identified QTL includes the LHCGR and FSHR genes as functional candidate genes. Precisely, a regulatory variant in the 5′ non-coding region of LHCGR is predicted as a potential causative mutation for the QTL region [122].

8. Conclusions

These studies covering the physiological mechanisms regulating ovarian follicular development through multiple births displayed us the reproductive traits are highly complex traits that involve a potential genetic background and significant contributions of various environmental factors. Several studies report that causative mutations in TGF-β pathway-related genes, including BMP15, BMPR1B, and GDF9, strongly affect the ovulation rates in prolific small ruminants, which tend to conceive and maintain multiple ovulation spontaneously. Even if the bovine is naturally low-ovulating mammals, some variations may still be observed in the ovulation rates, despite the low heritability; thus, there is a potential to make genetic progress through selection against reproductive traits by using multiple observations of ovulation rate as the indirect selection criterion in cattle. Recent fine-mapping studies that narrow the genomic region truly, influencing multiple ovulation, especially on BTA5, 10, 11, 14, and 24, give positive signals that causative mutation controlling high ovulation may be identified shortly.

The complete understanding of the complex process of follicular growth during the estrus cycle and the development of oocytes will undoubtedly improve the knowledge to maximize and control the efficiency of reproduction in livestock species, especially the existence of twin or triplet births since the fertilization of more than one oocyte after ovulation will be the main reason for multiple births. On the other hand, it should not be ignored that many factors, including genetic but primarily environmental sources, specifically breed differences, age and parity of dams, the season of calving, and the effect of feeding and management systems significantly affect ovulation and twinning rates importantly in especially cattle production systems. Therefore, the production of twin calves might be more profitable for the breeder by implementing appropriate management and feeding programs to cope with the reproductive problems faced by twin-bearing cows.

In conclusion, understanding the genetic background of high fertility in mammals, on the one hand, is extremely important for the designing of convenient genetic improvement and management programs in livestock; on the other hand, it provides the basic knowledge necessary to overcome fertility problems in humans by using the cow as the ideal model organism.

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