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# Cells: Segway for Genetically Desired Cattle through Embryotic Development Sequencings and Genetic Mapping

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Additional information is available at the end of the chapter

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## Abstract

Cells have a direct relation to understanding cattle genetic information aside from the cell's main role of housing genetic material. Gene presence being visible through phenotypic characteristics of living organisms allows for researchers to pinpoint specific genetic markers to access and evaluate the genetic information for desired visible traits. On a molecular level, through the interaction between cattle embryos and bovine cells, researchers can pair this information with current technological innovation to better understand on how genetics are manipulated naturally by environmental elements, which can have a direct effect on countries where cattle milk and meat production are the major contributors to economic stability. This overview of past, current, and potential future research involving cells can provide an understanding of the importance in cells and their relationship with biotechnology innovation. Through techniques involving cells and genetic analysis, various methods can be utilized to overcome limitations of cattle reproduction and increase the presence of desirable cattle traits, which in turn not only aid in the economic success of countries dependent on cattle products, but can also open the door to human therapeutics.

**Keywords:** genetics, cattle, cell, granulosa, embryo

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## 1. Introduction

Since the age of primitive man and of the hunter-gatherer, cattle has been the source of nutrition, clothing, culture, and innovation of tools to improve man's existence. As seen in **Figure 1**, an early view of animals through the eyes of cave dwellers in prehistory time allows today's modern

citizens to witness the existence of cattle (such as the ox) not only as a creatures of coexistence, but also as means of survival. As early as 8000 BC, the bovine species was domesticated as a means of a secure meat supply before becoming an essential object for clothes, tents, and even later becoming the main component in creating drums. Throughout history, cattles have been valuable not only as means of self-survival, but also played a major role in the art of agriculture and the conception of community. As the idea of growing food sources in one area (farming) grew more and more attainable the lifestyle of hunters and gathers phased into established communities with the help of cattle. The use of cattle as work tools for plowing fresh soil, as depicted in **Figure 2**, and the importance of manure for crop fertilization allowed the human race to settle and create civilized communities.



**Figure 1.** Untitled painting of ancient cave art. Retrieved from [https://en.wikipedia.org/wiki/Bison\\_priscus](https://en.wikipedia.org/wiki/Bison_priscus).



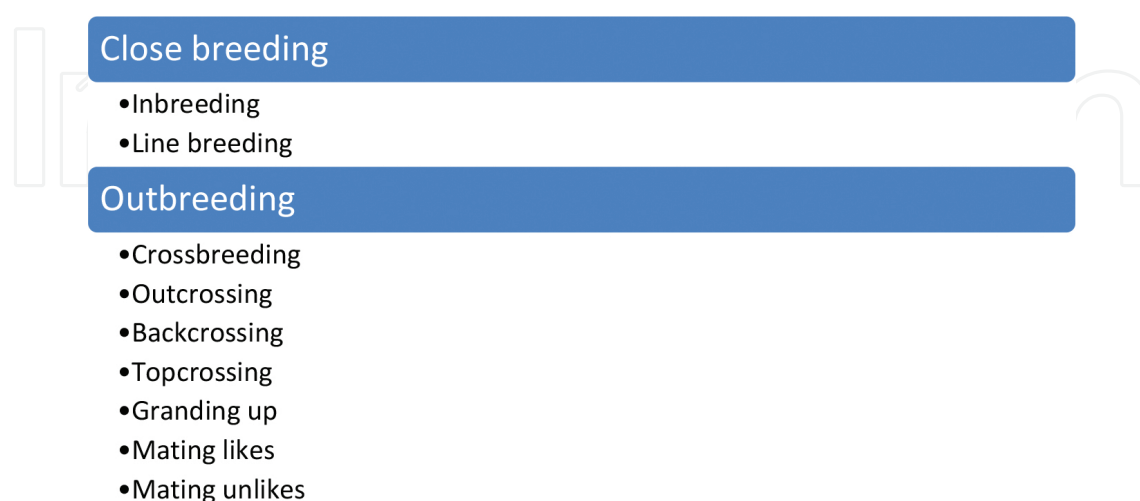
**Figure 2.** Untitled Egyptian art. Retrieved from <https://ucfant3145f09-04.wikispaces.com/Ancient+Egyptian+Food+%26+Agriculture>.

Today cattle remains a vital part in mankind's socio and economic progression through the same deliverables attained by their ancestors: food, clothing, and innovative tools. What worries the majority of cattle owners today is the pressure of supply and demand of meat and dairy products, which comes from sustaining high populations of cattle. Past and current

techniques for increasing cattle population include nutritional management and health practices to maintain or increase cattle production. As technological advancements and understanding of cattle genetics progress, methods including cell activity, genetic practices, and bioinformatics tools have been utilized to increase a deeper understanding of the cattle genetics not only to increase livestock numbers, which increase the production of desirable cattle that yields high economic profit, but also open new opportunities for novel human therapeutics.

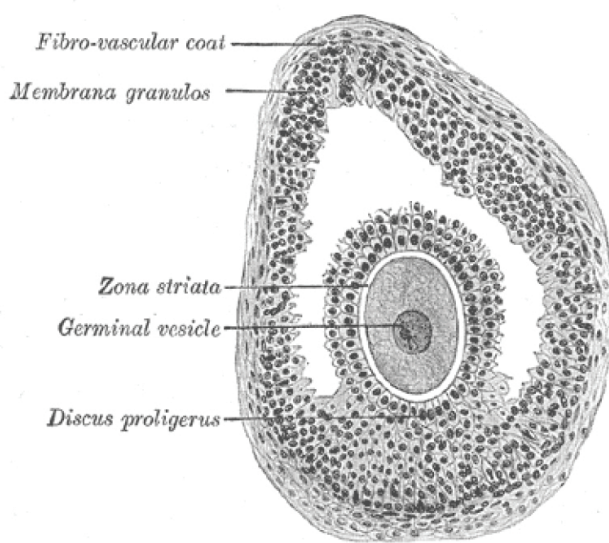
## 2. Breeding

One of the oldest methods for desirable cattle production involves breeding both close breeding and outbreeding (**Figure 3**). A popular and effective method of outbreeding, known as crossbreeding, involves breeding animals of pure breeds within each generation to produce desired animals. There are two main categories that fall under the crossbreeding umbrella: terminal- and rotational-crossing. Terminal-crossing is utilized more for meat animals and not dairy animals as it does not involve the progeny of breeding, but rather is slaughtered for commercialized meat. Whereas rotational-crossing involves the breeding of two breeds and utilizing the female offspring to breed with a sire of a different bred. The female offspring of this breeding sequence is introduced to one of the two breeds used in the original breeding scheme [1]. These techniques are particularly important when seeking heterosis. Heterosis is the improvement or enhancement of a specific trait that involves three types of improvement with respect to fertility: individual affecting the calf, maternal affecting the dam, and paternal affecting the sires. Individual heterosis increases live births and postnatal calf survival, while maternal increases conception rate, birth rates, and other economic traits such as milk product [2].



**Figure 3.** Types of cattle breeding methods.

These techniques are important in producing genetically desirable cattle to improve economic stimulating traits, but novel techniques are being studied to further the progression and overcome variables in increasing cattle. As previously stated nutritional management has been a means of sustaining current cattle numbers, but what if the nutrition in itself becomes an issue of calf abortions and still borns? This is an issue that is not new to those who raise cattle in the archipelago of the Azores. The Azores are made up of nine volcano-derived islands centered in the Atlantic Ocean roughly 2454 km from the coast of the United States and 1600 km from Portugal [3]. Bovine population and production are a vital source of economic stability for the archipelago with products ranging from butter, milk, and cheese to meat supplies for commercial fast food chains. Unfortunately, the Azores face two debilitating periods within the year where animal product is limited due to lack of grass growth. Cattle owners are forced to supplement their cattle with a native invader plant as an alternative feed, of which contains *Pittosporum undulatum* [4]. The compound *Pittosporum undulatum* can have a negative effect on an animal's immune, endocrine, and reproductive system, thus becoming a problem with cattle production even though it is a means of sustaining the current cattle population. A recent study (2016) performed at the University of the Azores provided an in-depth understanding of how this compound can directly affect embryotic development through coculturing with bovine granulosa cells (Figure 4) [5].



**Figure 4.** Depiction of ovarian follicle. The granulosa cells utilized in the previously discussed section of coculture utilized these cells that form the membrane granulose. Retrieved from: [https://en.wikipedia.org/wiki/Ovarian\\_follicle#/media/File:Gray1164.png](https://en.wikipedia.org/wiki/Ovarian_follicle#/media/File:Gray1164.png).

### 3. Coculturing

The idea behind coculturing cells with embryos allows for researchers to evaluate not only the progression of embryotic development, but also pinpoint interactions between the two entities to establish a better understanding of what cause and effects are taking place. Bovine granulosa



cells play a vital role in *in vitro* maturation (IVM) of coculture with oocytes as well as the development of embryos derived from *in vitro* fertilization (IVF) [6]. Granulosa cells express growth factor kit ligand (KL), which assist in follicular development and directly effects oocytes whenever gonadotropins, FSH- and LH-specific levels are present [7]. Through quantitative RT-PCR and specific primers one can analyze the gene expression level of KL. In the case involving *Pittosporum undulatum* previously mentioned, the study did not utilize RT-PCR, but rather relied on the expression of blocked embryo development. The idea behind the study at the University of the Azores stems from this hopeful possibility of understanding how and when embryotic development is affected when *Pittosporum undulatum* is present, which can have a relationship with KL production of the granulosa cells. The study showed that embryos cocultured with granulosa cells collected from cattle that were fed *Pittosporum undulatum* demonstrated higher embryo blocking when cocultured granulosa cells as opposed to the control group that were not exposed to the same feed (Figures 5 and 6).

Treatment	Nº of ovaries	No. of collected oocytes	No. of oocytes for maturation	Rate of		
				Maturation	Cleavage	Developed Embryo
Experimental	8	136	119	78.27±5.81 <sup>a</sup>	29.92±4.31 <sup>a</sup>	7.30±3.11 <sup>a</sup>
Control	8	186	148	90.46±3.02 <sup>a</sup>	41.86±5.58 <sup>b</sup>	21.88±6.85 <sup>a</sup>

Numbers in the same column with different letters (a, b) differ significantly at  $P < 0.05$ .

Data are expressed in percentage (%) as mean ± SEM, standard error of means.

% of developed embryos classified as morula, early blastocyst and blastocyst stages.

**Figure 5.** Rates of development embryonic *in vitro* of experimental and control groups.

### 3.1. Cells and transgenic techniques

Another interesting factor in applying cocultures with cells involves genetics directly by creating transgenic animals. A transgene is a genetic material that is naturally transferred from one organism to another through genetic engineering, which can change the genotype and phenotype of the transgenic organism. This process utilizes newly fertilized cells that are isolated and injected with specific DNA, the egg is then transferred into a host mother where it is able to develop into an embryo. Currently, a new method involves the removal of the nucleus from one egg and replaces with the nucleus of another cell; this is commonly known as nuclear transfer. This allows for complete transfer of genetic information of the donor cell into the recipient. Further yet the cells may be genetically modified with a laboratory before being transferred to the host. This type of cellular genetic transfer was conducted at the University of Georgia (Athens, Georgia) focused on the production of transgenic bovine

embryos through a granulosa cell theater [8]. This allows for the development of genetically desirable cattle through selection of genetic enhancement which can increase specific phenotypes of economically pleasing cattle. The study conducted at the University of Georgia showed that bovine granulosa cells can transfect desirable genes *in vitro* without negatively affecting embryo viability; essentially bovine granulosa cells are potentially useful passage cells to establish nuclear transfer of gene cloning [8]. Transgenic animals can add an advantage to the human therapeutic industry by providing preclinical theatres for vaccine production and tissue growth for human use.



**Figure 6.** Picture of bovine granulosa cells utilized at the University of the Azores. Photograph taken by Dr. Helder Nunes at the University of the Azores.

### 3.2. Cells and cryopreservation advancement

Further evidence suggests that the use of various bovine cell lines can assist in the cryopreservation of bovine embryos. This becomes essential when one applies genetically desirable crossbreeding *in vitro* to create embryo offspring in numbers, especially with the current technique of offspring sex selection. Studies show that cell culture systems with bovine oviduct epithelium cells (BOECs) can be utilized *in vitro* to increase embryo robustness [9]. BOECs are comprised of ciliated and nonciliated (secretory) cells. The ciliated cells aid in moving the ovum away from the ovary and toward the uterus, while the nonciliated cells release secretions needed to lubricate, provide nourishment, and protection for traveling ovum. Furthermore, it has been proven that BOEC can have a direct effect on embryo development when present during the first 4 days or the last 4 days of development by accelerating blastocyst development [10]. Prior to cryopreservation the bovine oocytes are matured and fertilized *in vitro* in culture medium supplemented with serum, as its presence provides a beneficial environment, such as energy substrate, amino acids, vitamins, and growth factors. The serum has a biphasic effect on embryonic development, on the one hand by inhibiting the first division, and on the other, by encouraging the development of blastocysts [11]. Thus, the use of BOECs and its metabolic entities with bovine embryos in simple medium can aid in furthering the embryo environment into a rich maturation media. The use of BOECs in a culture system with embryos

has not been exhausted and has not been studied in the comparison of “fresh” embryos and cryopreserved embryos. Second, according to Jang et al., it is highly recommended that BOECs be studied to investigate the specific mechanism of their actions with the development of embryos as well as provide a complete analysis of the somatic cell coculture system that supports high development rates *in vitro* as well as a potential increase in viability rates *in vivo* in both fresh and frozen embryos [12]. According to Schmaltz-Panneau et al., the mechanisms of BOECs have not been clearly clarified, but evidence shows that *in vitro* coculture of bovine embryos with BOECs can mimic the maternal environment which can improve the developmental quality of the embryo [15].

Previous studies suggest that tissue inhibitor of the enzyme metalloproteinase-1 produced in BOECs possess embryogenesis-stimulating activity, while another study demonstrated that BOECs contained luteinizing hormone (LH) receptors that are functional in increasing the synthesis of oviductal glycoprotein, which can increase the development of early embryos [13]. BOECs also control oxygen levels within the embryo environment, which is a vital factor is embryo success. To assist with embryo and maternal communication oviduct-secreted proteins (OSP) create a pathway for this communication as well as the maintenance of environment for successful embryo development [14]. There has been a progression in identifying many OSP, but two antioxidant enzymes seem to play a major part in continued embryo development: copper, zinc superoxide dismutase (Cu, Zn-SOD), and phospholipid hydroperoxide glutathione peroxidase (GPx-4) [14]. The metabolic exchange between SOD and GPx-4 maybe the explanation for the increase in the blastocyst rate of embryos when BOEC is present due to the gas regulation during development. BOECs also regulate the secretion of embryotrophic factors such as growth factors, component C3, oviduction, and osteopontin. The osteopontin protein is believed to have a direct effect on spermatozoa, oocytes, and embryos [15]. Osteopontin was first identified in the mineralized matrix of bovine bone, but it is also present in the testis and epididymis in males as well as within the female reproductive tissues including the oviduct. Further investigation of osteopontin determined that this protein is capable of enhancing bovine cleavage and blastocyst rates in culture [15]. This information sparks the question if a better genetic understanding of this occurrence is understood could researchers essentially pinpoint and modify this communication between embryo and its maternal surrounding (whether natural or synthetic) to aid in the fight against bovine miscarriages and to progress in successful postcryopreserved embryo development rates once they are introduced into carrying host. One way of using this information regarding cellular interaction is to understand the genetic phenomena that occur during specific phases of cellular expression. Another is to utilize this advancement in embryo cryopreservation in order bank transgenic embryos.

#### 4. Genetic mapping

Improving genetic understanding and innovative techniques have long evolved since the first recorded line of breeding performed by Robert Bakewell and the first freezing of semen in 1949. In 2001, genomic selection and genetic mapping became a popular method in improving



livestock genetics. Identifying and cloning genetically desirable traits can create a robust mapping of bovine genes. Genetic mapping is essential for researchers to identify where genes are located and how they function within cells and overall how it effects the phenotype of cattle. Genetic mapping of humans and cattle alike plays crucial roles in understanding chromosomal rearrangement through evolutionary progression. The understanding of cattle genome can shine a light on evolutionary changes but can also illuminate a path to pinpointing and selecting desirable and marketable phenotypes. Since it can be extremely costly to select specific desirable animals, it is beneficial to start the process as early as possible which makes embryo production of selective cattle not only more cost efficient, but also effective in ensuring that the trait is carried within the genetic information of the embryo.

#### **4.1. Synteny mapping**

A common approach to developing a genetic map is that of synteny mapping which utilized somatic cells. Synteny refers to two or more genomic regions within a set of chromosomes within a species that show evidence of deriving from one ancestral genomic region [16]. Synteny mapping consists of a method utilizing a generated list of well conserved genes, known as *syntentic blocks*, which are associated with the same chromosome for a specific species; whereas conserved synteny describes the location of homologous genes on the same chromosome, but for two different species [17]. Somatic cells are the primary component of synteny mapping, and they are living mammalian cells that are not gamete cells (i.e., ova and sperm cell) such as those that make up bones, organs, and blood cells. Theoretically, any gene product or sequence can be successfully mapped using the synteny method as long as the presence (or absence) of the gene of the species in question can be challenged against the fully retained rodent genomic background (through hybrid somatic cells) to confirm whether a concordance or discordance for the location of two genes is located on the same chromosome [17].

#### **4.2. Bioinformatics and genomic selection targets**

Today bioinformatics friendly programs such as BLAST are used to identify specific regions with similar genetic sequences for better understanding of characterizing organism and biological discoveries. Specific sites such as bovinegenome.org provide search and annotation tools to support bovine genome and those researchers seeking to expand bovine genetic mapping. Studies such as those conducted by Homer et al. touch upon genetic solutions to the increasing problem with milk production. Poor expression of estrus is considered to be a contributing factor to poor dairy cow fertility; by identifying where the genetic issue lies, Homer and his team believe that they can identify which cows display higher expressions of estrous, as well as identify the genetic issue associated with this lack of expression [18].

From a traditional standpoint, dairy cows are bred based on simple breeding selection, which primarily depends on the sire-side with the aid of worldwide semen distribution through artificial insemination. The use of genomic selection is used in dairy cattle to overcome limitation of traditional selection due to health and fertility problems. Genomic selection for dairy cattle owners becomes beneficial when comparing costs, reproduction, and generation

cycles associated with traditional breeding using progeny testing. There are two ways one can use genetic selection for breeding: one way is by preselecting young bulls to test or the other option involves selecting the bull based on already available genomic information, thus the use of genetic selection for future dairy cow reproduction can have a major positive impact on the dairy industry [19]. As for beef, cattle selection of desired animals is based on the targeted market, and due to the large population of beef cattle and lower accuracy of beef cattle genetic markers, the idea of using genetic selection is not as desirable and economically pleasing as it is for dairy cows [20].

## 5. Conclusion

Conventional breeding involves hundreds of unidentified genes, which require continued advancement in modern molecular biology with gene mapping. By identifying and isolating specific genes to create transgenic animals, as well as with the aid of cells to advance the understanding of environmental influences and their ability to improve cryopreservation of genetically desired embryos. Through better understanding of cellular interaction and genetic traits, researchers seeking answers for issues regarding animal reproduction can ultimately find solutions to not only maintain stable offspring numbers, but also produce genetically desirable progenies.

As mentioned, new phenotype genetic selection along with easier transfer of genetics can answer the social demand of meat and dairy products through preferable phenotype production. In the future, the dairy and meat industry will be faced with higher populations and not enough cattle products to sustain the increasing populace, as such the need for faster reproduction cycles and robust dairy cows will be necessary to meet demands. Future researchers are urged to expand their knowledge of the cattle genetic map, which allows for a better understanding not only of possible genetic selection for breeding purposes, but also aids in understanding the human genome itself. This is especially true when focusing on reproduction, when considering both human female and a cow, the only difference between the two is the length of the estrus cycle. Further studies on genetic relation to estrous expression can not only aid cattle owners in effectively increasing cattle head but also can aid in human infertility-related research as well.

A future capability of cellular participation in innovation is endless. Here a very small number of potential uses of cells for genetic selection are briefly introduced. There are numerous uses of cells to expand our knowledge of veterinary genetics. Even though cells house the information that makes everything what it appears to be, research progression in biotechnology has opened a portal to understanding how these fragments of information function and (to an extent) how to manipulate them to better understand their capabilities in creating their natural end product. A global perspective cells are minuscule in size but hold an enormous amount of information and material for researchers to utilize the information for animal and human therapeutic innovation.

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## References

- [1] FAO. Chapter 6 Crossbreeding [Internet]. 1982 [Updated: 2016]. Available from: <http://www.fao.org/docrep/004/X6536E/X6536E06.htm>
- [2] Greiner, S. Crossbreeding Beef Cattle [Internet]. May 1, 2009. Available from: <https://pubs.ext.vt.edu/400/400-805/400-805.html>
- [3] Visit Azores. The Archipelago [Internet]. 2016. Available from: <http://www.visita-zores.com/en/the-azores/the-9-islands/the-archipelago/geography>
- [4] Nunes, H., Faheem, M., Dinis, M., Borba, A., da Silva, F.M. Effect of feed with *Pitospodium undulatum* in vivo on bovine progesterone levels and embryo produced in vitro. Canadian Journal of Animal Science. Forthcoming. DOI: CJAS2
- [5] Nunes, H.P., Furnas, S., Dinis, M., Borba, A., Da Silva, F.M. Maturation, fertilisation and culture of bovine oocyte using chlorogenic acid in media. Animal Science. Forthcoming. DOI:
- [6] Pinkert, C.A., editors. A Laboratory Handbook. 3rd ed. Waltham, MA: Elsevier; 2014; p. 696. DOI: 978-0-12-410490-7
- [7] Parrot, J.A., Skinner, M.K. Thecal cell-granulosa cell interactions involved a positive feedback loop among keratinocyte growth factor, hepatocyte growth factor, and kit ligand during ovarian follicular development. Endocrinology. 1998;139(5):2240–2245.
- [8] Sezen, A., Rzucidlo, S.J., Gibbons, J., Kazuchika, M., Stice, S.L. Production of transgenic bovine embryos by transfer of transfected granulosa cells into enucleated oocytes. Molecular Reproduction and Development. 2001;(60):20–26.

- [9] Abe, H., Hoshi, H. Bovine oviductal epithelial cells: Their cell culture and applications in studies for reproductive biology. *Cytotechnology*. 1997;(23):171–183.
- [10] Cordova, A., Perreau, C., Uzbekova, S., Ponsart, C., Locatelli, Y., Mermillod, P. Development rate and gene expression of IVP bovine embryos cocultured with bovine oviduct epithelial cells at early or late state of preimplantation development. *Theriogenology*. 2014;(81):1163–1173.
- [11] Jonge, C., Barratt, C., editors. *Assisted Reproductive Technology*. New York: Cambridge University Press; 2002. 435 p.
- [12] Jang, H.Y., Jung, Y.S., Cheong, H.T., Kim, J.T., Park, C.K., Kong, H.S., et al. Effects of cell status of bovine oviduct epithelial cell (BOEC) on the development of bovine IVM/IVF embryos and gene expression in the BOEC used or not used for the embryo culture. *Asian Australasian Journal of Animal Science*. 2008;21(7):980–987.
- [13] Ian Gordon. *Laboratory Production of Cattle Embryos*. 2nd ed. Cambridge, MA: CABI; 2003. 549 p.
- [14] Mermillod, P., Schmaltz, B., Perreau, C., Tskis, G., Martinot, E., Cordova, A., et al. Early development of ruminant embryos, autonomous process or the result of a positive dialog with surrounding maternal tissues. *Acta Scientiae Veterinariae*. 2010;38(2):521–533.
- [15] Schmatz, B., Cordova, A., Dhorne, S., Hennequet, C., Uzbekova, S., Martinot, E., et al. Early bovine embryos regulate oviduct epithelial cell gene expression during in vitro co culture. *Animal Reproduction Science*. 2014;149:103–116.
- [16] Cogepedia. Synteny [Internet]. July 24, 2014. Available from: <https://genomeevolution.org/wiki/index.php/Synteny>
- [17] Womack, J.E. The cattle gene map. *ILAR Journal*. 1998;39(2):153–159.
- [18] Homer, E., Derecka, K., Webb, R., Garnsworthy, P. Mutations in genes involved in oestrous cycle associated expression of oestrus. *Animal Reproduction Science*. 2013;142:106–112.
- [19] Jonas, E., Koning, D. Genomic selection needs to be carefully assessed to meet specific requirements in livestock breeding program. *Frontiers in Genetics*. 2015;6(49):1–8.
- [20] Johnston, D., Tier, B., Graser, H. Beef cattle breeding in Australia with genomics: opportunities and needs. *Animal Production Science*. 2012;52:100–106.

