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Possibilities And Challenges Related to Norwegian Embryo Production in Cattle

Muligheter og utfordringer innen norsk embryo produksjon i storfenæringen

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Preface

As last year students at The Norwegian University of Life Sciences, Faculty of Veterinary Medicine, at the differentiation of "Production animal medicine and Food Safety" we have been tasked to write a specialization study.

Throughout our 6 years of education, the field of reproduction has been of great interest for both of us. Therefore, it was a great opportunity for us when we got the chance to write about embryo production in cattle. This is an exciting field in reproduction which is highly topical with Genos commercial offer, as it is the first in Norway to offer embryos commercial in large scale. Few studies have been done regarding embryo production in Norway last years. Therefore, we hope that this study will be of interest for veterinarians working in the field, students and others with interest for embryos as a tool to enhance breeding in Norway even further.

Abstract

Title: Possibilities and challenges related to Norwegian embryo production in cattle

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When the Norwegian breeding company, Geno, began its commercial work on embryo production in 2018, it was outdistanced compared to our neighboring countries. Thirty years earlier veterinarians at Geno established MOET in a minor scale and scientists at the Norwegian School of Veterinary Science conducted a lot of work as, among other things, resulting in the birth of "Anette", the first calf born after *in vitro* fertilization in Norway.

Throughout the 1990s the funding ended, and artificial insemination became again the main focus area in Norway. In the period between the 1990s and 2018 there has been a great development when it comes to reproductive technologies in the field of embryo production in many parts of the world. The introduction of genomic selection, evolvement of techniques for multiple ovulation and *in vitro* fertilization, have made embryo production relevant in Norway again.

Norway's agriculturstructure differs from many other countries, among others when it comes to herd size. In this study we have discussed some of the different ways of including embryo transfer in the Norwegian agriculture, like how to consider which animal to use from a genetic point of view. We have shown the opportunities embryo transfer might give, for example, when it comes to climate and the effort in reducing the greenhouse gas emission from production animal.

Also, we conducted a short survey among farmers, in which price and benefits are mentioned as causes by the farmers for not using embryo transfer. It appears that Geno has a job in convincing Norwegian farmers why to use embryo transfer in their herd.

Definitions and abbreviations

- **AI:** Artificial insemination
- **BCS:** Body condition score
- **CDE:** Carbon dioxide equivalents
- **CL:** Corpus Luteum
- **EEA:** The European Economic Area
- **ET:** Embryo transfer
- **EP:** Embryo production
- **EU:** The European Union
- **FSH:** Follicle-stimulating hormone
- **GS:** Genomic Selection
- **IETS:** The Internationale Embryo Technology Society
- **IVF:** In Vitro Fertilization
- **IVP:** In vitro production
- **MOET:** Multiple Ovulation and Embryo Transfer
- **NRF:** Norwegian Red (Norsk rødt fe)
- **NRK:** Norwegian Broadcasting Corporation
- **OPU:** Ovum Pick Up
- **PGF2α:** Prostaglandin F2α
- **RedX™:** Product consisting sex sorted semen of Geno.
- **SNP:** Single-nucleotide polymorphisms
- **TMI:** Total Merit Index (avlsverdi)

Introduction

Over the last decades there has been a huge technological development in our society. There is always a search for new ways to make things more efficient and practical. This applies also for agriculture. Embryo production is one of several new methods to drive agriculture forward regarding breeding. The first successful embryo transfer in cattle was conducted already in 1951, but the method has since then been improved substantially to be able to use it commercially. In Scandinavia it was not until 1980s that the first calf resulting from embryo transfer was born. Shortly after this, a lot of research was conducted in Norway regarding embryo production. However, for different reasons research in the field of embryo production was abandoned in Norway. Today there are a lot of countries that have done a lot of research and where embryo transfer has become a huge commercial business. After years with lack of research and progress in embryo production in Norway, breeding companies like Geno have now put in a great amount of resources to develop embryo production in Norway.

The genetic value of the Norwegian Red (NRF) regarding health- and reproductive traits, is already attractive in the global marked. This is because health- and reproductive traits have been of great focus in the breeding programs in Norway since the 1970s. Today more than 30 different countries import sperm from the NRF through Geno Global AS. This indicates that there is a great interest in the genetics of the NRF, and embryo could open new opportunities regarding export of genetics from the NRF.

Thus, the development of embryo production in Norway has a huge potential and veterinarians in the future should possess both theoretical and practical knowledge about the topic. However, the future progress of embryo production does not only depend on the veterinarians. The Norwegian conditions are of interest regarding further development of embryo production towards commercial business in Norway. Particularly farmers should play an active role for further development of EP. Therefore, this study also contains a brief survey on the interest among Norwegian farmers on the topic.

History

The first successful transfer of a mammalian embryo was already conducted in 1890 by Walter Heape when he was able to transfer rabbit embryos (2). Another successful transfer of an embryo was reported in 1920, again involving rabbit. It took another 31 years before the first calf was born after a successful embryo transfer at the University of Wisconsin credited E.L. Willet *et al* (3). The methods to conduct embryo transfer were developed during the 1960s, and included protocols for superovulation, appropriated medium, surgical recovery technique as well as the technique for transfer of embryos. In the early 1970s ET could finally become a commercial business (4). In the beginning the method of choice was surgical recoveries, but already by the late 1970s the nonsurgical techniques were used. Similar to beef cattle, this also opened the door for dairy cattle to become donor. It took another few years, by mid 1980s, before the nonsurgical techniques became prevalent. To become prevalent, the nonsurgical method had to overcome some problems. The main problem involved was that the pregnancy rate was significantly higher using surgical techniques. Another major obstacle involved the lack of proper tools to accomplish the transfers using nonsurgical methods. These problems were resolved after the introduction of the first ET gun in North America in 1984 to 1985, which led nonsurgical transfer to become the method of choice (5).
Another huge development in ET came already in 1973 when Wilmut and Rowson were the first to cryopreserve bovine blastocysts. The development of cryopreservation methods has taken huge steps, and has been important for the establishment of ET as a commercial business (5).
It took several years before the birth of the first calf resulting from ET took place in Norway in 1985 (6). Shortly after, 58 cattle, both beef cattle and Norwegian Red, where included in a project conducted by Geno between 1985 to 1987 at the Vevla farm, Stange. The purpose of this project included getting the first systematic trails of ET in NRF (7). There were several

projects in Norway during the 1990s describing different aspects in ET and embryo production. Anette Krogenæs conducted a lot of research at the Norwegian School of Veterinary Science in the 1990s. This resulted in the birth of “Anette”, which was the first calf born after *in vitro* fertilization (IVF) in Norway (8).

Compared to many other countries, like The United States of America and England, ET and EP were not further developed into commercial business in Norway. It was not until March 2018 Geno started the production of embryos, and thereby started the commercial business of EP in Norway (9).

Materials and methods

Literature

As mentioned in the introduction, limited research was conducted in Norway in the 1990s. It was natural to include some of this work in the present study because it discusses topics regarding Norwegian conditions.

Since only a few publications describe the Norwegian embryo production and embryo transfer, it was necessary to include literature from abroad. When reading literature published in other countries it is necessary to take into consideration that the conditions of that country differs from those of Norway.

The content of these publications where concluded to be relevant and credible, as it had to be approved before being published.

Also, some webpages were included in this study. These webpages are among others from organizations in Norway like Geno and Debio and were considered to give relevant information about conditions in Norway.

Meeting with Geno

During the work on this study we have been invited to Genos facilities at Store Ree several times. That gave us the opportunity to observe their work and routines when it comes to preparation of heifers used in embryo production, the process of flushing and the final steps with extraction of embryos from the flushing media and cryopreservation.

On 27th and 28th of February we were invited to the first Nordic Embryo Workshop, arranged by Geno. This gave us insight on the ongoing research in our neighboring countries, which are relevant to this study.

Survey

When looking into the Norwegian conditions regarding EP, we found it valuable and interesting to conduct a short survey among farmers to investigate their motivation to use ET. The survey was made using "Google Docs", and was posted in specific forums for farmers on "Facebook". It was also distributed to the members of "Storfekjøttkontrollen" (thanks to Animalia), and to members of TINE AS located in specific parts of Norway. A total of 930 farmers responded. In the survey the farmers had to answer questions about how old they are, which county they come from, which type of production they are into (milk, meet or both), herd size, whether ET is available or not in their district, whether they are using or want to use ET, and at last if they do not want to use ET, the reason why (Appendix nr 1) (10). The purpose of conducting the survey was to see tendencies and get farmers viewpoints regarding ET. Due to this, the survey was designed with the possibility of answering more than one alternative for the question about why they do not want to use ET. For three of the questions it was possible to write their own comment as an answer. As a result of this, we received many answers consisting of more than one alternative and with comments. Following, answers will not be presented in statistic form.

Techniques

There are several different techniques in use regarding EP. Due to the fact that this paper will discuss Norwegian conditions regarding EP, we will focus on the two techniques Geno is using today.

Multiple Ovulation and Embryo Transfer (MOET)

The main principle of MOET is to use a donor animal to produce transferable embryos which are thereafter transferred to chosen recipients.

MOET was already taken into use in the 1970s, but still decades later no remarkable progression regarding the development of this technique has been documented. For example, the number of transferable embryos produced per flushing has been nearly constant at proximately six during these years (11). There are many factors affecting the result of MOET. Management, animal related- and environmental factors are the main groups that will affect the outcome of MOET. These factors must be taken into consideration when working with MOET to achieve a good result. Of course, the skill of the practitioner conducting the MOET is also important for the outcome of MOET (5).

To make MOET more profitable and efficient, it is important to produce multiple embryos per flushing of one donor. This is achieved by superovulation of the donor. To provoke a superovulation, the donor is treated with hormones based on a specific protocol. There are many different protocols which can be used, but the main principle is the same.

At first the donor animal must be observed to determine the stage in the oestrus cycle. Alternatively, the donor can be the treated with Prostaglandin F2α (PGF2α) and thereby comes into oestrus (12). In this paper the protocol used by Geno will be described. The superovulation starts approximately 10 days after oestrus. The donor will now be given Follicle-stimulating hormone (FSH) twice a day, four days in a row as shown in *table 1*.

Normally the donor will receive a total of 10 ml with FSH, but this can vary from 7,5 ml to 12-13 ml depending on previous response, age and weight of the animal. The FSH will make sure that several follicles will grow, and potentially ovulate when oestrus is initiated. At day four with FSH treatment, the donor will also receive two doses with PGF2α. The treatment with PGF2α makes the donor to coming into oestrus in average 48 hours after injection. Usually the first artificial insemination (AI) will take place 48 hours after the first injection with PGF2α, but the heat behaviour monitored by Heatime® will be taken into consideration with regards to the point of first AI (13). To provide as many embryos as possible, multiple inseminations are common (11).

Seven days after fertilization the uterine horns are flushed transcervical, which will recover the embryos. After flushing the donor will be given an injection with PGF2α to prevent pregnancy resulting from potentially embryos left behind. The flushed embryos are then divided into groups based on quality. Then the embryos can be transferred fresh, or they can be cryopreserved.

Table 1: Genos protocol for superovulation (13)

TIME	MEDICIN	DOSEAGE (ML)		
Day 10 after estrus	PLUSET PLUSET	**1,5** **1,5**	MORNING EVENING	06:30 17:00
Day 11 after estrus	PLUSET PLUSET	**1,5** **1,5**	MORNING EVENING	06:30 17:00
Day 12 after estrus	PLUSET PLUSET	**1** **1**	MORNING EVENING	06:30 17:00
Day 13 after estrus	PLUSET & ESTRUMAT PLUSET & ESTRUMAT	**1** **2** **1** **2**	MORNING EVENING	06:30 17:00

Ovum Pick Up (OPU)

Ovum Pick Up (OPU) is also referred to as *in vitro* fertilization (IVF) and *In vitro* production (IVP). The main principle with this technique is to collect oocytes directly from the ovary, fertilize and cultivate them in a laboratory, and finally insert the fertilized embryo into a recipient. This is a technique that can be compared to what is used in humans, in Norwegian known as "*prøverørsbefruktning"* (14).

It is necessary to use an epidural anesthesia to facilitate the procedure and then place the cow so lateral movement is prevented. It is possible to pick up oocytes twice a week from cycling cows. The common technique is to use ultrasound-guided pick up. The vagina is entered with a specialized ultrasound and aspiration equipment. There are different types of equipment, but the main point is to fixate the ovary via rectum, lead the needle into the follicle transvaginally and then aspirate the fluid. The aspirate containing the oocytes is then sent through a filter. Immediately after, the oocytes will be located and there will be a quality control (15).

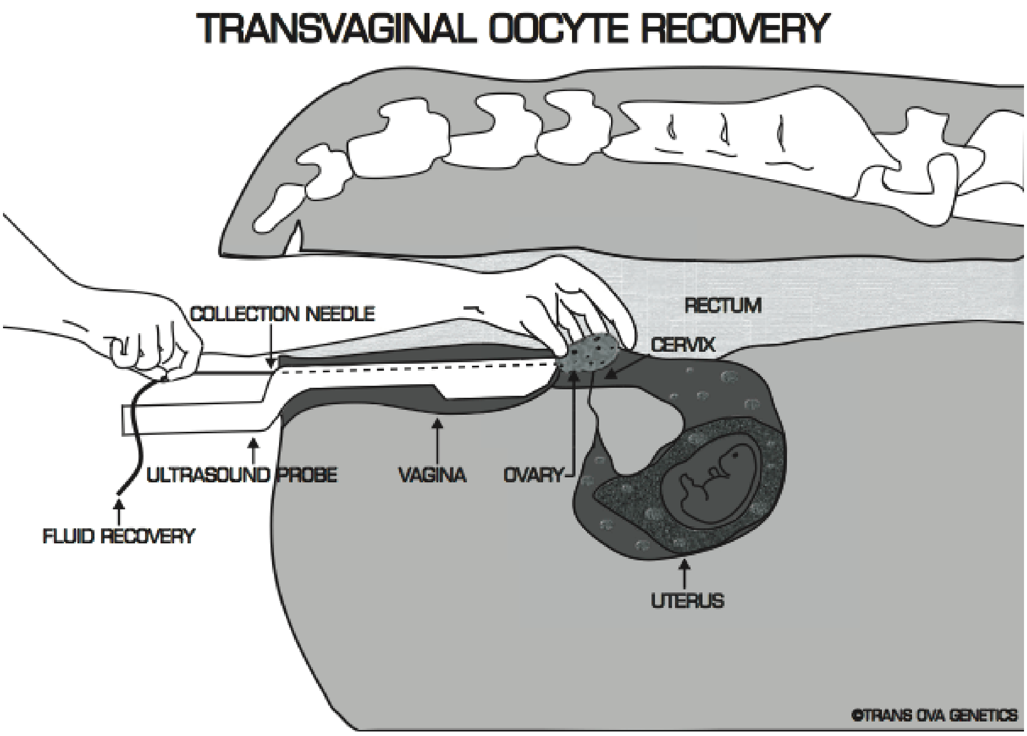


Figure 1: Technique for collecting oocytes (1)

Oocytes with satisfying quality should have at least 4 layers of cumulus cells surrounding them. This is important because the cumulus cells serve a nutrition role and contains hormone receptors. Oocytes which have characteristics known to cause apoptosis, are small or pale will not be chosen for maturation (12). When oocytes with satisfying quality have been sorted, they will be matured and then fertilized in an *In Vitro* Fertilization Lab (IVF-Lab).
After 7 days the embryos have reached the morula/blastocyst stage and are ready for transfer to cows/heifers, or they can be cryopreserved for later use (14).

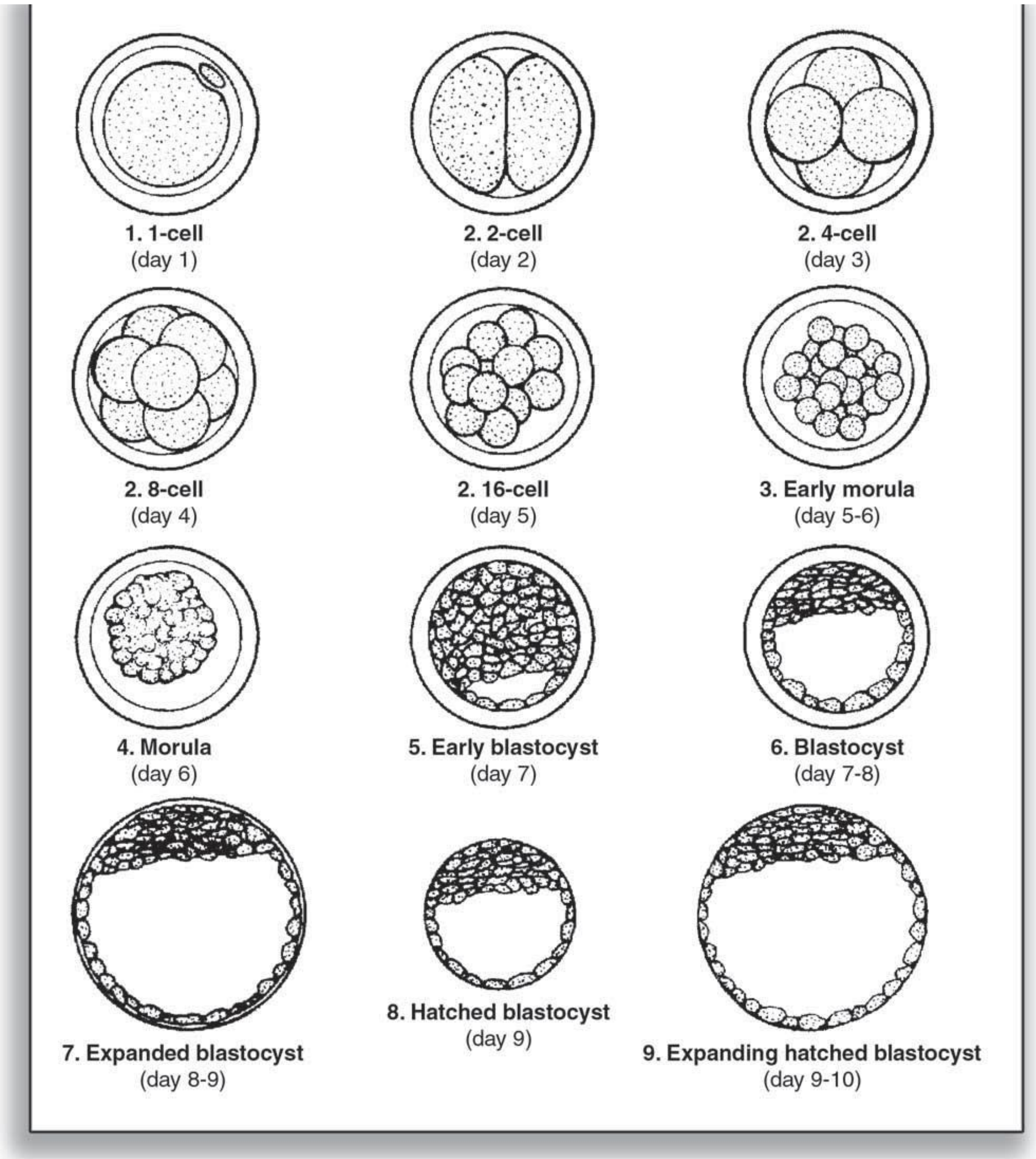
Embryo quality

To achieve a good result the evaluation of embryos is an important part. The standard scoring system provided by The International Embryo Technology Society (IETS), is based on morphology and developmental stage of the embryo (16). Every embryo is assigned with a code consisting of two numbers. The first number indicates the developmental stage of the embryo, while the other indicates the quality. The numeric code indicating the developmental stage is ranging from 1 to 9. Every number indicates a specific stage of development where 1 is an unfertilized oocyte or a 1-cell, and 9 is an expanding hatched blastocyst. At day seven after fertilization, when the flushing takes place, the developmental stage should be 5 (Early blastocyst) or 6 (Blastocyst) (16). However, it is important to be aware of the fact that in one flushing there can be embryos that are in different developmental stages. It is not uncommon that there are embryos in either stage 4 (Morula) or 7 (Expanded blastocyst).
Regarding the numeration of quality, the numeric scale ranges from 1 to 4. The IETS manual defines the codes as follow (16):

- **Code 1 – Excellent or Good:** Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. This embryo is consisting with its expected stage of development. Irregularities should be relatively minor, at least 85% of the cellular material should be an intact, viable embryonic mass.
- **Code 2 – Fair:** Moderate irregularities in overall shape of the embryonic mass or in size, color, and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass.
- **Code 3 – Poor:** Major irregularities in shape of the embryonic mass or in size, color, and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass.

- **Code 4 – Dead or degenerating:** Degenerating embryos, oocytes, or 1-cell embryos: nonviable.

Table 2: The numeric scale for stage of development from IETS manual. Reproduced with permission from the International Embryo Technology Association.



As the IETS manual points out, it is important to remember that this is a subjective evaluation and can therefore vary from person to person and depend on the person's experience. However, there are also a lot of other factors influencing a successfully pregnancy rate after a transfer, like environmental, recipients quality and the technicians experience (16). This will be further described.

Factors affecting pregnancy rate

As mentioned above, the quality of the embryo is very important for the success rate after an ET. However, one of the most important factors to take into consideration, and one of the most underestimated, is the recipient. The management of the recipient includes many aspects like selection of the right recipient, nutrition and estrus synchronization and determination. There are in principle two decision to make when the selection of a recipient in a herd takes place. The first is to choose between a cow or a heifer. Both have their advantages and disadvantages. With regards to heifer there are studies from North-America that show higher pregnancy rate, compared with cows (4). On the other hand, heifers are generally smaller than cows which leads to concerns regarding calving ease. However, perhaps more important is the genetics of the recipient. There must be an evaluation of the recipient's genetics, with regards to whether the animal has genetics that are favorable to keep in the herd. If that is the case, this animal should not be chosen and an animal with less favorable genetics should be chosen as recipient for embryo (17).

Another critical factor to success is nutrition. There are lacking Norwegian studies regarding this factor, but studies from abroad point out that high concentration of starch and fat diets before implantation may be factors that influence the success rate. Additives like organic trace minerals and mold may influence as well (5). An important factor that concerns feeding, is the body condition score (BCS) of the recipient. Fat or thin animals tend to have lower pregnancy rate then animals which have a proper BCS (5). A proper BCS for dairy cattle is approximately 3.5 in a scale from 1 to 5.

Another factor of importance is estrus detection due to the importance of transferring of the embryo with optimal timing. For many years the gold standard around the world was visual estrus detection. However, in the later years many practitioners has replaced this with pharmacological programs using progestogens and gonadotropins to synchronize the recipients (5). In Norway no studies concerning pharmacological synchronization have been performed.

Embryo transfer (ET)

To ensure a successful outcome, the process of embryo transfer requires that the recipients are in the same stage of the cycle as the donor. As already described, the embryos should reach the morula /blastocyst stage and be transferrable to a recipient at day 7 (14). The recipient, who can either be a heifer or a cow, must be examined to determine her stage in the cycle. This is done by rectal palpation of the ovaries and grading of the Corpus Luteum (CL). Recipients must have a palpable CL 3, approximately 7 days since last heat. One can either follow the natural cycle of the cow/heifer and wait until it is 7 days after the heat, or it is possible to use hormones (prostaglandin - PGF2α) for synchronizing. After administration of PGF2α, the recipient will come into heat three to four days later, then the embryo transferring process can be done 7 days after the heat, or about 10-11 days after administration of PGF2α (7).

The process of transferring embryos to recipients can be done surgically, but the most common technique is non-surgical. With the non-surgical technique, one uses a kind of modified inseminator – often referred to as an embryo-transferator, which is used for penetrating cervix. If necessary, the recipient will be sedated with Xylazine and an injection of Lidocaine/Procaine as epidural anesthesia. It is important to determine which of the ovaries that has the active CL, because the embryo must be placed in the associated uterine horn. This is controlled by rectal palpation. Vulva and the area around must be cleaned, then an embryo-transferator is lead through cervix and into the uterine horn associated with the active CL, and the embryo is deposited near the tip of the uterine horn (18).

Sex-sorted semen

Sex-sorted semen has been used for several years in relationship with *in vitro* fertilization as well as AI. Geno has a lab at Store Ree producing sex-sorted semen which they distribute in the Norwegian market as RedX™ (19). The technique of separation relies on the difference in DNA-content of X- and Y-chromosomes in the sperm. The principle for the technique is to fluorescence-stain the sperm DNA, and sort the sperm according to the fluorescence intensity. This procedure is done by using flowcytometry and detectors that measure the intensity of fluorescence from the X- and Y-chromosome in the sperm. To further separate the X- and Y-sperm, an electrical field is used and by using opposing charges for X- and Y-sperm, they will be separated. The sperm which cannot be sorted, for example due to problems with fluorescence-staining, will be discarded. As a consequence of this process the total amount of sperms are relatively lower than in "ordinary semen" (11).
As described, the technique used to sex-sort sperm includes a lot of mechanical stress. Combined with the relatively low number of sperms in a dose of sex-sorted semen, this may result in an impaired pregnancy rate compared to sex-sorted semen used in AI. Further, research has shown that both number and quality of transferable embryos decreases when sex-sorted semen has been used. Also, the pregnancy rate was reduced with 12% when embryos produced with sex-sorted semen were transferred (11). An interesting fact was shown in a study where a significantly higher mortality was documented for male calves when embryos were produced with sex-sorted semen. A plausible theory to explain the increased mortality relies on the fact that when an Y-chromosome mistakenly ends up being categorized as a X-chromosome, the length or shape of the Y-chromosome differs from a normal Y-chromosome. This can then lead to malformations or other problems with the fetus, which then can lead to mortality.

There was no difference in mortality among female calves from embryos produced with sex-sorted semen versus embryos produced with conventional semen (11).

Progeny testing and Genomic Selection (GS)

Traditionally progeny testing was used to calculate the breeding value, but modern technology like genotyping and genomic selection have become the main techniques for calculating breeding value today.

From the 1950s and up to 2015 progeny testing was the method in use in Genos breeding program. Progeny testing is a method based on testing the offspring regarding phenotype of different treats. Geno bought bulls from cows with high breeding value and these were sent to Genos testing station "Øyer testingsstasjon" (20). From these bulls a certain number was selected every year, which were sent off to "Store Ree Seminstasjon". At "Store Ree Seminastasjon" 2000 doses of sperm were produced from every young bull. These 2000 doses of sperm were sent out randomly throughout the country, to different herds with different production conditions. Then the young bulls were sent off to a waiting station pending the birth and growth of their offspring. After approximately 4-4½ years these, bulls got daughters that were finishing their first lactation. Throughout their first lactation a lot of information regarding different traits like milk production, health and fertility was registered. Based on these registrations a breeding value was calculated to each bull. To get an official breeding value of one bull there had to be from 140 – 350 daughters (varied through the years) that were evaluated. In order to get sufficient data, approximately 40% of all sperm used on NRF-cows in Norway was sperm from young bulls. Every year approximately 10-12 bulls were selected as elite bulls based on their breeding value (21).

The advantage of using progeny testing was the possibility to get a high level of certainty on traits, even those with low heritability. On the other hand, using this method resulted in long generation intervals. In addition, the fact that a large percentage of the population had to be used in testing sperm from young bulls at all time, was not advantageous (21).

After 2015 a new method was taken into use relying on evaluation of the genome. By using advanced techniques for genotyping based on samples collected at the farms, the variation in genetics is analyzed. The varying parts of the genome called single-nucleotide polymorphisms (SNP) are the parts that are evaluated in this analysis. To understand the point using GS, some background information will now be explained. The genome of an animal consists of two chromosomes. One chromosome from their father and one from their mother. When gametes are made there are taken some parts of each chromosome, which then are fused together to one chromosome. Before GS was available, one had to make the assumption 50% was taken from each chromosome when making a gamete. In fact, this is an incorrect assumption, but there was no method to check this. What is really happening is that there are usually more taken from one chromosome than the other, making a gamete with more genetic material from either the father or the mother. This means that, in theory, two siblings with the same mother and father can have from 0% to 100% (with a mean of 50%) identical genome. With GS, it is possible to check how much related animals really are based on genetics. For example, based on GS two calves with different mother but same father may be 15% related. Previously, one had to assume that they were 25% related due to that fact that they had same father but different mother (22).

Geno's ongoing breeding program on NRF is called "HD Genomics". In this program GS is used by taking a sample from the calf and analyzing the genetics, and for example find out how much related the calves are to their siblings. This is important regarding how much the sibling's phenotype must be emphasized. Information from farmers, veterinarians and slaughterhouses are then put together with the GS information in a system where all this is compared. Based on all this information it is possible for Geno to estimate both phenotype- and genotype-information. The result of this is that it is possible to estimate a genetic breeding value with higher safety, as well as shortening the generation interval considerably (23).

Genomic Selection of embryos

There are developed several techniques in order to select embryos based on GS. Today two different techniques are used to take a biopsy from the embryo.

One technique includes the use of a microblade attached to a micromanipulator taking out cells from a biopsy of the embryo at day 7 (blastocyst stage) after fertilization. This biopsy will then be analyzed to determine possible genetic defects, determine the sex and be used in GS (5) (24).

Another technique relies on the use of aspiration done at day 5 after fertilization. Using a biopsy aspiration capillary makes it possible to aspirate embryo cells. These cells are then used in the analysis. The benefits using this method are that there are only small holes made in the zona pellucida surrounding the embryo and that there is no cellular damage, compared with the use of a microblade. However, when using a microblade there are no requirement to special equipment, it is an easy technique to perform and the biopsies are larger and of better quality regarding SNP genotyping (24).

An issue when taking out biopsies from embryos is the damages applied to the embryos. There are less damages after using aspiration technique compared to the use of a microblade, but there are still some damages inflicted the embryo regardless technique (24). More studies are needed regarding the effect of these damages.

Norwegian conditions of interest

Herd size, prices and benefits using ET

According to statistics made by TINE, the number of herds producing milk has decreased from 22 433 in 1998 to 8 331 in 2017. In the same period cow equivalent per herd ("årsku") has increased from 13.5 in 1998 to 26.7 in 2017 (25). As we can see there is an ongoing change with fewer herds, but larger number of cows per herd.

The number of beef cattle is increasing. According to statistics made by Statistics Norway the number of beef suckler cattle counted 54 641 in 2008 and in 2017 there was 83 129 beef suckler cattle in Norway. At the same time the number of herds was decreasing, which means the heard size has grown bigger (26). However, the Norwegian herds are still small compared to our neighboring countries (27).

Economy in Norwegian agriculture

In March 2019 Statistics Norway published an article about farmers income in Norwegian agriculture. The average gross income was 662 000 NOK per farmer, but only 201 000 NOK was business income from agriculture. The rest included pension, other types of business income, subsidies and so on (28).

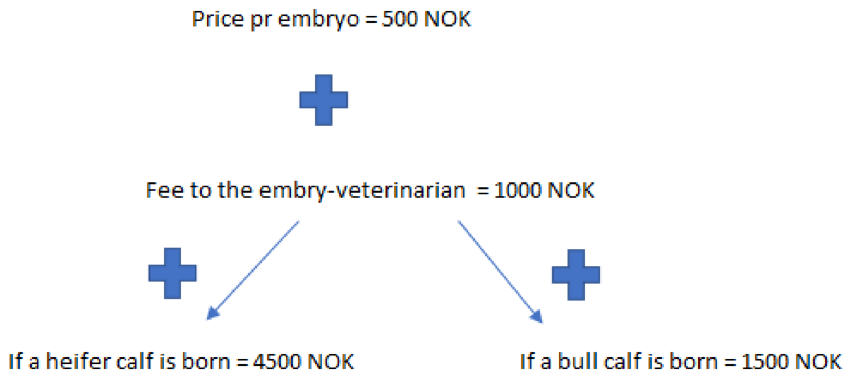


Figure 2: Illustration of price for ET based on information from Geno pr. March 2019

In the survey we conducted among farmers more than 25 % answered that price was a reason for not using ET (10). If we look at the price table GENO is using (pr. March 2019), we can see that the prices are depending on the outcome of ET. There is one price (1500 NOK) if the ET fails and no calf is born, and another price if for example a heifer is born (29). This is illustrated in *figure 2*.

The ET-veterinarians cost for driving is not included in the figure above and will vary by distance. To look further into cost-benefit by using embryo transfer, it is necessary to include prices for artificial insemination and the outcome you get by using AI or ET.

Artificial insemination

Table 3: Pricelist on AI based on information from Geno pr. March 2019

	Price in NOK
NRF elite. TMI up to 14	155,-
NRF elite. TMI 15 – 19	205,-
NRF elite. TMI 20 – 24	255,-
NRF elite. TMI 25 – 29	305,-
NRF elite. TMI 30 – 34	355,-
Addition to price for RedX™	300,-
AI done by veterinarian	365,-

As described in *table 3* the prices for artificial insemination varies among bulls depending on what breeding value the bull has. In addition, there might be a driving-fee that vary by distance. To summarize this, a calculation follows. According to Genos Norwegian Red sire catalog for semen available in Norway, most of the bulls have breeding value between 20 and 30 (30). For this example, we have chosen the bull named Ihle with number 11 949 and TMI at 29. The price for AI with Ihle will be 305 NOK + 365 NOK = 670 NOK. If the farmer chooses RedX™ instead of "ordinary semen" the total price for this example will be 970 NOK (31). To simplify this calculation the driving-fee to the veterinarian is not included. Standard AI, AI with RedX™ and ET all have their different advantages. If the cow or heifer is of good lineage and has favorable genetics this is an individual the farmer would like to carry on in the herd. This can be done by insemination with either standard semen or RedX™. 300 NOK is the difference between the two products. The advantage of RedX™ is that you will most likely get a heifer calf. Thus, if you want to bring genetics from a good cow further on in your herd, this might be the right decision. One of the disadvantages of using RedX™ is, as described earlier, that the pregnancy rates are lower. Geno recommends using RedX™ only on animals with high fertility. AI with standard semen is most common in Norway. The pregnancy rates are higher and as shown in the calculation above, it is cheaper. Thus, for most cows in the herd this will be the best choice.

Breeding programs

The price is varying depending on chosen technique as shown above. However, there are other aspects to be taken into considering before choosing ET, AI or AI with RedX™. Breeding organizations like Geno provide breeding plans and consulting to farmers based on the farmers goal for his herd and Genos breeding goal for NRF. It is possible to order different breeding plans from Geno, they are among others providing *avlsplan super* and *avlsplan enkel*. In short, *avlsplan super* include a breeding advisor to visit the farm and make an individual breeding plan for each animal. *Avlsplan enkel* is a standard breeding plan made by Geno, and will not include adjustments for the individual farm.

Breeding value can be defined by a curve of normal distribution *(figure 3*). One of the breeding goals is to achieve a value for the herd two standard deviations above the midpoint of the graph (32).

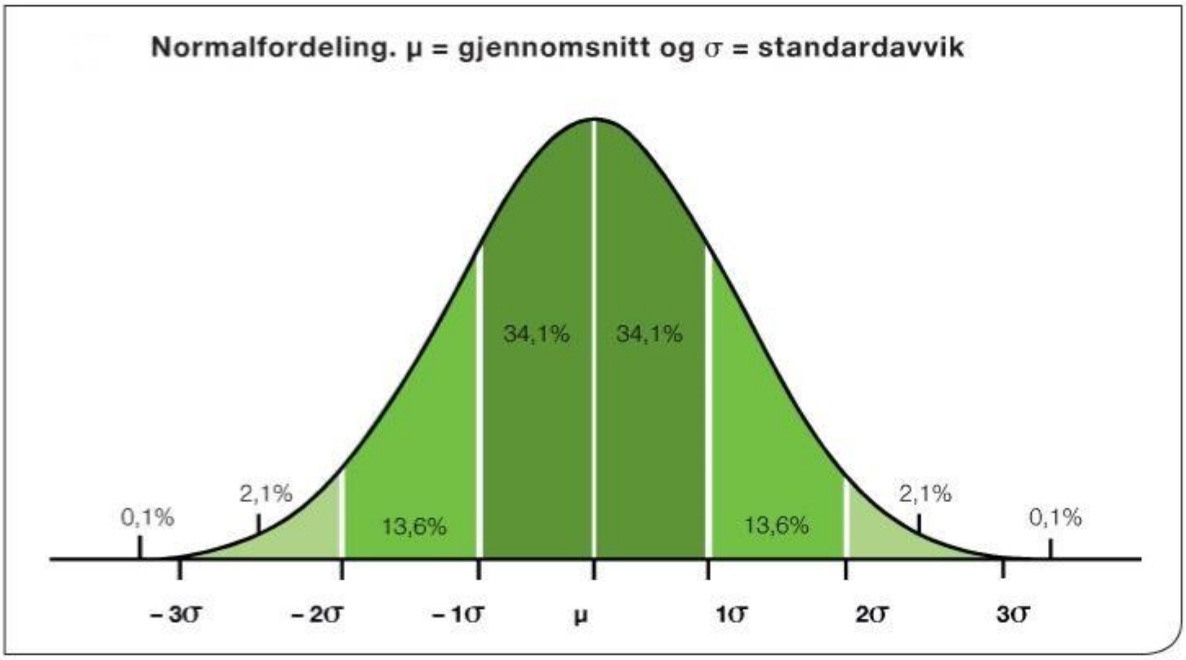


Figure 3: Curve of normal distribution from Geno (32).

Individuals at the right side of the middle should be considered inseminated with RedX™. The individuals with genetics and breeding value around the middle level can be inseminated with standard semen. Finally, the individuals with genetics and breeding value at the left side of the middle are those suitable for embryo transfer. These individuals will have genetics that the farmer might not want to carry on in his herd, which may be done either by using embryo transfer or inseminate the dairy cow with a beef cattle. When deciding on what approach to use, it is necessary to look at the following issue: Does the farmer need a calf with good genetics for recruitment to his herd? If so, ET is probably the best approach. If the answer to this question is no, then fertilization with semen from beef cattle might be the best. Then he will get a cow in milk production and a calf of mixed breed, which he can sell to slaughter for a higher price than a purebred NRF, or eventually as a livestock.

Embryo transfer VS beef cattle semen

When deciding what approach to choose, the farmer must take cost into consideration. If the farmer chooses ET, and it is successful, he will get an offspring with high breeding value, but at a price of approximately 6 000 NOK if it is a heifer calf, as presented above in *figure 2*. Fifteen months later this heifer can be inseminated with standard semen or eventually RedX™, and the farmer has most likely achieved great genetic progress for the price of 6 000 NOK.

Table 4: Pricelist on AI with beef cattle based on information from Geno

	Price in NOK
Beef cattle – young bull	247,-
Beef cattle – elite bull	320,-
Beef cattle – proven bull	285,-
AI done by veterinarian	365,-

If the farmer choses to inseminate the cow/heifer with semen from beef cattle instead of using ET, the following calculation applies. To simplify the calculation, we make an average of the prices presented in *table 4*, which will be 284 NOK ((247+320+285)/3). The total cost for insemination with beef cattle semen will then be 649 NOK (284+365) (31). If the pregnancy is successful, the result of this fertilization will be a cow in milk production and an offspring which can be fed to slaughter or eventually sold as a mixed breed livestock.

Conclusion

The right decision will vary among farmers. It is reason to believe that size of herds and the farmers income will affect the decision of using AI with standard semen, AI with RedX™ or ET. After all it is the economy, the farmers motivation of using new technology and taking part of the genetic progress which decides which method is preferred.

Animal welfare

A quick search in the news archive of Verdens Gang (VG) shows that there were over 40 articles in 2018 containing “dyrevelferd” (33). The interest and focus on animal welfare have been increasing over the last couple of years. The consumers are searching for information about the welfare and life of the animal producing the product they are consuming. The last years, companies like Nortura and organizations like Dyrevernalliansen have introduced different types of labels, which they provide to products produced from animals living under conditions that they have defined as good animal welfare (34). Consumers are asking questions about the mass production in the agriculture and traditional tie-stall is forbidden among others because there is great reluctance against animals being tied most of their life (35). Further, there is a growing interest for vegetarian- and vegan diets (36).

In the survey that we conducted among cattle-farmers about their interest in ET, several farmers responded that they would not use ET due to animal welfare reasons. One of them even answered that farm ox was the best choice considering animal welfare (10). If the farmers who are dependent on the industry are rising questions about the animal welfare in ET, most likely consumers will do the same.

Petyim et al. (2007) conducted a study on animal welfare during the procedure of OPU (37). Among others, they studied heart rate and level of cortisol in blood to measure the animal’s reactions to the different procedures. Reactions to each of the procedures like restraining of the animal, epidural-procedure and the OPU itself were closely observed. In their study, Petyim et al. (2007) refer to other studies conducted some years earlier. Among others, they are referring to a publication Greve and Jacobsen performed in 2001 regarding animal health and welfare during ET. They summarized the effects of OPU to the cow as injuries to the ovaries and area for epidural anesthesia, and stress during the OPU procedure (38). Petyim et al. showed that the heifers started eating, regurgitating and continued their ovarian cyclicity after the OPU procedure. They therefore concluded that the long-term response to OPU was minimal. After completion of the study all the heifers who participating were sent to slaughter, or the ovaries were taken out by ovariectomy for the purpose of analyzing the macroscopical changes on the ovaries after OPU. The result of this showed no signs of inflammation or adhesions. The only finding was a harder consistency of the ovaries (37). When analyzing the results from the blood samples and heart rate measuring, they found that the procedure with epidural gave the biggest elevation in both heart rate and level of cortisol in blood. Petyim et al (2007) therefore concluded that the procedure with administration of epidural caused most discomfort to the heifers and not the OPU itself. They suggested the solution of administering the epidural in a less painful way, for example, by using a smaller needle for the injection or administration of an anesthetic cream on the site of injection. It is worth noticing that they in their study showed that it is possible to conduct OPU without general sedation of the animal, but only using epidural anesthesia (37).

From the publication mentioned above it may be concluded that the most stressful part of OPU is administration of epidural anesthesia. There were minimal signs of damage to internal organs, like vaginal wall and ovaries. Despite these facts, organizations for animal welfare may still rise questions about the welfare and have concerns buying products from animals which have undergone ET/OPU. Therefore, it is important that breeding organizations like Geno and companies like Nortura are providing more information concerning animal welfare during the procedure of OPE, for example, by using results from the study done by Petyim et al. (2007).

To get a professional review of ethical dilemmas concerning animal welfare, the “The Council of Animal Ethics” are often asked to give a review. “The Council of Animal Ethics” are appointed by the Ministry of Agriculture and Food, and one of their main tasks are to assess the ethical aspect regarding modern breeding and animal husbandry (39). By virtue of this, they were asked by Geno to give a review concerning EP and ET in Norway. In January 2019 they published their review. In this review they concluded that, even though they acknowledge that EP includes some challenges concerning animal welfare at an individual level, the method can be defended because of the positive outcome. Therefore, they give their acceptance using ET in Norway, but recommend that the Ministry of Agriculture and Food should work out regulations including criteria for the use of ET to ensure the animal welfare (40).

Climate changes

The year of 2018 was measured as the fourth warmest year since 1901, and nine of the ten warmest years that have been measured after 2005. The average temperature globally has increased 1,05 degrees Celsius since the pre-industrial society (41).
This is a topic that engages a lot of people and has become an important political issue. Also, agriculture has been evaluated with regards to greenhouse gas emission and there has been a broad opinion that agriculture must reduce their emission. This was evaluated in the report "Agriculture and climate change" from the Ministry of Agriculture and Food in 2016.

It has been commonly known that cows do contribute with emission of e.g. methane gas and, a lot of people have pointed out that emission from agriculture is an important contribution to greenhouse gas emission. The public engagement was expressed for example through the "Norwegian Broadcasting Corporation" (NRK) when they published an episode in "Folkeopplysningen" called "Kjøtt".
In the report from the Ministry of Agriculture and Food it is documented that the emission from cows has decreased a lot since 1990. If the numbers from the years before 1990 are included the reduction is even greater. The main reason for this reduction is the great reduction in numbers of cows in Norway. From 1960 to 1979 the number of cows went from 603 000 to 400 000. Both breeding and feed has improved a lot over the years, resulting in a greater milk production from a single cow. This means that fewer cows can produce the same amount of milk than cows in earlier days (42). Nevertheless, there is a broad opinion that there is still a lot of work to be done with regards to reduction of methane gas from cows. In the report form the Ministry of Agriculture and Food one of the main priorities was concluded to be a systematic work to reduce the total greenhouse gas emission from cows with 20%.

This is equivalent to 350 000 carbon dioxide equivalents (CDE), or approximately equivalent to the yearly emission of 150 000 cars (42).

As previously mentioned, a considerable contribution to the reduction in emission from cows has been improved breeding. To get a further reduction, breeding must be a part of the solution. The potential EP has with regards to further improvement of breeding, may be an important tool to reach the Ministries goal achieving 20% reduction of the total greenhouse gas emission from cows. However, according to statistics from Geno, the pregnancy rate after an ET is approximately 50% (43). In comparison when using AI, the pregnancy rate is significantly lower using ET. This will result in longer time from calving to next pregnancy, resulting in less efficient production. Cows will therefore use more energy, food and resources, and produce less when using ET, which again will lead to emission without producing anything.

Organic farming

Organic production in Norway

In 2017 the total number of dairy cattle was 219 265 (44). The same year the production of milk was 1 495 million tons (45), and 3,4% of all the milk production came from organic farms (46). Thus, a considerable amount of all the milk production comes from organic farms. The numbers of beef suckler cattle in 2017 was 93 689 (44), and the total meat production (including sheep, cattle, goat and pig) was 249 000 tons. 1,1% of all the meat production in 2017 came from organic farms (46).

To become an approved organic farm in Norway certain guidelines must be followed. These guidelines are enshrined in Norwegian law. Because Norway has an agreement with The European Union (EU) called The European Economic Area (EEA) agreement, Norway has committed to implement decrees coming from EU. This applies also for the laws regulating organic productions (47). In Norway the task to supervise and control the laws and regulations regarding organic farming has been given to Debio (48). When a farmer has met the requirements that must be fulfilled to become certified as an organic farmer, he will receive additional payouts for his products.

TINE SA is the largest producer, distributor and exporter of dairy products in Norway. The company is owned by its 11 400 members, and has 9 000 cooperative farms (49). TINE has an ambition that 6% of all the milk should be organic. To achieve this, TINE pays 0,75 NOK additional per liter milk (50). The conditions to qualify for organic production are thoroughly described by The Norwegian Food Safety Authority. These covers many aspects among others that the feeding must only consist of organic food mainly produced on their own farms, and that the calf must go with the cow minimum 3 days after calving. Also, when using medications with retention time, this time is doubled.

Reproduction in organic farms

The regulation for organic production also includes a section about reproduction that points out the importance of animals breeding and giving birth naturally. This rules out using ET in organic farming. However, despite the fact that animals should breed naturally AI is still allowed as an exception (47). In correspondence with Debio it was concluded that the use of sperm from bulls originating from ET and to buy animals that have ET in their heritage is permitted in organic production in Norway (51). It may sound strange that ET is not permitted in organic farming, while it is permitted to use sperm and animals that are a result of ET. From a breeding point of view, it is a good idea using genetic materials from ET animals to enhance breeding.

Also, the use of AI in breeding as an exception, rather than using natural breeding, raises some questions when other artificial breeding techniques are forbidden. The consequence of the exception is that almost everyone chooses to use AI instead of natural breeding, as it enhances the breeding in general. Therefore, the important point underlined in the law, stating that animals in organic farms should breed natural, is not really followed in practice. The main point using AI is to enhance breeding, which also is the main argument for using ET.

Another problem relating to the use of ET in organic production is the use of hormones. It is not permitted to use hormones in organic farming today, with some exceptions regarding treatment of certain diagnoses (47). Hormones are widely used to synchronize recipients before conducting ET. This is not an option in organic farming. However, it is an option to observe the natural estrus cycle and conduct ET 7 days after observed estrus. There are not performed proper studies in Norway on what is the most common protocol, but to conduct ET without use of hormones on recipients is in use today.

Another argument is that hormones are used in the production of embryos. Even though the production of embryos is taking place outside organic farms, the use of hormones is not in line with the whole idea behind organic production. In any case, by accepting the use of sperm from bulls resulting from ET it seems like this issue has already been considered in Sweden. After all, these bulls are a result of embryos produced using hormones and still they are permitted to be used in organic farming due to their genetic material.

Response from farmers

A total of 930 farmers answered the survey on ET. The purpose of the survey was to get feedback on the interest among Norwegian farmers to implement ET in their breeding programs. The form that was used is included as appendix nr.1.

Results

Question 1: Age. The answers vary between 15 and 74 years of age.

Question 2: County. We got responders from all counties, except Oslo, and the distribution are shown in table 5.

Table 5: Distribution of responders

County	Number of responders
Akershus	33
Aust-Agder	5
Buskerud	42
Finnmark	3
Hedmark	91
Hordaland	71
Møre og Romsdal	13
Nordland	71
Oppland	126
Rogaland	160
Sogn og Fjordane	12
Telemark	10
Troms	5
Trøndelag	248
Vest-Agder	5
Vestfold	6
Østfold	29

Question 3: Type of production. The answers are shown in table 6.

Table 6: Type of production

Type of production	Number of responders
Ammeku	206
Begge deler	106
Melkeku	618

Question 4: Herd size. The herds were ranging from 2 to 520 animals.

Question 5: Is embryo transfer available in your district? The answers are presented in table 7.

Table 7: Is embryo transfer available in your district?

Alternatives	Number of responders
Ja	370
Nei	145
Vet ikke	415

Question 6: Do you use, or do you want to use embryo transfer? The answers are presented in table 8.

Table 8: Do you use, or do you want to use embryo transfer?

Alternatives	Number of responders
Har ikke bestemt meg	322
Ja	222
Nei	386

Question 7: If no, why not? On this question we set up three alternatives: Price, cannot see the point, know too little about it. The fourth alternative was open, so the farmers could write their own comments. In table 9 we will present the number of responses for the four alternatives. We emphasize that on this question there was possible to answer more than one alternative.

Table 9: If no, why not?

Alternatives	Number of times the alternative was chosen
Andre kommentarer	97
Pris	146
Ser ikke poenget	205
Vet ikke nok om det	192

Question 8: If used, which embryo (breed) have you used? The answers to this question vary between different breeds, both milk- and beef breeds. This question was misunderstood by many farmers, and therefore the answers will not be presented. Further discussion about this will follow in the chapter “Reflections on the survey”.

Question 9: This was a question named "Other comments". On this question the farmers could write their own comments, and these comments will not be presented here. Some of the comments will be discussed in next chapter "Discussion".

Discussion

From the answers given in the survey, we see a slight tendency that those who only conduct with dairy production were more negative to use ET, than farmers who are into both dairy- and beef cattle production. Nevertheless, with 42% stating that they were negative towards using ET versus 24% that were positive, it was a clear tendency towards a negative attitude against ET regardless the type of production.

An interesting observation is the reason to why many farmers have chosen not to implement ET in their breeding program. In the survey the farmers could choose between three alternatives to why they do not want to use ET, or they could write their own explanation. A total of 526 farmers answered, and it is important to point out that it was possible to choose more than one alternative.

"Do not see the point using ET" was the alternative that most of the farmers answered. More than 200 chose this alternative. This may indicate that there has been too little or insufficient information from those selling embryos, about why using ET in their breeding program. Another explanation may be that improved breeding has been so successful that farmers do not see the benefits of using relatively more expensive methods to proceed in their already successful breeding.

"Do not know enough about it", with more than 190 choosing this alternative, was the number two reason why not using ET. This indicates more or less the same that the option "Do not see the point using ET". However, that so many farmers are choosing this alternative gives raise to the question if information about ET reaches out to those farmers that are not actively

seeking information about new reproductive technologies.
More than 140 chose the third alternative “Price”. By choosing this, many farmers stated that it was too expensive using ET in their production and they claimed that it was less expensive and worked just as well as using standard AI.

As mentioned earlier, it was also possible to write down their own comments in addition to choose among the alternatives in the question about why they did not use ET. A lot of the farmers chose to write their own comment in addition to choosing one of the alternatives. This gave us an insight in some other reasons to why some farmers did not want to use ET. Approximately 2% commented that ethics was an important factor for not using ET in their breeding program. In varies ways farmers stated that either the ethical aspect of ET was not acceptable, or that they wanted an official assessment of the ethical aspect before considering using ET. Based on these comments it is reason to believe that consumers might raise questions as well when so many farmers are questioning the ethical aspect using embryos. Therefore, it might be necessary to communicate research regarding animal welfare and ethical aspects using ET to both consumers and farmers if ET are to be widely accepted in Norway.
Also, The Council of Animal Ethics published a comprehensive review regarding ethical aspects using embryos in Norwegian breeding programs in January 2019. In this review they concluded that EP was acceptable to use in Norway from an ethical point of view. It will be interesting to see the effect of an official review regarding ethical aspects, on the opinions among both consumers and farmers.

One of the questions in the survey was whether the farmers knew about any veterinarians conducting ET in their area. 45% answered that they did not know. Many of these are dairy cattle farmers and are located in areas were Geno are providing embryos. This may indicate that there is insufficient information to farmers in those areas that are selected by Geno as areas were embryos are available.

In the chapter about organic farming the different regulations regarding ET was presented, with the conclusion that ET is not permitted in organic farming today. Some of the farmers answered that they cannot use ET in their breeding program due to the regulations in organic farming. This can be interpreted in two ways. Either the farmer can be positive and willing to use ET in his breeding program but is prevented by the regulations in organic farming, or he can be negative to ET and support the regulations.

Sources of error

This survey was a short and quick survey to get some viewpoints from Norwegian farmers. Off course, it may include some sources of error due to the short time perspective and the lack of statistical method. To reach out to as many farmers as possible, it was decided to use two forums on “Facebook” dedicated to farmers called “Norsk Melkeku Forum” and “Ammeku”. By using such forums some farmers will be excluded from the survey. Only those who have a Facebook-account, and are relatively active on Facebook, will therefore be part of the population in this survey.

The rest of the population consists of farmers who are either part of “Storfekjøttkontrollen” or TINE, because we got help from Animalia and TINE to distribute the survey. This excludes those who are not part of either of this, but it also gave a great number of farmers who answered.

Another source of error is that the survey was conducted anonymously. Therefore, the survey was relaying on that fact that those who answered was actually farmers and that they answered honestly.

Reflections on the survey

Retrospectively, the form of the survey could have been different. When the survey was planned, we estimated around 50 responses. After Animalia and TINE distributed it to their members, 930 responded. Because the purpose was to get viewpoints from a selection of farmers, we designed the survey with possibility of writing their own comments and answer more than one alternative for some of the questions. With 930 responses it could have been interesting to present the numeric material in statistic form, but due to our choice of design it is difficult to present such statistics.

Question 8 in the survey was: If ET is used, which breed has been used. The purpose of this question was to get an insight on which breed the farmers who are into ET have used. We got a lot of different answers, both milk and beef cattle breeds. It is obvious that many farmers misunderstood this question, because some of the comments was articulated as “Wagu is most actual right now”. Due to this misunderstanding we cannot distinguish between farmers who actually have used for instance Wagu-embryo and the one who are planning to use it. The results from this question are therefore of little use, and retrospectively this question should have been articulated in a different way.

Conclusion

EP has been commercially available around the world for many years. In 2018 EP became available in Norway by Geno. This gives many new opportunities when it comes to further progress in breeding. It means that Geno can now use both the elite females as well as the elite male animals in their breeding. Additionally, the use of EP and ET gives raise to new technologies that can be used in breeding like GS. The use of GS makes it possible to considerably shorten the generation interval.

These techniques are already in use in Norway, but can it be included in breeding programs throughout Norway? A survey among farmers indicates that they in general are slightly negative to EP, mostly because the price is too high and the benefits are too small. To become effective and widespread in use in Norway, it is important that farmers do want to use it. Also, some farmers indicated that the ethical aspect should be more considered. For example, ET is forbidden in organic farming in Norway because it contradicts to normal breeding. Nevertheless, genetics from these EP animals are allowed in organic farming through, among others, sperm in Sweden who follows the same EU decrees as Norway. Therefore, maybe it should be taken into consideration if it should be allowed with ET in organic farming, when it already is considered favorably using genetics from EP animals.

Implementing EP, ET and GS also give raise to the opportunity of other new technologies, among others cloning. In Norway there is a broad focus by the people on ethical aspects, and it remains to see how people will react to these new techniques used in agriculture. And, where should the boundaries been drawn? Should techniques such as cloning, which is more applicable because of EP, be allowed now that EP is allowed? Or should actors in the Norwegian agriculture take more ethical consideration? Ultimately, it is all up to the Norwegian population where these boundaries should be drawn.

Respects

Anne Cathrine Whist and the rest of TINE SAs Department for Counseling and Membership Service.

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Sammendrag

Tittel: Muligheter og utfordringer innen norsk embryo produksjon i storfenæringen

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Veiledere: Anette K. Krogenæs og Irma C. Oskam, Institutt for produksjonsdyrmedisin

Da Geno startet kommersiell embryoproduksjon i Norge i 2018, var nabolandene allerede langt fremme på feltet. 30 år tidligere hadde veterinærer i Geno utarbeidet teknikker for MOET og forskere ved Norges Veterinærhøgskole gjennomført mye arbeid innen embryoproduksjon, som blant annet resulterte i at den første kalven i Norge etter *in vitro* fertilisering ble født.

I løpet av 1990-tallet ble embryoproduksjon nedprioritert innen forskningen i Norge, til fordel for kunstig inseminering. Dette står i kontrast til store deler av verden hvor det i perioden 1990 til 2018 har blitt fokusert mye på forskning innen dette feltet, noe som har resultert i betydelige fremskritt. Introduksjonen av genomisk seleksjon, utvikling av teknikker for superovulasjon og *in vitro* fertilisering de senere årene har igjen gjort embryoproduksjon aktuelt i Norge.

Strukturen i det norske landbruket skiller seg i stor grad fra strukturen man ser i mange andre land. For eksempel gjelder dette den gjennomsnittlige besetningsstørrelsen, som er betydelig

mindre i Norge enn i andre land. I denne oppgaven er det diskutert ulike måter embryooverføring kan brukes i dagens landbruk. Det være seg at man blant annet burde legge inn embryo på de dyrene med genetikk som bonden ikke ønsker å avle videre på.

Andre aspekter rundt bruken av embryo har blitt diskutert, som betydningen det kan ha innen klimautviklingen og hvordan det kan brukes for å fremme avl av dyr med lavere utslipp av klimagasser.

Utbredelsen av embryo i avlsarbeidet i Norge er naturligvis veldig avhengig av bøndenes interesse. Det ble derfor gjennomført en kort undersøkelse blant bønder i Norge. Pris og nytteverdi ble lagt frem som argumenter for at mange bønder ikke ønsket å bruke embryo i sitt avlsarbeid. Dette viser at Geno har en stor jobb foran seg med å markedsføre embryo til norske bønder.

References

1. Genetics T. Transvaginal oocyte recovery. Transova.com: TransOva Genetics; 2017.
2. Heape W, Foster M. III. Preliminary note on the transplantation and growth of mammalian ova within a uterine foster-mother. Proceedings of the Royal Society of London. 1891;48(292-295):457-8.
3. Willett EL, Black WG, Casida LE, Stone WH, Buckner PJ. Successful Transplantation of a Fertilized Bovine Ovum. Science. 1951;113(2931):247.
4. Hasler JF. Forty years of embryo transfer in cattle: a review focusing on the journal Theriogenology, the growth of the industry in North America, and personal reminisces. Theriogenology. 2014;81(1):152-69.
5. Phillips PE, Jahnke Marianna M. Embryo Transfer (Techniques, Donors, and Recipients). Veterinary Clinics of North America: Food Animal Practice. 2016;32(2):365-85.
6. Velle WM. embryo-overføring Store Norske Leksikon: Store Norske Leksikon; 2009 [updated 10.09.2018. Available from: https://snl.no/embryo-overf%C3%B8ring.
7. Refsdal AO, T. Vatn, K. Landsverk, E. Kommisrud. Embryotransplantasjon hos storfe under norske forhold. Norsk Veterinærtidsskrift. 1988;100:11-9.
8. Krogenæs A. *In vitro* fertilisering hos storfe. Norsk Veterinærtidsskrift. 1995;4:463-70.
9. Embryoprosjektet vedtatt i styret [press release]. Geno.no, 23.10 2017.
10. Borge Sigurd HO. Embryooverføring - fordypningsoppgave. In: docs g, editor. Facebook, Stofekjøttkontrollen, Kukontrollen: Animalia, Tine; 2019.
11. Mikkola M. Superovulation and embryo transfer in dairy cattle: effect of management factors with emphasis on sex-sorted semen. Suomen eläinlääkärilehti 123 (2017): 5. 2017.
12. Nernæs AM, Steinar. Embryo Transfer and Related Techniques in Cattle. 2003.
13. Mikkola M. Superovulasjon Protokoll. In: Hovelsrud O, editor. 2019.
14. Oskam I. Embryoteknologi som del av avlsarbeidet. Buskap. 2016:23-6.
15. Galli C, Crotti G, Notari C, Turini P, Duchi R, Lazzari G. Embryo production by ovum pick up from live donors. Theriogenology. 2001;55(6):1341-57.
16. Stringfellow DA, Givens MD, International Embryo Transfer S. Manual of the International Embryo Transfer Society : a procedural guide and general information for the use of embryo transfer techology emphasizing sanitary procedures2009.
17. Geno. Embryo - Ofte stilte spørsmål Geno.no: Geno; 2019 [updated 21.01; cited 2019 02.04]. Available from: https://www.geno.no/Start/Avl/embryo-i-nrf-avlen/embryo--ofte-stilte-sporsmal/#hva_er_riktig_dyr.
18. Farstad W. Embryooverføring hos storfe. Lecture; NMBU Veterinærhøgskolen. Canvas2018.
19. Geno. REDX™ – Kjønnsseparert sæd Geno.no: Geno; 2018 [updated 13.11; cited 2019 13.03]. Available from: https://www.geno.no/Start/Brunst/Produkter/Redx-kjonnsseparert/.
20. Vangen O. ku-indeks. Store Norske Leksikon. Store Norske Leksikon2009.
21. Geno. Avkomstgransking geno.no: geno; 2017 [updated 13.10.17. Available from: https://www.geno.no/Start/Avl/avlsteori/avkomsgransking/.
22. Nordbø Ø. Genomisk seleksjon del 1: Hva er GS? youtube.com: geno; 2016.

23. Geno. Genomisk seleksjon i NRF-avlen Geno.no2018 [updated 15.05.18. Available from: https://www.geno.no/Start/Avl/Genomisk-seleksjon/genomisk-seleksjon-i-nrf-avlen/.
24. Mutikainen M, editor Genomic selection in embryos. Nordic Embryo Workshop; 2019; Hamar.
25. Tine. Statistikksamling fra ku- og geitekontrollen 2017. Tine.no; 2018.
26. Bjørlo B. Fleire kyr og oksar SSB: Statistisk sentralbyrå; 2018 [updated 05.06. Available from: https://www.ssb.no/jord-skog-jakt-og-fiskeri/artikler-og-publikasjoner/fleire-kyr-og-oksar
27. Nortura. Storfehold i Norge Nortura.no [Available from: http://www.nortura.no/naturlig-kvalitet-fra-norske-bonder/storfehold/.
28. Sentralbyrå S. 1 av 3 gårdbrukere uten jordbruksinntekt SSB.no: Statistisk sentralbyrå; 2019 [updated 04.03; cited 2019 11.03]. Available from: https://www.ssb.no/jord-skog-jakt-og-fiskeri/artikler-og-publikasjoner/1-av-3-gardbrukere-uten-jordbruksinntekt.
29. Geno. Kjøp av NRF-embryo Geno.no: Geno; 2019 [updated 11.03; cited 2019 12.03]. Available from: https://www.geno.no/Start/Brunst/Produkter/kjop-av-nrf-embryo/.
30. Geno. Norsk Rødt Fe - Eliteoksekatalog Oksekatalogen.geno.no: Geno; 2019 [updated 03.03; cited 2019 12.03]. Available from: https://oksekatalogen.geno.no/?breed=nrf&category=eliteokse.
31. Geno. Priser - Sæd og inseminering Geno.no: Geno; 2019 [updated 01.01; cited 2019 12.03]. Available from: https://www.geno.no/Start/Brunst/Produkter/Sad--og-inseminasjonspriser/.
32. Geno. Avlsverdi geno.no: Geno; 2018 [updated 26.03.18. Available from: https://www.geno.no/Start/Avl/avlsteori/avlsverdi/.
33. Archive search vg.no: Verdens Gang; 2019 [Available from: https://www.vg.no/sok/?q=dyrevelferd.
34. Dagligvarehandelen. Lanserer eget dyrevelferdsmerke Dagligvarehandelen.no2016 [updated 02.09. Available from: https://dagligvarehandelen.no/2016/lanserer-eget-dyrevelferdsmerke.
35. Larsen E. Norske bønder må bygge om for 13 milliarder Nrk.no: NRK; 2015 [updated 23.04. Available from: https://www.nrk.no/rogaland/norske-bonder-ma-bygge-om-for-13-milliarder-1.12325391.
36. Redaksjonen. Økning i vegetar på 60 prosent Convenience.no2018 [updated 19.10. Available from: http://www.convenience.no/okning-i-vegetar-pa-60-prosent/.
37. Petyim S, Båge R, Madej A, Larsson B. Ovum Pick-up in Dairy Heifers: Does it Affect Animal Well-being? Reproduction in Domestic Animals. 2007;42(6):623-32.
38. Greve Torben JH. New embryo-technologies: imphcations for animal health and welfare. Arch Tierz, Dummerstorf 44. 2001(Special Issue):80-90.
39. dyreetikk Rf. Om oss radetfordyreetikk.no2019 [Available from: https://www.radetfordyreetikk.no/om-oss/.
40. dyreetikk Rf. Embryooverføring på storfe. In: Food MoAa, editor. radetfordyreetikk.no: Rådet for dyreetikk; 2019.
41. Klima Eo. En varmere klode: Energi og Klima; 2019 [updated 13.02. Available from: https://energiogklima.no/klimavakten/global-temperatur/.
42. Food MoAa. Agriculture and climate change regjeringen.no, Food MoAa; 2016.

43. Geno. Metoder for produksjon av embryo geno.no2018 [updated 15.08.18. Available from: https://www.geno.no/Start/Avl/embryo-i-nrf-avlen/metoder-for-produksjon-av-embryo/.
44. Norway S. Strukturen i jordbruket Statistics Norway: Statistics Norway; 2019 [updated 12.02.19. Available from: https://www.ssb.no/jord-skog-jakt-og-fiskeri/statistikker/stjord/aar.
45. Buskenes P. Nedgang i melkeproduksjonen i 2017 Tine: Tine; 2018 [updated 09.01. Available from: https://medlem.tine.no/aktuelt/nyheter/statistikk/nedgang-i-melkeproduksjonen-i-2017.
46. Anne Snellingen Bye PAA, Anne Ingun Løvberget,, Storbråten ORoB. Jordbruk og miljø 2018. 2019.
47. Mattilsynet. Regelverksveileder Økologisk landbruk. In: Food TMoAa, editor. Mattilsynet2019.
48. Agency NA. Om økologisk landbruk Norwegian Agriculture Agency: Norwegian Agriculture Agency; 2010 [updated 29.06.18. Available from: https://www.landbruksdirektoratet.no/no/miljo-og-okologisk/okologisk-landbruk/om-okologisk-landbruk#aktuelle-lenker-til-meir-informasjon-om-oekologisk-landbruk.
49. Tine. About Tine Tine.no: Tine; 2019 [cited 2019. Available from: https://www.tine.no/english.
50. Ebbesvik M. Priser agropub.no: agropub; 2013 [updated 10.10.2018; cited 2019 12.03]. Available from: https://www.agropub.no/nyheter/priser.
51. Nilssen L. Embryooverføring. Mail ed2019.

Appendix

Appendix nr 1: The Survey

Embryooverføring - Fordypningsoppgave

Vi er to studenter på sisteåret ved Veterinærhøyskolen, NMBU. I den forbindelse skriver vi en fordypningsoppgave som handler om embryooverføring. Vi er derfor veldig interessert i å kartlegge interessen for embryooverføring blant norske bønder.
Takk for at du tar deg tid til å svare på denne undersøkelsen.

*Må fylles ut

1. **Alder** *

____

2. **Fylke** *
Markér bare én oval.

- ◯ Finnmark
- ◯ Troms
- ◯ Nordland
- ◯ Trøndelag
- ◯ Møre og Romsdal
- ◯ Sogn og Fjordane
- ◯ Hordaland
- ◯ Rogaland
- ◯ Vest-Agder
- ◯ Aust-Agder
- ◯ Telemark
- ◯ Vestfold
- ◯ Buskerud
- ◯ Oppland
- ◯ Hedmark
- ◯ Oslo
- ◯ Akershus
- ◯ Østfold

3. **Type besetning** *
Markér bare én oval.

- ◯ Melkeku
- ◯ Ammeku
- ◯ Begge deler

4. **Antall dyr** *

____

https://docs.google.com/forms/d/1ypuWrm7YDR0aoanqUDEbAwhOObEFfU-8JrWU0KQikiA/edit 1/2

5. **Er embryooverføring tilgjengelig i ditt distrikt?** *

Markér bare én oval.

- Ja
- Nei
- Vet ikke

6. **Benytter du, eller ønsker du å benytte, embryooverføring?** *

Markér bare én oval.

- Ja
- Nei
- Har ikke bestemt meg

7. **Hvis "Nei", hvorfor ikke?**

Merk av for alt som passer

- [ ] Pris
- [ ] Vet ikke nok om det
- [ ] Ser ikke poenget
- [ ] Andre: ____________________

8. **Hvis benyttet, hvilke embryo er lagt inn (rase)?**

____

9. **Andre kommentarer**

____
____
____
____
____

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