UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA

USE OF FTAI AND MOET REPRODUCTIVE TECHNOLOGIES IN AN ABERDEEN ANGUS HERD

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Resumo

UTILIZAÇÃO DAS TECNOLOGIAS REPRODUTIVAS IATF E OMTE NUM EFETIVO ABERDEEN ANGUS

As tecnologias reprodutivas desempenham um papel fundamental na indústria pecuária, oferecendo inúmeros benefícios que contribuem para o aumento da produtividade, o melhoramento genético e a sustentabilidade global.

Este estudo tem como objetivo fornecer informações sobre a integração de tecnologias reprodutivas, especificamente a inseminação artificial (IA) e a ovulação múltipla e transferência de embriões (OMTE), num efetivo da raça Aberdeen Angus, contribuindo para orientar os criadores e produtores de gado na tomada de decisões fundamentadas sobre a utilização da IA e da OMTE, nos seus programas de reprodução. Os dados relativos a 499 IATF e 107 TE foram analisados para determinar se alguma das variáveis tinha impacto no resultado desejado - um diagnóstico de gestação positivo - que se traduz, em última análise, no nascimento de um vitelo saudável.

Não foram encontradas associações estatisticamente significativas entre as variáveis relativas à IATF e o diagnóstico de gestação, enquanto que foram encontradas algumas associações significativas entre as variáveis da TE e o diagnóstico de gestação (qualidade do procedimento de TE, qualidade do CL, idade embrionária, paridade e dias pós-parto). As variáveis independentes não se revelaram preditores significativos de um diagnóstico de gestação positivo, quer com as variáveis relacionadas com a IATF, quer com as da TE.

Palavras-chave: IATF, OMTE, REPRODUÇÃO, ABERDEEN ANGUS

Abstract

USE OF FTAI AND MOET REPRODUCTIVE TECHNOLOGIES IN AN ABERDEEN ANGUS HERD

Reproductive technologies play a pivotal role in the cattle industry, offering numerous benefits that contribute to enhanced productivity, genetic improvement, and overall sustainability.

This study aims to provide insights into the integration of reproductive technologies, specifically artificial insemination (AI) and multiple ovulation and embryo transfer (MOET), in an Aberdeen Angus herd, contributing to guide breeders and cattle producers in making informed decisions regarding the use of AI and MOET into their breeding programs. Data on 499 FTAI, and 107 ET were analysed to determine whether any of the variables had an impact on the desired outcome - a positive pregnancy diagnosis, which ultimately translates to a healthy calf being born.

No statistically significant associations were found between the variables regarding FTAI and the pregnancy diagnosis, while some significant associations were found between ET variables and pregnancy diagnosis (ET procedure quality, CL quality, embryonic age, parity, and days postpartum). The independent variables did not prove to be significant predictors of a positive pregnancy diagnosis in neither FTAI nor ET related variables.

Key-words: FTAI, MOET, REPRODUCTION, ABERDEEN ANGUS

Resumo Alargado

UTILIZAÇÃO DAS TECNOLOGIAS REPRODUTIVAS IATF E OMTE NUM EFETIVO ABERDEEN ANGUS

Este trabalho teve como objetivo a análise da utilização de tecnologias de reprodução assistida num efetivo de bovinos da raça Aberdeen Angus de elevado mérito genético.

A raça Aberdeen Angus é uma raça de bovinos de aptidão cárnica, cuja presença mundial a tem colocado como líder nos principais países produtores de carne. Em Portugal teve um crescimento a partir de 2008, e é reconhecida, por um lado, pelos consumidores – que a associam a carne de elevada qualidade, e por outro, pelos produtores, que a associam a interessantes características produtivas. A rusticidade, a capacidade de adaptação a diferentes sistemas de produção, bem como diferentes climas, associadas à docilidade e fácil maneio tornam esta raça interessante para diferentes tipos de explorações. Estas vantagens são ainda mais potenciadas pelas excelentes características maternas típicas da raça (precocidade, fertilidade, facilidade de parto). Posto isto, espera-se que a expansão da raça se mantenha e que seja uma das raças de bovinos relevantes do futuro.

No que diz respeito às tecnologias reprodutivas, sabe-se que se caracterizam por ser o conjunto de técnicas que, de alguma forma, manipulam acontecimentos e/ou estruturas reprodutivas, com o objetivo de alcançar uma gestação e produzir descendentes saudáveis. São ferramentas cruciais na vida diária dos produtores por serem um meio de alcançar melhorias genéticas, otimizar características relacionadas com a fertilidade, reduzir a vulnerabilidade das explorações à introdução de agentes patogénicos externos, entre outros. Neste trabalho analisaram-se a utilização de dois tipos de técnicas: a inseminação artificial em tempo fixo (IATF) e a ovulação múltipla e transferência de embriões (OMTE). A IATF surgiu como resposta para ultrapassar a dificuldade que a deteção de cios representa em efetivos em regimes extensivos, e apresenta-se como um método de sincronização e indução da ovulação. Por outro lado, a OMTE é o conjunto de técnicas efetuadas para recolher embriões viáveis de uma dadora e transferi-los para recetoras.

Foram analisados os resultados de 499 inseminações, que ocorreram no âmbito de 20 protocolos de sincronização de cios no decorrer de 5 anos de atividade. Os protocolos tiveram por base o CO-synch 7d com adição de ECG, com utilização de um PRID ou CIDR, em que os animais eram inseminados cerca de 48 horas após a remoção do dispositivo intravaginal. No que diz respeito à ovulação múltipla os dados disponíveis refletem os resultados de 27 protocolos de superovulação, no decorrer de 3 anos e meio. Estes protocolos foram caracterizados por terem um dia zero com colocação de CIDR e administração de GnRH, e depois doses decrescentes de FSH e LH a partir do dia 4, terminando com doses luteolíticas de PGF2a no dia 7, deteção de cios nos dias 8 e 9 e duas

inseminações, nos dias 9 e 10 com 12horas de diferença. A recolha das estruturas foi feita no dia 16. Os dados da transferência de embriões são relativos a 106 transferências, divididas em 20 protocolos de indução da ovulação, no decorrer de 5 anos. Estes protocolos, semelhantes ao CO-Synch 7d com adição de CIDR, têm a diferença de não existir inseminação, uma vez que se espera até ao dia 16 do protocolo para que seja transferido o embrião para as recetoras.

No que diz respeito à estatística, procurou-se perceber se existia alguma associação entre as variáveis analisadas e o sucesso destas técnicas, ou seja, associações entre as variáveis e o diagnóstico de gestação positivo. Para isso usaram-se testes estatísticos como o Chi-quadrado para comparação das taxas de gestação e diferenças de proporções entre os diagnósticos de gestação no caso das variáveis qualitativas e o teste T-de student para a comparação das médias das variáveis quantitativas. Recorreu-se ainda à regressão logística binária para perceber se alguma das variáveis poderia ser preditor do resultado desejado. As variáveis analisados são dados obtidos a partir dos registos de campo dos procedimentos que se concretizaram e são fatores relacionados com as fêmeas ou com a técnica propriamente dita.

No caso da IATF tentou-se perceber se alguma das variáveis estava associada a uma maior taxa de gestação. No caso dos fatores associados à fêmea - idade, paridade, e intervalo pós-parto - não foram encontradas diferenças significativas entre a proporção de diagnósticos de gestação positivos e negativos. Para os fatores associados ao procedimento - protocolo de indução e sincronização da ovulação, local de deposição do sémen, técnico responsável pelo procedimento e avaliação do procedimento não se encontraram diferenças significativas, embora fosse expectável existirem diferenças entre as taxas de gestação em protocolos onde foram usados PRID comparando com os protocolos onde foram usados CIDR. Também não houve diferenças entre as IAs traumáticas e atraumáticas, embora a larga maioria destas tenham sido atraumáticas, o que terá causado, como seria de esperar, um enviesamento dos dados. Quando se incorporaram as variáveis na equação estatística da regressão logística binária, nenhuma delas se revelou preditor significativo do desfecho desejado.

No caso da ovulação múltipla analisaram-se fatores relacionados com a fêmea e com o procedimento, contudo, uma vez que a amostra era pequena, não foi possível passar para a fase da estatística inferencial e fez-se apenas estatística descritiva. Os fatores relacionados com a fêmea – idade, paridade e intervalo pós-parto – foram descritos, bem como os fatores relacionados com o procedimento – sémen utilizado nas inseminações e a resposta ovárica- onde se concluiu que a média da resposta ovárica ficou abaixo da média expectável.

No caso da TE analisaram-se fatores relacionados com a fêmea – idade, paridade, intervalo pós-parto, qualidade do corpo lúteo; com o embrião - tipo de fertilização, estádio de desenvolvimento, qualidade embrionária, idade embrionária e sincronia dadora-recetora; e

com o procedimento - avaliação do procedimento, técnico responsável pelo procedimento. Não houve diferença nas médias de idade das fêmeas com diagnósticos de gestação positivos e negativos, contudo, existiu diferença significativa (p=0,021) na paridade, sendo que as taxas de gestação para as vacas foram mais baixas (33%) que as das novilhas (59%), resultado pode ter sido afetado pelo facto de existirem poucos casos (17%) de fêmeas primíparas e multíparas (18/106). As médias dos IPP diferiram estatisticamente (p=0.028), sendo que os diagnósticos de gestação positivos estavam associados a animais com menores IPP (M=106,5; DP= 12,42) quando comparados com a média dos IPP dos diagnósticos negativos (M=1171,2; DP=87,44). Existiu maior proporção (p=0,026) de diagnósticos de gestação positivos em TE onde a recetora tinha um corpo lúteo maciço (61,5%) quando comparado com os diagnósticos das fêmeas um corpo lúteo cavitário (35,7%). No que diz respeito aos fatores relacionados com o embrião, não se encontraram diferenças significativas nas taxas de gestação das transferências feitas com embriões fertilizados *in vitro* ou produzidos *in vivo*, nem com embriões em diferentes estádios de desenvolvimento. As taxas de gestação relativas às transferências embriões qualidade 1 também não diferiram significativamente das taxas de gestação envolvendo embriões de qualidade 2 e 3, embora a maioria (86,8%) dos embriões transferidos tenham sido de qualidade 1, o que poderá ter influenciado os resultados. A idade embrionária mostrou uma diferença de proporções significativa (p=0,035), com as transferências que utilizaram embriões de 7 dias a terem maiores taxas de gestação (64,6%), quando comparado com as taxas de gestação dos embriões com 6,5 dias e 7,5 dias (43,8% e 36,0%, respetivamente). A análise da sincronia dadora recetora, não mostrou diferenças significativas nas taxas de gestação. Por fim, houve diferenças significativas (p=0,013) nos procedimentos que não resultaram em trauma, como seria de esperar, com os procedimentos com “boa colocação do embrião” e “razoável colocação do embrião” sem indícios de hemorragia, a terem taxas de gestação superiores (62,8% e 60,0%, respetivamente) aos procedimentos onde houve registo de indícios de hemorragia (29,4% e 16,7%, respetivamente). O fator técnico responsável pelo procedimento não mostrou diferenças significativas nas taxas de gestação. Quando se incorporaram as variáveis na equação estatística da regressão logística binária, e embora houvesse diferenças de proporções estatisticamente significativas, nenhuma das variáveis se revelou preditor significativo do desfecho desejado.

O presente estudo permitiu concluir que, para os dados analisados, não foram encontradas associações significativas entre as variáveis em questão e o resultado da FTAI; não foi possível fazer estatística inferencial com os dados relativos à ovulação múltipla; e na TE, foram encontradas associações estatisticamente significativas entre o diagnóstico de gestação e as seguintes variáveis: paridade, IPP, qualidade do CL, idade embrionária, avaliação do procedimento.

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List of Abbreviations

AI – artificial insemination

AMH - anti-Müllerian hormone

BCS – Body condition score

CIDR- controlled internal drug release

CL – *corpus luteum*

EED – expected delivery date

ET – embryo transfer

FSH – follicle-stimulating hormone

FTAI - fixed time artificial insemination

GEP – good embryo placement

GEP BS – good embryo placement, bloodstained

GNRH - gonadotropin-releasing hormone

IETS - International Embryo Technology Society

IM - Intramuscular

IVD – *In vivo* derived

IVF- *In vitro* fertilization

IVP – *In vitro* produced

LH - luteinizing hormone

MO – multiple ovulation

MOET - multiple ovulation and embryo transfer

P_4 – progesterone

PD – pregnancy diagnosis

$PGF_{2\alpha}$ – prostaglandin F2alpha

PRID - progesterone releasing intra-vaginal device

REP – reasonable embryo placement

REP BS – reasonable embryo placement, bloodstained

SOV – superovulatory/superovulation

Internship Activities report

Internship activities took place from early September 2019 to mid-March 2020.

These activities consisted in accompanying Prof. João Nestor das Chagas e Silva in his role as a theriogenologist service provider on behalf of ACIVET and being involved in the daily activities of the company AgriAngus. The focus of the internship at AgriAngus was to take part in the events that surround the reproductive season of the herds owned by the company, therefore I was closely involved in daily operations such as observing and performing physical and gynaecological examinations, performing reproductive tract ultrasounds, assisting with CIDR placement and removal, drug administrations, watching and performing andrological exams, preparation of semen doses and assisting in artificial inseminations and embryo transfers, etc. Other activities not mentioned in the table below included implementation of vaccination schemes, oestrus detection for AI and ET, daily routine activities such as regrouping and steering animals and relocating the herd, ear tagging calves, grooming young bulls for sale, doing ultrasound examination of the composition of fat in young animals and data management and sorting.

Activities	Number of Cases	Species
Andrological Examination	38	Bovine
Embryo collection	4	Bovine
Embryo collection	3	Ovine
Embryo transfer	5	Ovine
Embryo transfer	24	Bovine
Embryo freezing	3	Bov/Ov
Ultrasound examination of the reproductive tract	312	Bovine
CIDR placement	260	Bovine
Artificial Insemination	268	Bovine
Pregnancy Diagnosis	207	Bovine
Pregnancy Diagnosis	14	Ovine
Dystocia	1	Bovine
Uterine Prolapse	1	Bovine
Vaccination	146	Bovine

1. Introduction

This thesis was carried out as part of the Integrated Master's programme at the Faculty of Veterinary Medicine of the University of Lisbon. The main goal of this thesis was to study the integration of assisted reproductive technologies (ARTs) in an Aberdeen Angus breeding operation, focusing on fixed-time artificial insemination (FTAI), and multiple ovulation and embryo transfer (MOET), through the analysis of overall success rates and investigation of factors that could be associated with the success of these ARTs. This culminated in a retrospective observational study where data collected by the theriogenologist practitioner regarding FTAI and MOET programs was analysed, in order to assess if there was an indication of a relationship between the different variables and a positive pregnancy diagnosis – the ultimate desirable outcome.

Information shared in chapter two is intended to provide the necessary context for the analysis of the results obtained in this study. It begins with an overview of the Aberdeen Angus breed, exploring its historical significance, genetic characteristics, and overall position in the beef industry. Subsequently, the focus shifts towards assisted reproductive technologies, exploring the details of fixed-time artificial insemination and embryo transfer techniques, as well as a review of the oestrus cycle and of oestrus synchronization protocols, offering insights into their potential applications in Aberdeen Angus breeding programs.

The following chapter aims to provide an account of FTAI, multiple ovulation, and ET procedures, data collection methods and statistical analyses employed to expose the relationships between ART variables and pregnancy diagnosis outcome. Lastly, chapters four and five will present and discuss the relevant findings subsequent to the descriptive and inferential statistical results.

2. Literature review

2.1. The Aberdeen Angus Breed

The Aberdeen Angus, native to Scotland, is one of the oldest breeds of cattle in the world. It is believed this breed is a descendant of the aboriginal cattle of northeast Scotland, common in the mid-1700s (Origem e História – Aberdeen-Angus Portugal, 2020). The conception, consolidation, dissemination, and success of the breed can be attributed to the three founding breeders - Hugh Watson, William McCombie, and Sir George Macpherson - whose talent and dedication to the selection of a superior breed of cattle lives on in an outstanding legacy. In 1842, the “Polled Herd Book” was published as the first herd book, which included both Aberdeen Angus and the Galloway breeds. From the 4th edition onwards,

each of the previously mentioned breeds were registered separately. The first breeders' association, dubbed the "Polled Cattle Society", was created in 1879 and has continued until present day; it is currently known as the "Aberdeen-Angus Cattle Society" (Origem e História – Aberdeen-Angus Portugal, 2020).

The breed gained momentum after it was exported to the United States in 1873, and by 1901, there were more animals registered in the U.S. than in the United Kingdom (Origem e História – Aberdeen-Angus Portugal, 2020).

The early recognition of the superior traits of polled animals, namely good temperament, medium structure, easy maintenance of body condition, and excellent characteristics of the meat in terms of the quality of its "grain", tenderness, flavour, and succulence, led to an early selection of this breed (Origem e História – Aberdeen-Angus Portugal, 2020).

This thorough selection process led to a breed that is currently known for its adaptability and good nature and is recognized by breeders and beef producers worldwide. Due to the high carcass yield and marbling of its meat, this breed is often used in crossbreeding, with the objective of improving both carcass yield and meat quality. Furthermore, when crossbred with other breeds, its characteristically easy calving and good rearing traits make Aberdeen Angus an excellent means to improve milking and mother abilities (Carne Angus – Aberdeen-Angus Portugal, 2020).

The quality of the meat is unquestionably the main reason for the breed's recent rise in popularity. Even unfamiliar consumers often perceive the "Angus brand" as a quality product. It has gained recognition not only due to the marketing strategies aimed at promoting the quality of the meat, but also due to its potential to satisfy consumer's needs and concerns relating to sustainable animal production systems and animal welfare. In addition to offering the potential for a grass-based production product, the meat is also advertised as a form of contributing to a more balanced diet, due to its significant omega-3 and conjugated linoleic acid (CLA) fatty acids content. The production of carcasses with an adequate fat cover contributes to a quicker cooling of the carcass and easier maturation process. This, in turn, leads to the tender meat normally associated with the "Angus brand". The high intramuscular fat deposition gives its typical marbling, flavour, and succulence (Carne Angus – Aberdeen-Angus Portugal, 2020).

The Aberdeen Angus Cattle Society advertises the use of the breed for breeding, commercial and dairy operations. The main reasons advertised are strong and growing demand (backed by the premium price the breed commands); high growth rates (perfect blend of production, quality, and fertility); versatility; great maternal ability; cost efficiency; and breed longevity, amongst others (The Breed - Aberdeen-Angus Cattle Society, 2020).

Animals of this breed (Figure 1) are typically medium sized, with mature cows weighing approximately 680 kg and mature bulls weighing approximately 1050 kg (Krupová et al. 2020). Characteristically, the animals are naturally polled and may have black or red coats, with the latter being a recessive gene. Generally, Aberdeen Angus animals are well balanced in terms of size – reasonable length, wide posterior quarters, and a suitably developed muscle mass. The animals typically reach sexual maturity at younger ages and at lighter weights compared to other beef breeds; they have docile temperament and easily adapt to diverse soil and climatic conditions. Lastly, their polled characteristic is extremely evident, with no visible hint of horns. This gene is dominant and prevails in crossbred offspring, which is an advantage in certain settings (A Raça – Aberdeen-Angus Portugal,2020).

Overall, the breed's precocity results in an exceptional conversion of pasture into meat, and its good temperament and adaptability result in easily managed herds under field conditions, both of which are primary requirements of beef producers (A Raça – Aberdeen-Angus Portugal,2020).

Figure 1 - Aberdeen Angus cattle (A Raça – Aberdeen-Angus Portugal, 2020)

2.2. Assisted Reproductive Technologies

Assisted reproductive technologies (ARTs) are techniques that in some way manipulate reproductive-related events and/or structures in order to achieve pregnancy and produce healthy offspring (Velazquez 2008). When applied to animal production, their most important goal is to improve it by increasing efficiency and productivity (Schultz et al. 2020).

ARTs play a crucial role in research related to genetic improvement and reproduction and their rapid evolution has allowed for the growth of genetic improvement in livestock (Schultz et al. 2020). Their value is also noteworthy when it comes to safeguarding germplasm from threatened species and domestic breeds (Ferré et al. 2020).

Biotechnology available currently offers beef producers an unparalleled opportunity to improve herd genetics, by giving them access to more accurate tools for genetic selection (Johnson et al. 2017). These technologies allow them not only to introduce superior genetics into their herds but also reduce vulnerability to transmission of diseases, improve overall fertility and fertility-related traits (Fontes et al. 2020), utilize donors with anatomical disabilities and sub-fertile conditions (Ferré et al. 2020), and overcome natural barriers to reproductive success (Schultz et al. 2020).

The use of ARTs in production settings generally coincides with the moment they are made feasible economically, which happens when their efficiency and/or outcome outweighs costs, especially when compared to conventional breeding approaches (Schultz et al. 2020). Use of a defined breeding season, oestrus synchronization methods, breeding soundness evaluations of bulls, artificial insemination (AI), use of sex-sorted semen, multiple ovulation, and embryo transfer (MOET) and *in vitro* fertilization (IVF), are some of the technologies that have been proven to provide gains when compared to traditional methods (Fontes et al. 2020).

Breeding programmes are used in most modern approaches to optimize decisions related to the breeding process, where the primary goal is achieving the shortest possible interval from calving to the next successful pregnancy (Findlay et al. 2019). Reproductive management strategies play a crucial role in increasing profitability of beef operations. Cow-calf operations depend on the production of a calf per year/per healthy female to remain profitable, as less than that represents a waste of ever more important resources that could be channelled elsewhere (Fontes et al. 2020). In breeding operations focused on obtaining genetically elite animals, embryo technologies offer producers attractive possibilities (Findlay et al. 2019).

It appears to be obvious to modern-day herd owners that the widespread use of these technologies is crucial to maximize herd efficiency and maintain a financially sound cattle enterprise (Baruselli et al. 2018)

2.2.1. Fixed Time Artificial Insemination

Artificial insemination was first used successfully over 200 years ago and was widely adopted as a tool in agriculture in the mid-20th century. Growth happened predominantly through Europe and North America, particularly focused and applied to dairy cattle production. Because it allowed for gains such as genetic improvement, elimination of geographical constraints and limitation of disease spread, it was soon regarded as a beneficial technology, even when taking into account the time and technical ability required (Taponen 2009). AI is currently the most widely used ART for the genetic improvement of livestock in the world (Day

2015). It has grown to such an extent that approximately 130 million cattle worldwide are submitted for AI every year (Moore and Hasler 2017).

The successful implementation of an AI program depends on the correct detection and management of the oestrous cycle in the recipient females. This means that accurate heat or oestrus detection paired with correct timing of AI are absolutely critical steps in achieving desired efficiency and efficacy in a production setting (Schultz et al. 2020). Some of the methods available for this purpose are visual cues, mount detectors, pedometers, and other similar emerging wireless technologies (Schultz et al. 2020). However, where beef herds are concerned, the need for oestrus detection to perform AI becomes tricky. These animals are usually managed in big herds, and time spent on observation, manipulation and contact is generally far less when compared to dairy cattle. This translates into a difficulty, and in some cases, it is not feasible to detect oestrus, which ultimately results in poor pregnancy rates (Taponen 2009).

Fixed-Time Artificial Insemination (FTAI) was introduced as an alternative to AI, to allow it to be used without the need for oestrus detection - and therefore increasing the number of cows inseminated at a given time (Bó et al. 2013). For example, Baruselli et al. (2011) reported that the Brazilian FTAI market represented U$175 million and involved an estimated 3,500 veterinarians, with FTAI performed on approximately 8.2 million beef cows, leading to an 8% increase in calf production – resulting in around 656,000 more calves as compared to natural service breeding.

Research has evolved significantly since then, allowing for a deeper understanding of ways to induce and synchronize ovulation in females, being them replacement heifers or postpartum cows. As a result, it has become possible to inseminate cows at specific predetermined times, without compromising pregnancy rates, while reducing the number of days spent on heat detection. Many options exist for synchronization of oestrus and ovulation (Johnson et al. 2017).

2.2.2. Embryo transfer

The first successful transfer of a mammalian embryo, attributed to Walter Heape, was performed in 1890. Heape transferred two four-cell Angora rabbit embryos into an inseminated Belgian doe that then gave birth to four Belgian and two Angora young. The next documented record of successful transfers dates back to the 1920s, also performed on rabbits. This period marks the expansion of knowledge regarding the relationship between the pituitary gland and the ovaries, which ultimately led to noteworthy progress in reproductive technologies. The 1930s and 1940s saw even more advances in superovulation, oestrous synchronization and AI techniques that would make it possible to expand the successful use of embryo transfer

(ET) in other species, although not in cattle. The first ET calf was born in 1951, and in the early 1970s commercial ET programs gradually became accepted, particularly in the UK and North America (Mikkola et al. 2019). In 1983, Nikkola and Smith suggested the use of a breeding scheme for dairy cattle that involved the systematic use of MOET, with several schemes being developed later that decade and used in production settings (Callesen et al. 1996).

Embryo transfer, although not as widespread as AI, provides an analogous tool for the dissemination of superior genetic traits. Similarly to AI, the use of ET offers an opportunity to expand superior genetic characteristics of both male and female animals, which would not otherwise be feasible (Schultz et al. 2020). It allows for more offspring to be obtained from valuable females as well as from infertile females, for the export and import of animals as fresh or frozen embryos, the introduction of new genetic material into specific pathogen-free farms and an increase in the population of rare or endangered breeds or species (Wakchaure and Ganguly 2015).

ET comprises the process of retrieving embryos of a known developmental stage from the reproductive tract of a donor female, and subsequently transferring them to a recipient female. The recovered embryos may be transferred fresh or undergo thawing after being cryo-preserved (Schultz et al. 2020). Embryos may be produced *in vitro* (IVP embryos) or *in vivo* derived (IVD embryos) - normally after a superovulation protocol followed by AI (Findlay et al. 2019).

Even though the number of IVD embryos collected and transferred worldwide seems to have stabilized in recent years (Ferré et al. 2020), MOET will continue to be an important method for producing embryos for transfer because fewer laboratory resources are required and pregnancy rates are superior to those achieved with IVP embryos (Hansen 2020). The IETS “2021 Statistics of embryo production and transfer in domestic farm animals” Report noted that in Europe, a total of 121,376 bovine *in vivo*-derived embryos (beef and dairy) had been transferred, of which 5,604 were fresh embryos, 15,523 were frozen domestically-produced embryos and 2,288 were frozen imported embryos. This same document further reported that the collection of bovine *in vivo*-derived embryos in Europe equated to 4,306 flushes, yielding 42,638 ova and 25,698 transferrable embryos in beef cattle. Data reported in Portugal for this same period showed 22 flushes from conventional semen in beef cattle, 0 flushes from sexed semen, a total of 289 ova collected and a total of 117 transferable embryos. Transfers of IVD fresh embryos added up to 38, while frozen domestic and imported embryos accounted for 31 and 24 transfers respectively. No IVP production of embryos was reported for beef cattle in Portugal, neither with abattoir-derived oocytes, nor with OPU-collected oocytes (Viana 2022). Although IVD embryos still represent a notable proportion of the total embryo production, the proportion of IVP embryos represents less than 10% in Asia, 27% in Europe and 49% in Oceania (Mikkola et al. 2019). Nevertheless, the transfer of IVP embryos

is growing at average annual rate of 12% (Ferré et al. 2020) and the 2021 IETS Report celebrated the achievement of a new milestone, reaching the one-million mark for IVP embryos transferred worldwide (Viana 2022). For the first time ever in history, in 2016, the International Embryo Transfer Society (IETS) recorded that the number of viable IVP embryos surpassed the number of transferable *in vivo*–produced embryos (Ferré et al. 2020). IVP programs have the advantage that donors can have ovum pickup (OPU) performed every 12 to 18 days, up to 100 plus days of pregnancy. This is done using an ultrasound-guided follicular aspiration unit, yielding high numbers of oocytes recovered, which are then sent to laboratories where maturation and fertilization (IVF) and culture (IVC) are performed. IVP embryos can be transported back to the farm using a specially designed temperature-controlled incubator, following which the embryos can be transferred into synchronized recipients or undergo cryopreservation. In recent years, frozen IVP embryos have had pregnancy rates similar to those for conventional ET embryos, when using grade I embryos (Phillips and Jahnke 2016).

Embryo transfer represents a means to overcome pregnancy failure caused by issues in the fertilization or early embryonic development before the 7-day mark, including problems caused by intrinsic defects in the gametes or embryo and reproductive tract. However, it cannot reduce losses caused by the intrinsic inability of the transferred embryo to develop to term or by the incapability of the reproductive tract to support development after day 7 (Rodrigues et al. 2018).

2.2.3. Oestrus cycle

The reproductive cycle is divided into three stages: the follicular phase, oestrus, and the luteal phase (Smith et al. 2015).

It involves two main components: the anatomical structures such as the hypothalamus, pituitary gland and ovaries, and the hormones that allow interaction between these different anatomical structures (Deguettes et al. 2020). Hormones act as chemical messengers that target specific tissues and receptors, providing the necessary foundation for events to take place (Smith et al. 2015).

Gonadotropin-releasing hormone (GnRH) is produced by the hypothalamus and interacts directly with the pituitary gland. In turn, the pituitary gland produces follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones migrate via the bloodstream and act on the ovaries causing the growth of several immature follicles. Production of oestradiol by the follicles generates positive feedback on the pituitary gland, and results in a surge of LH production, which causes the maturation of a single follicle and consequently the selection of the dominant follicle, which will be described in further detail below. After 21 days,

which is the average duration of the oestrus cycle, if no fertilization occurs, ovulation of the dominant follicle is observed (Deguettes et al. 2020).

Each oestrus cycle has two to three follicular waves, which consist of the synchronous emergence of a group of antral follicles followed by the selection of a dominant follicle and atresia of the other ones. The emergence of the first follicular wave occurs on the day of ovulation; however, the presence of a mid-cycle corpus luteum (CL) causes the dominant follicle of the first wave to regress, allowing for the emergence of a second wave. In two-wave cycles, the second wave emerges on days 9 or 10, and in three-wave cycles, around days 8 or 9, with the third wave emerging on days 15 or 16. Follicular waves also occur in heifers before puberty and in postpartum cows, before the first ovulation (Mapletoft et al. 2018).

There is a surge of circulating FSH that is followed by the appearance of a group of follicles that are 4 to 5 mm in diameter. Follicles, especially the future dominant follicle, produce oestradiol and inhibin, which inhibit FSH. Dominant follicles are selected based on the difference in responsiveness of antral follicles to FSH and LH. As it matures, the dominant follicle acquires more LH receptors allowing it to shift its gonadotropin dependence to LH during the period of low FSH. The result is that it continues to grow while subordinate follicles, that did not shift, still require FSH, and therefore regress. This sequence of events leads to the selection of a dominant follicle approximately 3 days after wave emergence (Mapletoft et al. 2018).

As mentioned before, the presence of a CL means there is production of progesterone (P_4) which supresses LH. Low LH causes the dominant follicle in the first wave - and second wave, in 3-wave cycles - to ultimately cease its metabolic functions and regress, thus leading to the surge of FSH, which supports the emergence of a new follicular wave. When luteolysis occurs, there is an increase in LH pulse frequency, that allows for growth of the dominant follicle, elevated oestradiol concentrations, an LH surge, and ultimately, ovulation. The dominant follicle present at the time of luteolysis becomes the ovulatory follicle, and emergence of the next wave is delayed until the ensuing ovulation (Mapletoft et al. 2018). The following illustration (Figure 2) depicts the hormonal secretion patterns described above, as well as the sequence of follicular growth and selection in a three-wave cycle.

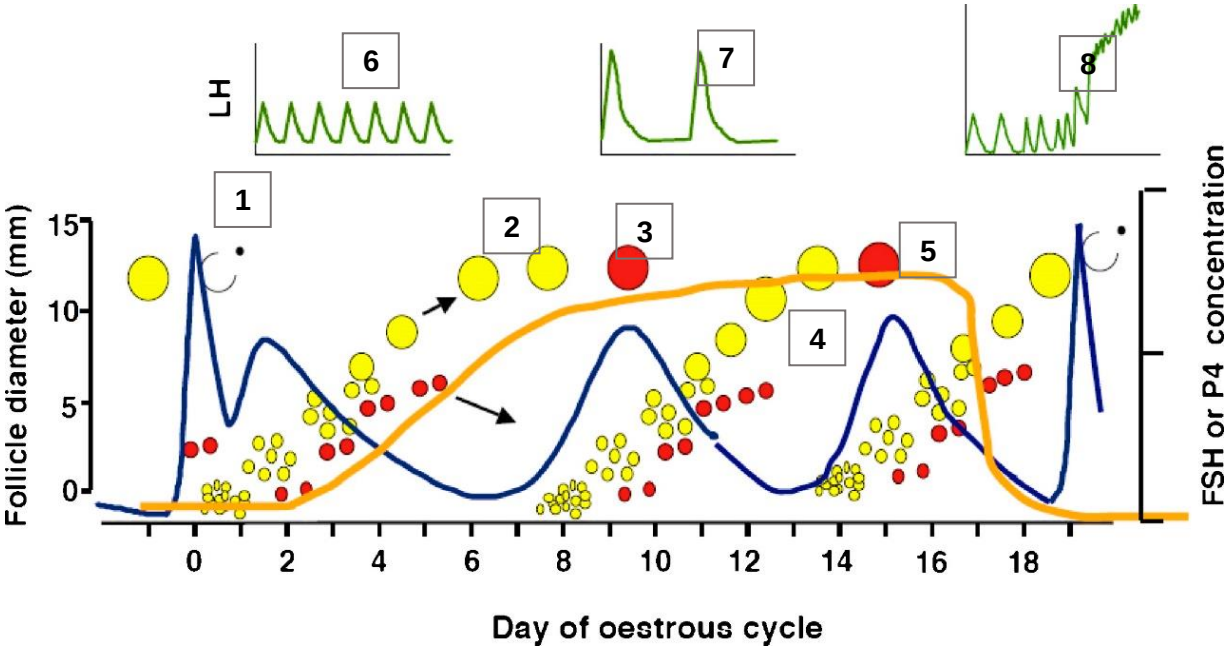


Figure 2 - Schematic Representation of the oestrus cycle (Forde et al. 2011)

Caption: 1- Ovulation; 2- Growing follicles; 3- Atretic follicles; 4- FSH concentration; 5- P4 concentration; 6- Pattern of LH secretion in the early luteal phase; 7 – Pattern of LH secretion in the mid-luteal phase; 8- Pattern of LH secretion in the follicular phase.

Furthermore, as stated by Hansen (2020), the "birth of a live, healthy calf requires ovulation of an oocyte capable of being fertilized and supporting the development of the resultant embryo, deposition of sperm in the reproductive tract capable of fertilizing the oocyte, formation of an embryo with the genetic and nongenetic inheritance from the oocyte and sperm that allow it to develop to term, and a reproductive tract competent to support gamete transport, fertilization, and development of the conceptus to term".

2.2.3.1. Oestrus synchronization protocols

There are certain physiological changes that need to occur before and after fertilization, in order to maximize pregnancy rates. Firstly, there must be high circulating concentrations of P_4 for several days prior to the onset of corpus luteum regression. Secondly, the regression must be rapid and complete and must lead to low P_4 concentrations at the time of AI. Thirdly, a physiologically mature follicle must increase its secretion of oestradiol, before and at the time of AI, while ovulation must be followed by an increase of adequate amounts of circulating P_4 secreted by the newly formed corpus luteum (Smith et al. 2015).

There are three general approaches when it comes to synchronization protocols in cycling animals: inhibition of ovulation following spontaneous corpus luteum regression; induction of corpus luteum regression; or a combination of both. Long-term progestin treatments are used to achieve the former and $PGF_{2\alpha}$ is used to achieve the latter (Smith et

al. 2015). However, fertility in long-term P_4 treatment is frequently reduced due to the presence of persistent follicles (old age follicles), and females that happen to be until the 5th or 6th day of their cycle are not responsive to the $PGF_{2\alpha}$ treatment, therefore resulting in decreased synchronization response. Thus, the combination of these approaches allows for a more effective synchronization of oestrus that does not compromise fertility and can be used regardless of the stage of the cycle. The addition of a GnRH injection at the beginning of the progestin treatment results in ovulation of the dominant follicle or its luteinisation, and a synchronized new follicular wave – consequently reinforcing the positive effects of combining the two approaches (Smith et al. 2015).

The main advantage of an oestrous synchronization protocol is to enable the use of ART such as AI and ET, as a result of better oestrous prediction, while reducing labour expense associated with its detection – therefore making them more practical (Patterson et al. 2011). In addition, procedures that facilitate synchronization of oestrous in cycling females, and induce ovulatory oestrus in peripubertal heifers and postpartum cows, will consequently boost reproductive rates and accelerate genetic progress (Patterson et al. 2011).

In beef operations, where profitability is most important, implementation of defined breeding seasons significantly impacts these by allowing producers to match cattle to the available resources, refining nutrient delivery to groups of cattle, concentrating labour resources, and by consequently increasing the sales price of calves (Knox 2014). In this kind of operation, the main indicator of success is the annual calf crop - total kilograms of calf weaned divided by the number of cows in breeding. Therefore, overall season pregnancy rates and pregnancy achieved early in the breeding season are critical factors (Kasimanickam et al. 2014). For example, with the use of FTAI, approximately fifty per cent of the herd could potentially become pregnant at the beginning of the breeding season (Bó and Baruselli 2014). Therefore, oestrus synchronization plays a relevant role in increasing uniformity of calf crops, and shortening the breeding season, since females that conceive to a synchronized oestrus calved earlier in the calving season and their calves are consequently older and heavier at weaning (Patterson et al. 2011). Use of semen from genetically superior bulls will not only result in an improvement in herd genetics but will also play a role in achieving heavier weights of calves at weaning (Bó and Baruselli 2014).

Oestrous synchronization protocols and products have changed and evolved over time, however, the basic physiological principles fundamental to their performance remain the same – so, an understanding of the bovine oestrous cycle and how oestrous synchronization products work will facilitate the application of these technologies (Smith et al. 2015).

Effective oestrous synchronization protocols are designed to synchronize follicular maturation with the onset of corpus luteum regression (Smith et al. 2015).

The most widespread method in beef cattle is the CO-Synch approach, in conjunction with or without a CIDR insert (Bridges et al. 2012). CIDR (Figure 3) consists of a “T” shaped nylon backbone that is coated with a silicone layer containing 10% progesterone by weight. The devices are inserted deep into the vagina with the help of an applicator and removed by pulling the flexible nylon tail-like structure (Smith et al. 2015).

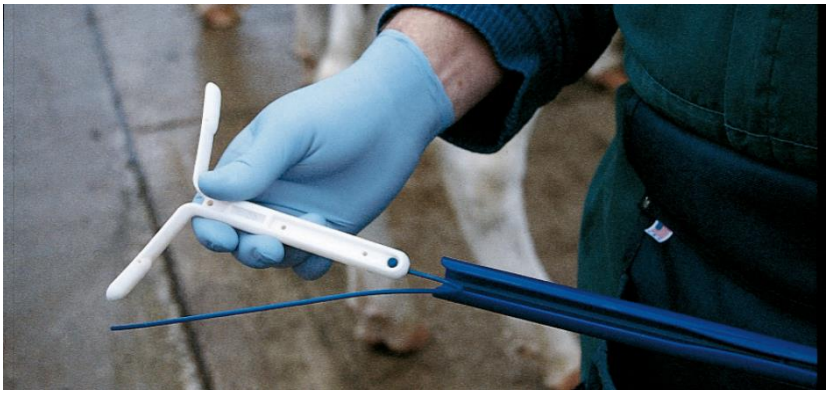


Figure 3 - CIDR insert (ZOETIS n.d.)

Studies have shown that due to the prevention of early ovulation, the addition of a progestin-releasing device to a 7-day GnRH protocol improved pregnancy rates both in heifers and in beef cows (Bó and Baruselli 2014). Comparison between conception rates of protocols with and without an exogenous source of P_4 also corroborated that fertility improved when P_4 was utilized. Furthermore, the addition of a CIDR to protocols for anoestrus cows achieved pregnancy rates comparable to the ones for cyclic cows (Lamb and Mercadante 2016). This ultimately means that it is very common for cows and heifers to be fitted with a device between the administration of the first GnRH dose and the $PGF_{2\alpha}$ dose (Bó and Baruselli 2014).

Recently, it has been demonstrated that a decrease in the interval between the GnRH + CIDR insertion and CIDR removal + $PGF_{2\alpha}$ administration by two days, from seven to five, and the increase in the time between CIDR removal and FTAI from sixty-six to seventy-two hours, leads to higher pregnancy rates in FTAI. However, the current recommendation for the 5d CO-Synch+ CIDR protocol is that two doses of $PGF_{2\alpha}$ are administered twelve hours apart at CIDR removal, to ensure complete regression of the luteal body. This recommendation of two doses of $PGF_{2\alpha}$ increases the number of times animals are handled and will in many cases limit adoption of this protocol (Bridges et al. 2012). Because they only require animals to be handled at three different occasions, 7d CO-Synch + CIDR protocols are used more frequently amongst beef cattle producers(Colazo and Mapletoft 2014). The following table (Table 1) summarizes some of the available protocols for FTAI for cows and heifers.

Table 1 – Some available FTAI protocols for cows and heifers

FTAI protocols for heifers (Patterson et al. 2013)	7-day CO-Synch + CIDR	GnRH is administered at CIDR insertion on day 0, followed by removal of CIDR 7 days later, and $PGF_{2\alpha}$ administration. Insemination is performed 54 hours after CIDR removal, with GnRH administered at AI.
	5-day CO-Synch + CIDR	GnRH is administered at CIDR insertion on day 0, followed by removal of CIDR 5 days later, and $PGF_{2\alpha}$ administration. A second injection of $PGF_{2\alpha}$ is administered 8 hours after the first $PGF_{2\alpha}$ injection. Insemination is performed 60 hours after CIDR removal, with GnRH administered at AI.
	CIDR Select	CIDRs are inserted on day 0 and removed on day 14, GnRH is administered on day 23 and $PGF_{2\alpha}$ is administered on day 30. Insemination is performed 72 hours after $PGF_{2\alpha}$ injection with GnRH administered at AI.
	14-day CIDR- $PGF_{2\alpha}$	CIDRs are inserted on day 0 and removed on day 14 with $PGF_{2\alpha}$ administered on day 30. Insemination is performed 66 hours after $PGF_{2\alpha}$ injection with GnRH administered at AI
FTAI Protocols for cows (Patterson et al. 2016)	5-day CO-Synch + CIDR	GnRH is administered at CIDR insertion on day 0, followed 5 days later with CIDR removal, and $PGF_{2\alpha}$ administration. A second injection of $PGF_{2\alpha}$ is administered 8 ± 2 hours after CIDR removal and the first $PGF_{2\alpha}$ injection. Insemination is performed 72 hours after CIDR removal, with GnRH administered at AI.
	7-day CO-Synch + CIDR	GnRH is administered at CIDR insertion on day 0, followed 7 days later by CIDR removal and $PGF_{2\alpha}$ administration. Insemination is performed 66 hours after CIDR removal, with GnRH administered at AI.

A successful synchronization program is confidently achieved by understanding the principals behind the bovine oestrous cycle, the biological actions of oestrous synchronization products such as progestins, $PGF_{2\alpha}$, and GnRH, and the basics for the selection of heifers and cows that have a high chance of responding suitably to these actions/products (Smith et al. 2015). Good candidates that are likely to respond must be carefully identified and it is therefore recommended that heifers need to reach puberty prior to oestrous synchronization and postpartum cows must have a body condition score (BCS) of at least 2.5 (scale 1 - 5) and be beyond the 40-day postpartum mark at the beginning of the program, as it is important to

consider that longer recovery period between calving and the beginning of the breeding season leads to a greater proportion of cows cycling at the start of the breeding season (Smith et al. 2015).

The correct process to determine whether it is beneficial to introduce the use of a synchronization and AI program should begin with an analysis of the pregnancy rates after a 60 to 80-day breeding season. If pregnancy rates are less than 85%, it is an indicator that there may be underlying management issues that therefore need to be resolved before considering these procedures. Nevertheless, even with higher pregnancy rates, there should always be an evaluation process to determine if heifers and cows are good candidates for these protocols (Perry and Smith 1995).

Unsuccessful inseminations occur not only due to incorrect insemination methods, but also to other technical causes such as inaccurate detection of oestrus or inadequate storage of semen (Hansen 2020b).

2.2.3.2. Superovulation protocols

Superstimulatory treatments are used to retrieve the maximum number of viable embryos through stimulation of growth and consequent ovulation of competent oocytes (Mapletoft et al. 2018).

Treatments most frequently used nowadays involve the use of purified FSH extracts, either from porcine or ovine pituitaries (Mikkola and Taponen 2017). The first reports of an improved superovulatory response following administration of FSH appeared in the late 1970s, eventually replacing the use of equine chorionic gonadotropin (eCG), which had been standard until then. The main reason for the preference in the use of pituitary FSH over eCG was linked to the downside of prolonged stimulation of the ovaries by the latter, due to its long circulating half-life. Prolonged ovary stimulation with eCG treatments resulted in un-ovulated follicles, abnormal endocrine profiles, and reduced ova/embryo quality (Bó and Mapletoft 2020).The most commonly used protocols use a decreasing dose of FSH and an administration of $PGF_{2\alpha}$ at the end of the protocol. Due to its short half-life (5 hours), porcine FSH must be administered twice daily (Phillips and Jahnke 2016).

The two main factors influencing variability in superstimulatory response are the intrinsic number of antral follicles of donor ovaries, and the stage of follicular development at the time of initiating FSH treatments (Mapletoft et al. 2018). Because this approach is contingent on the cyclicity of the female, protocols with the addition of a CIDR have recently been embraced, as a way to increase flexibility to begin a superovulatory schedule independently of the phase of the oestrous cycle. Elimination of the dominant follicle allows for a new wave of antral follicles to emerge and respond to the FSH treatment in larger

numbers. This can be accomplished by a variety of ways such as ultrasound-guided dominant follicle aspiration or GnRH administration in combination with a CIDR (Phillips and Jahnke 2016).

Although the number of embryos recovered from a donor may range from 0 to 50, the average number of transferable-quality embryos is six to seven. Approximately 15–20% of all embryo collections result in non-viable embryos. Superovulatory response has been shown to be limited by high individual variation and low repeatability, and this characteristic seems to have remained relatively unchanged throughout the evolution of the technique. This has ultimately resulted in an embryo population where two-thirds of the embryos originate from one-third of superovulated donors. (Mikkola et al. 2019).

There are various factors that could potentially influence the outcome of superovulation, namely: cattle breed, genetics, age, parity, reproductive history, lactational status, season, climate, weather, management system, nutrition, stress, stage of cycle, number of follicles before start, anti-Müllerian hormone (AMH) level, method of synchronisation, gonadotrophin, gonadotrophin diluent, gonadotrophin dose, route of injections, frequency of injections, insemination, semen quality, unsexed vs sexed semen, number of spermatozoa, and embryo recovery method (Mikkola et al. 2019).

Although recipient management is an essential part of a fruitful ET program, it is frequently neglected. Recipient health and nutrition play a vital role in this management strategy. Beef recipients should have BCS between 2.5 and 3.5, and health programs must be fully effective before the animals are used as a recipient. Diseases such as BVD, enzootic bovine leukosis, neosporosis, Johne's disease and brucellosis should be controlled and eradicated prior to the inclusion of the animals in these programs (Phillips and Jahnke 2016).

It is common practice to use heifers as recipients because they normally have higher pregnancy rates than cows. However, heifers might not necessarily carry the genetics selected for calving ease and, therefore, calving management must be a primary concern when using heifers as recipients (Phillips and Jahnke 2016).

"When high-quality embryos are transferred, with or without cryopreservation, high rates of pregnancy can be achieved if suitably prepared recipients are available, and animals are managed appropriately" (Findlay et al. 2019b).

Determining the embryo quality grade (Table 3) is achieved through a visual assessment of the embryo's morphologic characteristics. Because the visual assessment is, to some extent, a subjective evaluation, the best predictor of an embryo's viability is its stage of development relative to the given day after fertilization. Some of the characteristics taken into account when determining the quality of an embryo include uniform size and colour of the blastomeres, presence or absence of vacuoles amongst the cells, presence or absence of

extruded cells, and shape of the zona pellucida (Phillips and Jahnke 2016) as described further in the Table 2 and Table 3.

Table 2 - Embryonic stages

Morula	The cellular mass of the embryo occupies most of the perivitelline space. A mass of 16 blastomeres or more. Individual cells are difficult to distinguish.
Compact morula	The embryo mass occupies 60 to 70 % of the perivitelline space. A compact mass formed by the coalescence of individual.
Early blastocyst	The embryo occupies 70 to 80% of the perivitelline space. An embryo that has a blastocele and resembles a signet ring. The inner cell mass and trophoblast are difficult to differentiate in early blastocyst stages, making it appear of questionable quality.
Blastocyst	Embryo occupies most of the perivitelline space. Blastocele is highly prominent. Pronounced differentiation of the outer trophoblast layer and the darker, more compact inner cell mass is evident. Visual differentiation between the trophoblast and the inner cell mass is possible at this stage of development
Expanded blastocyst	The overall diameter of the embryo dramatically increases, with a concurrent thinning of the zona pellucida to approximately one-third of its original thickness
Hatched blastocyst	Embryos recovered at this developmental stage can be undergoing the process of hatching or may have completely shed the zona pellucida. Hatched blastocysts may be spherical with a well-defined blastocele or may be collapsed. Identification of hatched blastocysts can be difficult unless they're expanded when the signet ring appearance is again obvious.

From: Bó and Mapletoft (2013)

Table 3 – Embryo quality codes

Code 1 - Excellent or Good	Embryos have a spherical symmetrical mass with individual blastomeres that are uniform in size, colour, and density. The embryo is consistent with its expected stage of development. Irregularities are minor, and at least 85% of the cellular material is an intact, viable embryonic mass. These embryos withstand the freezing/thawing procedure.
Code 2 - Fair	The embryo has moderate irregularities in the overall shape of the embryonic mass or in size, colour, and density of individual blastomeres. At least 50% of the embryonic mass is intact. Survival of these embryos to the freezing/thawing procedure is lower than with Code 1 embryos, but pregnancy rates are adequate if embryos are transferred as fresh into suitable recipients.
Code 3 – Poor	Poor. These embryos have major irregularities in shape of the embryonic mass or in size, colour, and density of individual blastomeres. At least 25% of embryo mass is intact. These embryos do not survive the freezing/thawing procedure and pregnancy rates are lower than those obtained with fair quality embryos if transferred fresh into suitable recipients.
Code 4 – Nonviable structures	Dead or degenerating embryos, and oocytes, or 1-cell embryos. All are nonviable and are to be discarded.

From: Bó and Mapletoft (2013)

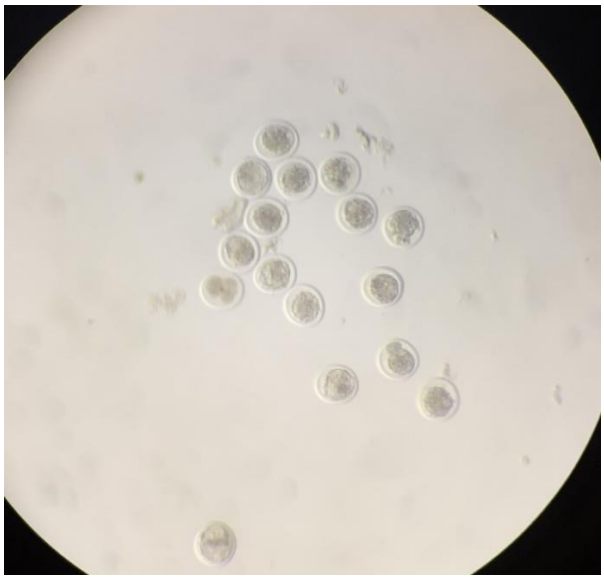


Figure 4 - Microscopy imaging of retrieved embryos (Day 7) at different stages

3. Materials and methods

3.1. Herd description

During the time of this study, the herd was managed in different groups according to age and phenotypical appearance. Heifers were grouped together while red cows and black cows were separated. Calves were kept with their mother until the age of 6 months, at which point they were weaned. Heifers were introduced to their groups according to their phenotypical appearance, once a positive pregnancy was achieved. Bulls and young bulls were kept separated from the rest of the herd, except for during the mating season, when they were placed with the different groups, included in the ARTs to act as “sweeper” bulls after FTAI and ET programs were complete. The herd was kept in a semi free-range system. Adequate nutrition was guaranteed through a combination of grazing on available pastures and complemented with silages and concentrate, according to energy requirements. Pastures were kept in irrigated areas and the animals rotated between the available fields. Prophylactic management was equal in all groups. Young calves were first vaccinated at the time of tagging and regular vaccination was maintained throughout the life of all animals.

3.2. FTAI

A total of 251 Aberdeen Angus beef cows were included in the study and FTAI was performed 499 times in all breeding seasons between December 2013 and December 2019, adding up to 20 synchronization programs. For the purposes of this study, we will consider each synchronization program as the time between the beginning of the synchronization protocol and the insemination, starting on the day the group was first evaluated and treated. The beginning and end of overall breeding season for each group is noticeably similar for the whole herd, as all groups started their breeding season with a synchronization program, varying only a few days each year.

All animals included in the synchronization programs were submitted to a thorough physical evaluation by the clinician in charge. This preliminary evaluation served to assess the body condition score, reproductive status, history, and gynaecological exam of the females. A portion of reproductive tract pre-breeding exams (n=376) were done by ultrasonography. Ultrasonography was performed with an *Easi-Scan* (BCF Technology, Scotland) ultrasound scanner and linear 128 elements probe. The remaining were performed via transrectal palpation and considered size of cervical and uterine structures, presence of palpable ovarian structures and position of the reproductive tract relative to the pelvis.

Females that did not meet the minimum requirements concerning BCS and reproductive tract health were regrouped either to be sent for slaughter, or to undergo treatment for recuperation.

Each protocol (Table 4) was initiated nine days prior to the date of insemination, with the above-mentioned physical exam. If regarded as a suitable candidate, each female was then submitted to synchronization.

GnRH (100 µg, IM; Gonestin, Spain) was administered to each suitable candidate and either a CIDR or PRID was inserted on day zero. On day seven, the insert was removed, and animals were injected with d-Cloprostenol (150 µg, IM; Veteglan, Spain) and eCG (400UI, IM; Intergonan, Netherlands). Fixed-time AI occurred on day nine (48 h after insert removal) for all females (heifers and cows), at which time a second dose of GnRH (100 µg, IM; Gonestin, Spain) was administered. Table 5 provides a description of the substances used in the protocols. Commercial labels indicated are solely for product identification purposes.

Table 4 - FTAI synchronization protocol: CO-Synch+CIDR7d

Day 0	Gynaecological exam (RP or ultrasonography) CIDR insert + GnRH (100 µg, IM)
Day 7	CIDR removal + d-Cloprostenol (150 µg, IM) + eCG
Day 9	FTAI + GnRH (100 µg, IM)

Table 5 – Substances used and Routes of administration of CO-Synch+CIDR7d Synchronization Protocol

	Commercial name	**Active Substance**	**Dosage**	**Route of administration**
CIDR	CIDR 1.38 g – For *cattle (cows and heifers, Zoetis Portugal)*	1.38 g of P_4	n/A	Intravaginal application
PRID	Prid Delta 1.55 g – For cattle *(France)*	1.55 g P_4	n/A	Intravaginal application
GnRH	GONESTIN, 50 µg/mL, injectable solution for cattle	Gonadorelin (as gonadorelin acetate) 50 µg	2 mL (100 µg)	Intramuscular

	Commercial name	Active Substance	Dosage	Route of administration
$PGF_{2\alpha}$	Veteglan 0.075 mg/mL – For cattle, pigs and equine	d-Cloprostenol 0.075 mg (d-Cloprostenol as Na salt 0.079 mg)	2 mL (150µg)	IM
eCG	INTERGONAN 6000 UI, (powder and solvent) *Injectable solution for cattle, sheep, rabbits, and dogs*	Equine chorionic gonadotrophin	2 mL 400 IU	IM

All intravaginal devices were carefully handled and increased care was taken to avoid the contamination of the devices. Recommendations regarding cleaning the vulva, and hygiene at the moment of insertion of the device and applicator were strictly followed. A light spray of oxytetracycline aerossol [Oxymycin Aerossol 32.1 mg/mL, for cattle and sheep*;* Oxytetracycline hydrochloride 32.1 mg (3.57% m/m)] was applied in order to reduce vaginitis.

All animals were immobilized through the squeeze chute to ensure that administration of substances was correctly executed.

Semen from 33 different sires was used, and each female was only submitted to one synchronization program per breeding season. All semen was stored in private liquid nitrogen containers, kept with rigorous records, and appropriate maintenance. Semen straws were thawed in a temperature-controlled recipient at approximately 37 ºC, for 25 seconds. Clean-up bulls were introduced to the herd 15 to 20d after FTAI, for a period of 60d.

AI was done by recto-vaginal method of insemination. Disposable sheaths and sleeves were used, insemination guns were disinfected between each usage and overall appropriate sanitation and hygiene measures were followed. At the end of each procedure a verification step was carried out by lightly taping the insemination gun on a clean paper towel and assessing if there was blood on the remaining fluid, meaning there was some degree of trauma to the genital tissues. AI was performed by three different technicians, all of whom duly trained and certified to perform it.

Pregnancy status was determined at a median of 40 days (range from 27-160) post-FTAI, using the scanner mentioned above. Expected delivery dates and actual delivery dates were compared and used to confirm that the pregnancy was correctly diagnosed, and that the indicated progeny was correct. Conception rate attributable to FTAI was calculated as the number of bovine females diagnosed pregnant following FTAI, divided by the total number of females treated.

Because, in general, only one pregnancy diagnosis was performed on each group, embryonic and foetal losses were clustered and are hereinafter further referred as to intrauterine mortality. The intrauterine mortality rate is defined as the proportion of cows that were diagnosed pregnant at the initial pregnancy evaluation and did not give birth to viable calves. It therefore includes stillbirths and abortions.

3.3. MO

This study included 27 superovulation protocols, completed between February 2016 and October 2019.

The protocols started with a thorough evaluation to assess the body condition score, reproductive status and history, and gynaecological exam of each female. Assessment of reproductive tract health was performed with an ultrasound using the equipment mentioned above (n=27) and via transrectal palpation, where size of cervical and uterine structures, presence of palpable ovarian structures and position of the reproductive tract relative to the pelvis were considered.

Each female considered to have met the requirements received a CIDR insert and a GnRH injection on the morning of day 0 of the protocol. On days 4 through 7, each donor received a dose of FSH+ LH injection (Pluset, 20 mL, Calier Portugal, S.A.), in decreasing dosages, every twelve hours. On day 7, CIDR inserts were removed and two doses of $PGF_{2\alpha}$ were administered 12-hours apart. Heat detection was performed on days 8 and 9, by a trained technician, for half an hour in the morning and half an hour at dusk. AI was performed on day 9 (pm) and on day 10 (am) as shown in Table 6.

Embryo retrieval was performed on day 16 (6.5 - 7 days post-insemination) and marks the end of each protocol. Recovery procedure was done via non-surgical transcervical uterine flushing (Figure 5) following the standard protocol including epidural anaesthesia (flushing each uterine horn at least four times with 450-500 mL of PBS solution and collecting embryos into a 1L collecting flask). Upon filtration (EMCon filter) the following process included assessment of embryo morphology and grading of embryos in terms of quality and developmental stage with a stereomicroscope. Embryo recovery, handling and evaluation was performed according to strict procedures by an experienced theriogenologist.

Table 6- Multiple ovulation and embryo retrieval protocol

	AM	PM
Day 0	Gynaecological exam (RP + ultrasonography) CIDR insert 2 mL buserelin acetate 0.0042 mg	-
Day 4	4 mL Pluset - porcine pituitary extract (PPE), which contains both gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH)	4 mL Pluset
Day 5	3 mL Pluset	3 mL Pluset
Day 6	2 mL Pluset	2 mL Pluset
Day 7	1 mL Pluset CIDR removal luteolytic dosage of $PGF_{2\alpha}$	1 mL Pluset luteolytic dosage of $PGF_{2\alpha}$
Day 8	Heat detection	Heat detection
Day 9	Heat detection	1st AI
Day 10	2nd AI	-
Day 16	Embryo retrieval, transfer and freezing	

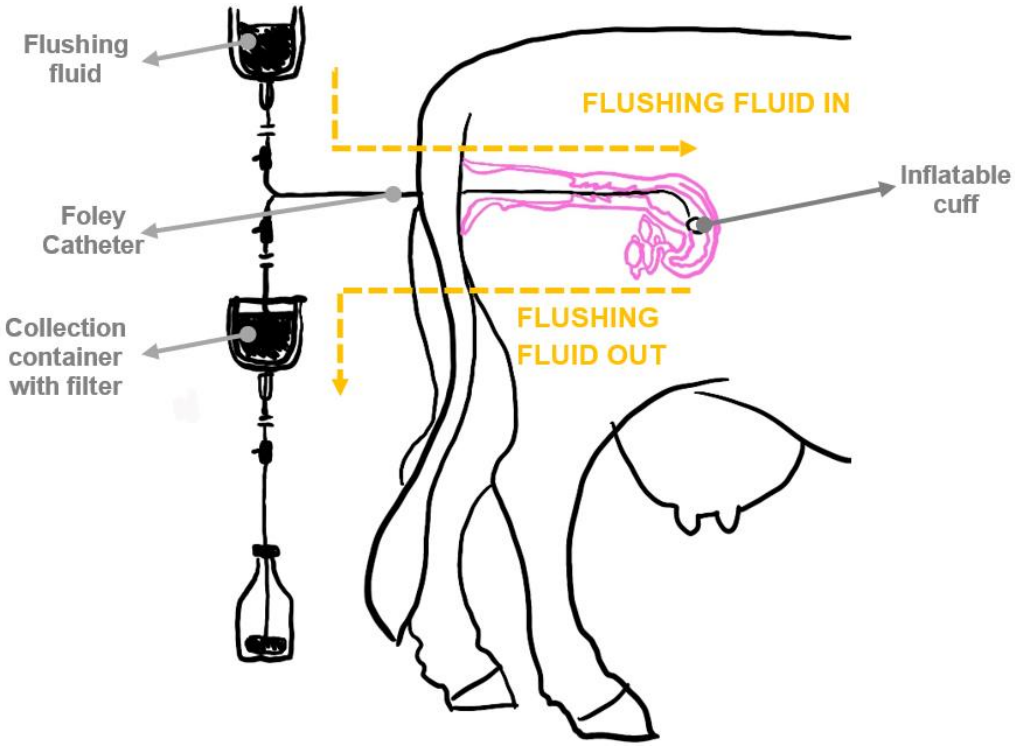


Figure 5 - Non-surgical transcervical uterine flushing technique for embryo retrieval (Adapted from: Embryo Transfer (ET))

3.4. ET

Embryo transfer was performed 106 times, between 2014 to 2019. A total of 81 females were included in 20 synchronization protocols.

As described above, each synchronization protocol started with the assessment of reproductive tract health. This was performed using ultrasound (n=107) and via transrectal palpation, where size of cervical and uterine structures, presence of palpable ovarian structures and position of the reproductive tract relative to the pelvis were considered.

Each female considered to have met the requirements of an appropriate recipient received a dose of GnRH (100 µg, IM; Gonestin, Spain) and a CIDR (Zoetis Portugal) insert (day 0). On day seven, the insert was removed, and animals were injected with d-Cloprostenol (150 µg, IM; Veteglan, Spain)). Heat detection was performed on days 8 and 9, by a trained technician, for half an hour in the morning and half an hour at dusk. Embryo transfer was completed on day 16 with donor-recipient synchrony ranging from -24 to +24 hours. All embryos were stored in private liquid nitrogen containers, kept with thorough records, and appropriate maintenance. Embryo transfers followed standard procedures and rigorous hygiene measures. At the end of each procedure a verification step was carried out by lightly taping the transfer syringe on a clean paper towel and assessing if there was blood on remaining fluid, meaning there was some degree of trauma to the tissues. The transfer was then categorised accordingly taking into account not only this information but also the technician's feedback as to where the embryo was placed anatomically (good embryo placement refers to placement in uterine horn ipsilateral to the corpus luteum close to the apex of the horn, reasonable embryo placement refers to placement in the middle part of the horn). All transferred embryos were cryopreserved IVD or IVP embryos.

Table 7 - ET synchronization protocol

	AM	PM
Day 0	Gynaecological exam (RP + ultrasonography) CIDR insert + GnRH (100 µg, IM)	-
Day 7	CIDR removal + luteolytic dosage of $PGF_{2\alpha}$	
Day 8	Heat detection	Heat detection
Day 9	Heat detection	
Day 16	Embryo transfer	

3.5. Data collection methods

Data related to all events was collected, by the author of this study, based on records kept by the assisting theriogenologist and in the online archive used by the company (*Rural Bit e-Exploração*). All information was cross-checked with its corresponding field annotations and, whenever necessary, edited for consistency and accuracy to reduce errors. The information was combined and organized into three excel sheets, one for FTAI, one for MO, and one for ET.

Information registered for FTAI included date of AI, female identification number, birth date, first calving date, number of calvings, previous calving date, type of device inserted (CIDR or PRID), duration of CO-Synch protocol, AI technician, AI sire, deposition site, insemination score (atraumatic/traumatic AI), pregnancy diagnosis date, pregnancy status, expected calving date, calving date, calf's sex, and calf viability.

Information registered for MO include ID number of the donor, date of birth of the donor, age at MO program, number of calvings, SOV protocol, SOV hormone dosage, starting date of the MO program, end date of the MO program, date of oestrous, date of 1st AI, No. of semen doses, sire, date and time of flushing, size of right ovary, size of left ovary, No. of CLs in right ovary, No. of CLs in left ovary, total retrieved structures, No. viable embryos, No. degenerated embryos, No. of oocytes and No. of frozen embryos.

Information registered for ET include ET date, ID number of the recipient, breed of the recipient, birth date of the recipient, age at ET, number of calvings, days postpartum, synchronization protocol, type of fertilization of embryo, embryo parents, stage code, quality code, embryonic age, donor-recipient synchrony, recipient CL quality, duration of ET, transfer score, technician, date of pregnancy diagnosis, pregnancy status, expected delivery date, real delivery date, calf's sex and calf viability.

Available data was assessed in order to filter which variables were relevant to the present study, and information such as birth date, number of calvings or previous calving date were transformed into variables like age, parity, and days postpartum in order to be correctly investigated. Relevant variables included in the study will be described in the results section. Data regarding post-pregnancy diagnosis events, such as expected delivery dates, real delivery dates, calf's sex and calf's viability were not included in the current study, as they were considered out of the defined study scope.

3.6. Statistical analysis

Statistical analysis was conducted using the IBM® SPSS® Statistics 27 (Statistical Package for the Social Sciences) and Microsoft Excel. The significance level accepted for rejecting the null hypothesis was 5%; that is, a level of significance <0.05 was considered to be the effect of variables and their interactions.

The qualitative variables will be characterized through absolute and relative frequencies, and the quantitative variables through averages and respective standard deviation.

For the inferential statistics stage the qualitative variables were analysed through the Pearson Chi-square test of independence. This was used to understand if there is any association between categorical variables and PD. The Chi-Square requirement that there should be no more than 20% of cells with expected frequencies of less than 5 was analysed. In situations where this assumption was not met, the Chi-Square test was used by Monte Carlo simulation. Proportions of positive and negative pregnancy diagnoses within each variable were analysed through z-tests for independent proportions, and significant differences identified and discussed below. The quantitative variables were analysed through the t-test for independent samples to compare the means of two independent groups in order to determine whether there is statistical evidence that the associated group means are significantly different.

Binary logistic regression was used in order to assess a possible relationship between a binary dependent variable (PD positive or negative) and the independent variables in each equation. The qualitative variables were coded into dummy variables.

4. Results

4.1. FTAI

Pregnancy rates (number of animals that had positive pregnancy diagnosis divided by number of animals inseminated) was 49.1% (245/499). Pregnancy rates for heifers and cows was calculated at 42.5% (74/174) and 51.7% (168/325), respectively. Differences were not considered significant at the 0.05 level (P=.051). Number of females included in FTAI programs varied from 5 to 59, and pregnancy rates between protocols varied from 34% to 80%, as shown in Figures 6 and 7.

Fifteen females were excluded in order to correctly calculate the birth rate, since 14 were sold before their expected delivery date and one died before the EDD. That said, the

calculated birth rate was 44.8% (217/484). The intrauterine mortality rate was 5% (12/242), in which ten cases consisted of stillbirths and two of abortions.

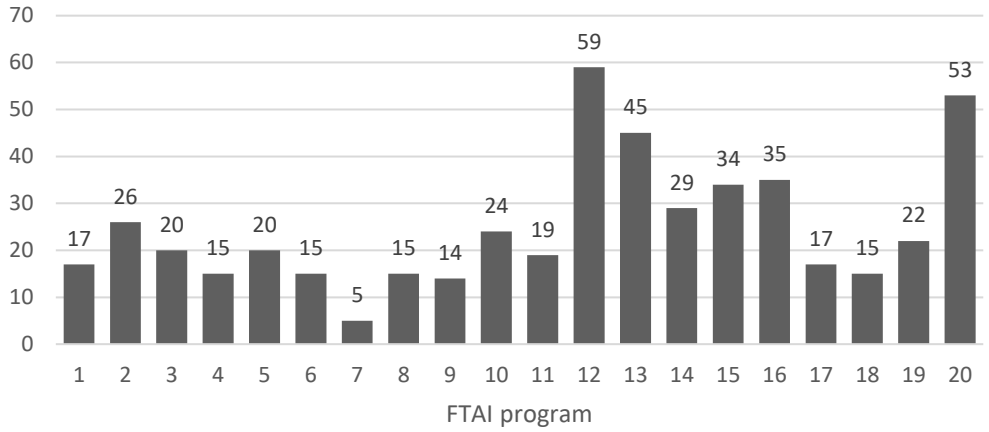


Figure 6 - Number of females included in each FTAI program

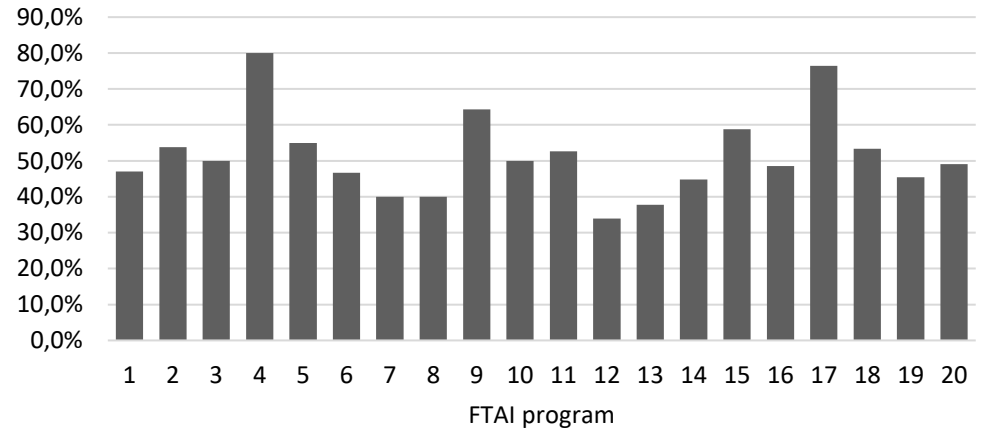


Figure 7 - Pregnancy rates for each FTAI Program

Variables described below were grouped in 2 sections (female related variables and procedure related variables) as the effect of age, days postpartum, parity, synchronization protocol, semen deposition site (uterine body/intra-uterine horn/cervical), procedure verification score (traumatic/atraumatic), and the technician that performed the AI, on the successful outcome - a positive pregnancy diagnosis - was analysed. No statistically significant associations were found between the variables under analysis and the pregnancy diagnosis (PD).

4.1.1. Female related variables: Age, Parity, and postpartum interval

Age at AI was calculated for all inseminations (n=499) and ranged from 1 to 10 years (Table 8, Figure 8). The comparison of age throughout the study timeline (Figure 9) allows for

further understanding of the herd dynamic. As it evolved, the herd grew older and age range was therefore broader.

Table 8 - Descriptive Statistics on Age at AI

	Age (years) n=499
Mean	3.3
Median	2.9
Minimum	1.2
Maximum	9.8
Standard Dev.	1.66
Coef. of Variation	0.51

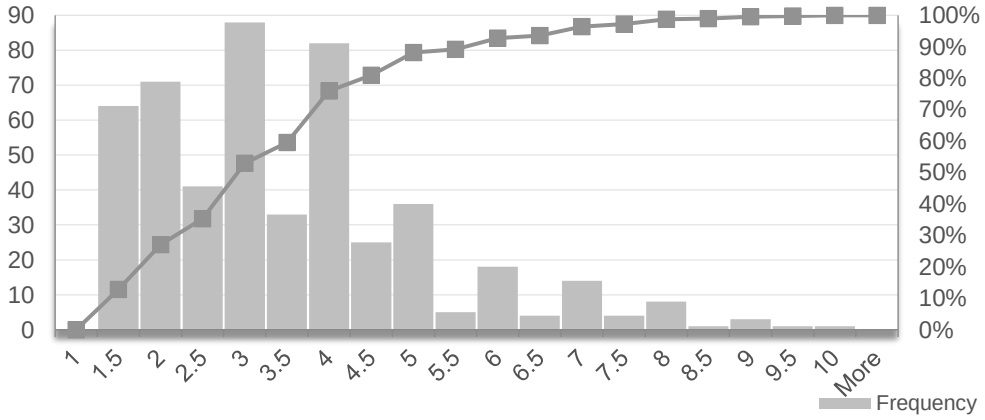


Figure 8 - Age at FTAI (years)

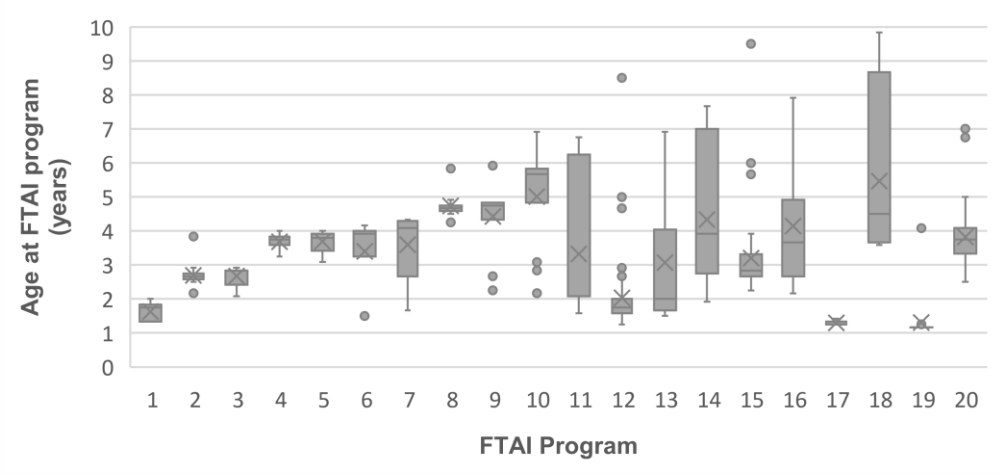


Figure 9- Comparison of age at FTAI between synchronization programs

In order to compare the means of the two independent groups (negative PD and positive PD) and assess if there is statistical evidence that the associated means are significantly different, an independent t test was performed. As the means where the same,

and p=.994, it was not possible to reject the null hypothesis that stated that the mean age is the same between the two groups (Table 9).

Table 9 - Association between quantitative FTAI variables – age –and PD: the independent samples t-test results

	Negative PD			Positive PD			Total	Significance
	n	Mean	Std. Deviation	n	Mean	Std. Deviation	n	
Age	257	3.28	1.77	242	3.28	1.52	499	.994

Parity was calculated for all inseminations (n=499); postpartum interval refers to n=325 inseminations done on primiparous and multiparous females (Table 10). It is important to note that there is no information relating calving for 46% (n=115) of the total number of females. This number is explained by the fact that it is common practice to sell heifers once a positive pregnancy diagnosis has been confirmed.

Table 10 - Descriptive Statistics on Parity and Postpartum interval at FTAI

	Parity n=499	Postpartum interval (days) n=325
Mean	1.34	124.72
Median	1.0	81.0
Minimum	0	11
Maximum	8	859
Standard Dev.	1.41	113.63
Coef. of Variation	1.05	0.91

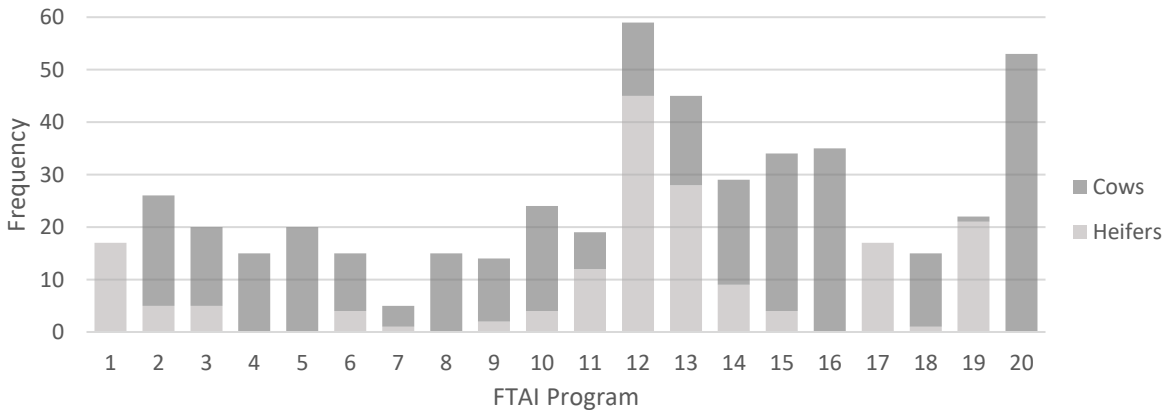


Figure 10 - Distribution of parity at each FTAI program

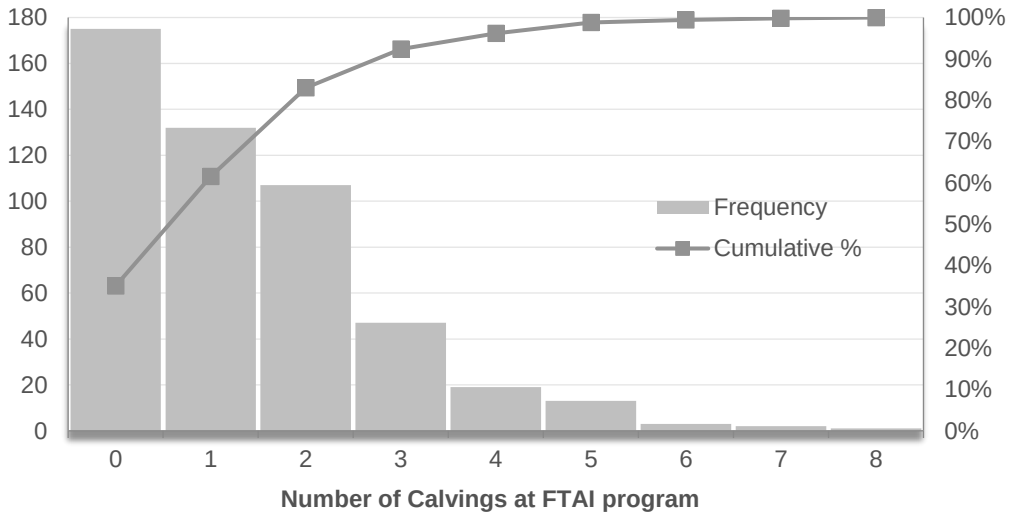


Figure 11 - Number of calvings at FTAI program

The figure below (Figure 12) represents the distribution of days postpartum of cows included in each of the 20 FTAI programs.

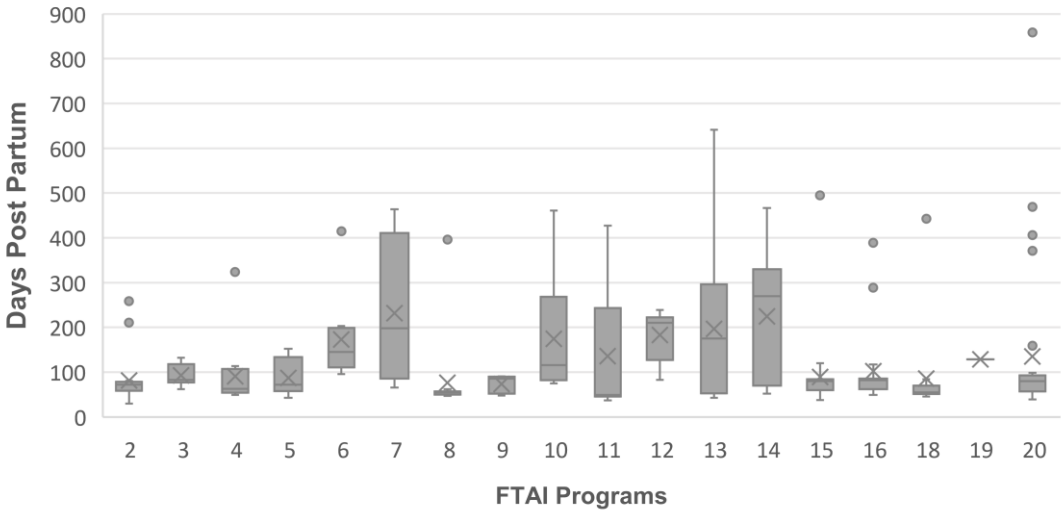


Figure 12 – Distribution of Postpartum interval in each FTAI Program

The effect of parity on the successful outcome - a positive pregnancy diagnosis - was analysed. No statistically significant associations were found between the variable under analysis and the pregnancy diagnosis (PD), so it was not possible to reject the null hypothesis that stated that the two variables are independent (Table 11). While it may seem that nulliparous females have lower pregnancy rates (Figure 13), it was not possible to conclude that the proportions are significantly different.

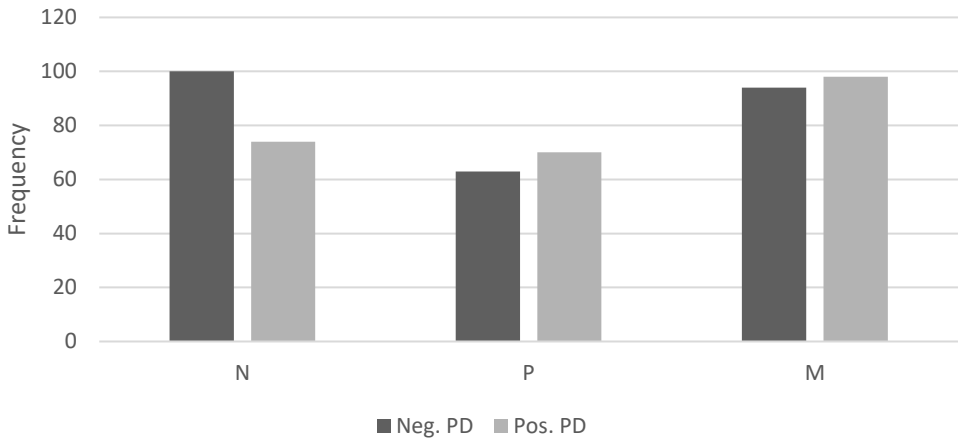


Figure 13 - Comparison of FTAI outcomes between parity categories

(M – Multiparous; N – Nulliparous; P- Primiparous)

Table 11 - Association between categorical FTAI variable Parity and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	n	%	n	%	n	%	
Parity							0.143
Nulliparous	100 a	57.5 (100/174)	74 a	42.5 (74/174)	174	34.9 (174/499)	
Primiparous	63 a	47.4 (63/133)	70 a	52.6 (70/133)	133	26.7 (133/499)	
Multiparous	94 a	49.0 (94/192)	98 a	51.0 (98/192)	192	38.5 (451/499)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level.

In the interest of understanding if there is an association between the postpartum interval and the PD, the statistical analysis started with a null hypothesis stating that means between two groups were not different. As means were comparable, (134 vs 117 days) and p=.160 the null hypothesis was not rejected, and it can be assumed that there is no association between the two variables (Table 12).

Table 12 - Association between quantitative FTAI variables – Postpartum interval - and PD: the independent samples t test results

	Negative PD			Positive PD			Total	Significance
	n	Mean	Std. Deviation	n	Mean	Std. Deviation	n	
Postpartum interval (days)	157	134.39	123.23	168	116.57	103.13	325	.160

4.1.2. Procedure related variables: synchronization protocol, semen deposition site, technician, and procedure verification score

Approximately 60% of protocols included a CIDR device, while the rest (40%) included a PRID. When analysing the device used in each synchronization protocol, the null hypothesis stated that there is no association with the pregnancy outcome, and, as it was not possible to reject this (p=.83), data shows that pregnancy rates between the two protocol categories do not differ significantly, as shown in Figure 14 and Table 13.

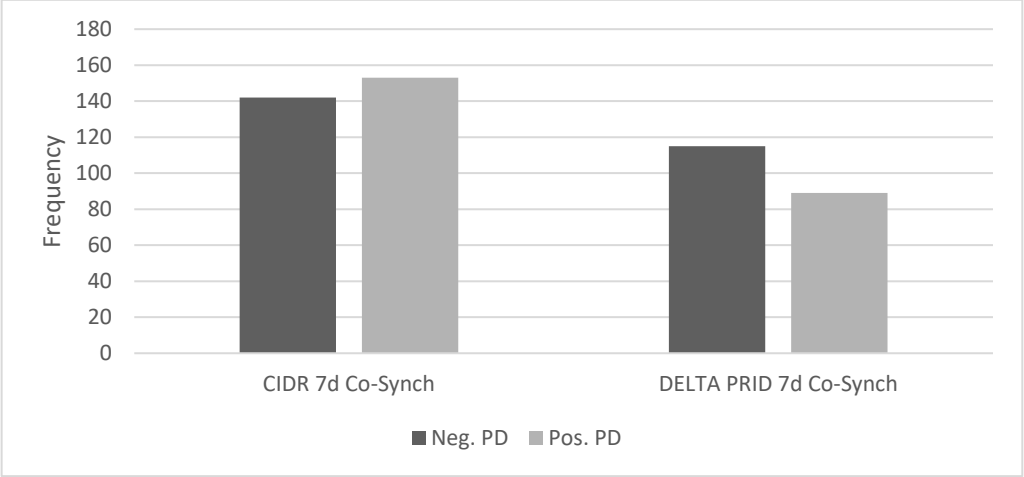


Figure 14 - Comparison of FTAI outcomes between protocol categories

Table 13 - Association between categorical FTAI variable - Protocol - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	n	%	n	%	n	%	
Protocol							.83
CIDR 7d Co-Synch	142[a]	48.1 (142/295)	153[a]	51.9 (153/295)	295	59.1 (295/499)	
DELTA PRID 7d Co-Synch	115[a]	56.4 (115/204)	89[a]	43.6 (89/204)	204	40.9 (204/499)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level.

Semen deposition site was classified as uterine body (n=416) cervical (n=5) and ipsilateral uterine horn (n=78), and in these cases, the side of the pre-ovulatory follicle was determined by transrectal palpation or ultrasonography. When looking at the impact of semen deposition site on the pregnancy outcome it was hypothesized that the two variables were

independent. Although it is noteworthy to mention that the vast majority of inseminations had semen deposited in the uterine body (83%), and a reduced number of inseminations had semen deposited in the cervix (1%), so the samples for each category vary heavily in size. Nevertheless, the statistical test showed that there is no significant difference, therefore not only it leads to the conclusion that the two variables are in fact independent but also that the pregnancy rates between uterine body, intra-uterine and cervical depositions site do not differ, even with high numerical differences (50% vs 20%), as shown in Table 14.

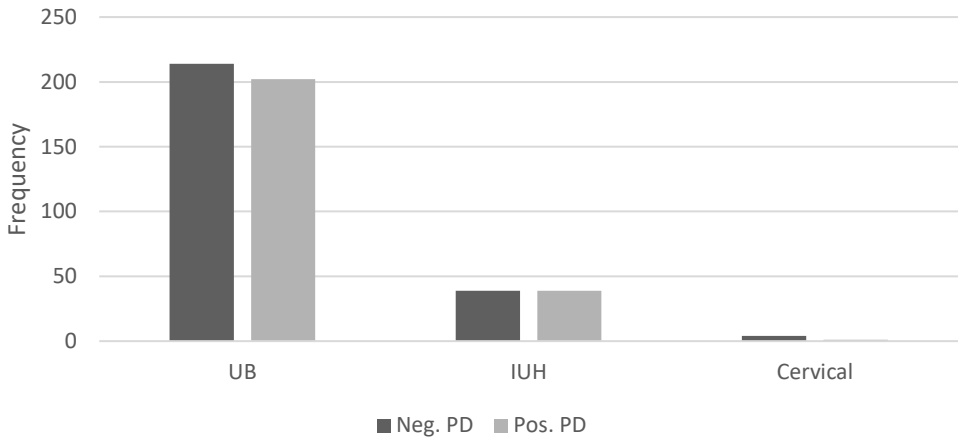


Figure 15 - Comparison of FTAI outcomes amongst semen deposition site categories

(UB – Uterine body; IUH – intra-uterine horn)

Table 14 - Association between categorical FTAI variable - Semen deposition site - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	n	%	n	%	n	%	
Semen deposition site							.490[b]
UB	214 [a]	51.4 (214/416)	202 [a]	48.6 (202/416)	416	83.4 (416/499)	
IUH	39 [a]	50.0 (39/78)	39 [a]	50.0 (39/78)	78	15.6 (78/499)	
Cervical	4 [a]	80.0 (4/5)	1 [a]	20.0 (1/5)	5	1.0 (5/499)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level; [b]- more than 20% of cells have expected counts less than 5

As expected in any production setting, the vast majority (96%) of procedures were carried through correctly, which meant that procedure verification scores were most often recorded as atraumatic (Table 15). However, even with this relevant difference in samples, the statistical testing was conducted based on the assumption that the procedure verification

score could not affect the pregnancy rates. With this disparity in mind, it was not possible to reject the null hypothesis, therefore the data show the two variables are independent. On the other hand, it was also possible to understand that pregnancy rates were similar between categories (49% vs 45%), as represented in the figure below (Figure16).

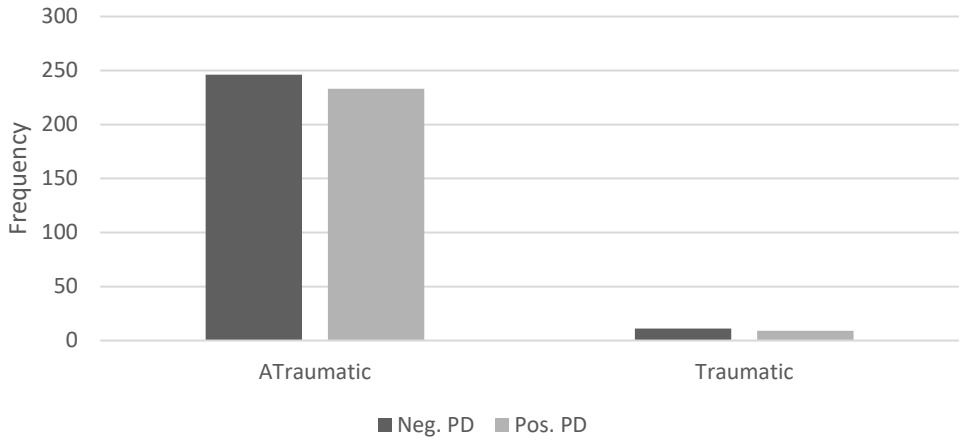


Figure 16 – Comparison of FTAI outcomes between procedure verification score categories

Table 15 - Association between categorical FTAI variable - Procedure verification score - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	n	%	n	%	n	%	
Procedure verification score							.822
Atraumatic	246 a	51.4 (246/479)	233 a	48.6 (233/479)	479	96.0 (479/499)	
Traumatic	11 a	55.0 (11/20)	9 a	45.0 (9/20)	20	4.0 (20/499)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level.

The vast majority of inseminations were done by two technicians: B (76%), followed by technician A (19%); and finally C, the third one (5%). In order to identify if there is an association between the technician that performs the AI procedure and the successful outcome, statistical testing was conducted based on the null hypothesis that there is no association between the two variables, and that pregnancy rates do not differ between technicians. As seen in Figure 17 and Table 16, although there is a difference in sample size for each technician, the results (p=.444) show that pregnancy rates tend to be similar (54%, vs 47% vs 44%) and that there is indication that the two variables are independent.

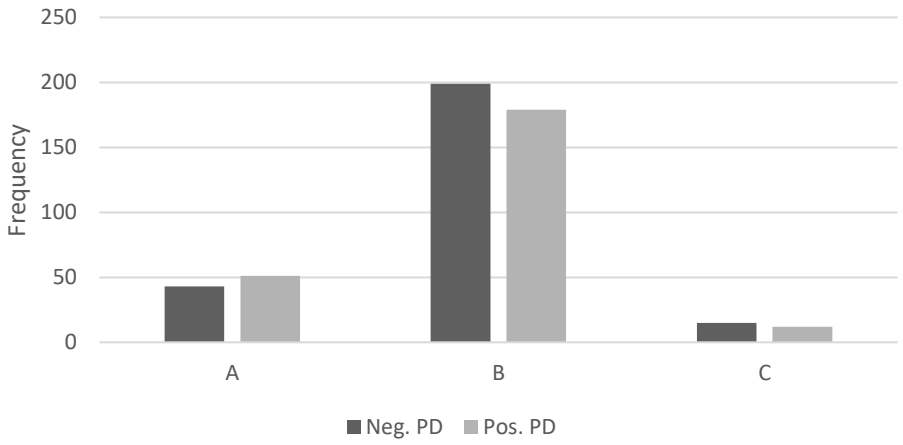


Figure 17 - Comparison of FTAI outcomes between technicians performing AI

Table 16 - Association between categorical FTAI variable - Technician - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	n	%	n	%	n	%	
Technician							.444
A	43 a	45.7 (43/94)	51 a	54.3 a (51/94)	94	18.8 (94/499)	
B	199 a	52.6 (199/378)	179 a	47.4 a (179/378)	378	75.8 (378/499)	
C	15 a	55.6 (15/27)	12 a	44.4 a (12/27)	27	5.4 (27/499)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level.

4.1.3. Logistic Regression

The independent variables did not prove to be significant predictors of a positive pregnancy diagnosis ($p > .05$).

As seen in Table 16, the likelihood ratio difference test (omnibus tests of model coefficients; $p = .208$) indicates that the difference between the constant-only equation and the equation with added explanatory variables is not statistically significant. Therefore, the equation with added explanatory variables does not offer an improvement over the baseline equation. According to Nagel-Kerke's coefficient of determination, 4.9% of the total variation of the dependent variable (pregnancy diagnosis) can be explained by independent variables in the current equation, and the value of the Hosmer and Lemeshow goodness of fit test is $\chi 2 (8) = 7.675$, $p = 0.466$, showing an adequate fit to the data.

Table 17 - Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	12.099	9	.208
	Block	12.099	9	.208
	Model	12.099	9	.208

Table 18 - Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	438.075	.037	.049

Table 19 - Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	7.675	8	.466

Table 20 - Variables in Equation

	B	Sig.	Exp(B)	95% C.I. for EXP(B)	
				Lower	Upper
Synchronization protocol	-0.239	0.382	0.787	0.460	1.347
Semen deposition site		0.721			
Intra-uterine Horn	0.323	0.419	1.382	0.631	3.026
Cervical	19.260	1.000	231374589.056	0.000	
Procedure verification – Traumatic	1.727	0.133	5.625	0.590	53.644
Technician		0.790			
Technician B	-0.241	0.498	0.786	0.392	1.577
Technician C	-0.254	0.668	0.776	0.244	2.470
Age	-0.186	0.068	0.830	0.679	1.014
Days postpartum	-0.001	0.424	0.999	0.997	1.001
Parity	-0.076	0.803	0.927	0.510	1.683
Constant	1.142	0.040	3.133		

Categories of variables not shown were considered redundant or linearly dependent upon preceding variables and thus excluded by the statistics software.

4.2. Multiple Ovulation

A total of twenty-four females were submitted to multiple ovulation protocols. Of these, twenty were nulliparous, two were primiparous and two were multiparous (Figure 18). Age of females ranged from approximately one to eight years (Figure 19), and the number of births ranged from zero to six; all cows were more than 55 days postpartum (Table 21).

Statistics regarding MO will be predominantly descriptive, as the small sample size may lack the ability to detect true effects or associations in the data variability of the sample and may not accurately reflect the variability in the population, and the only measurable success outcome would be the retrieval of viable embryos, specifically grade 1 embryos. Of the 27 SOV protocols, 3 (11.1%) had negative SOV response, however, only 55.5% (15/27) of embryo recovery resulted in the retrieval of one or more viable embryo, as represented in Figure 20.

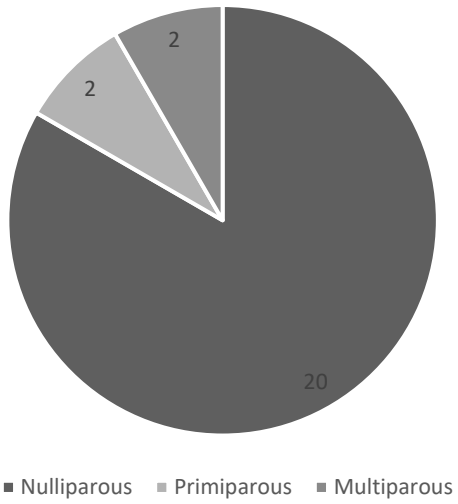


Figure 18 - Parity status at SOV protocol

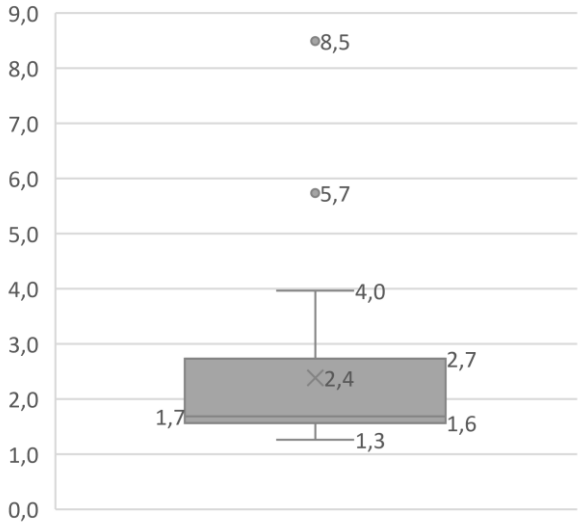


Figure 19 - Age of females at SOV Protocol (in years)

Table 21 - Descriptive Statistics on Age at MO, Number of calvings and Days Postpartum

	Minimum	Maximum	Mean	Std. Deviation
Age (n=27)	1.25	8.42	2.3407	1.61165
Parity (n=27)	0	6	0.41	1.279
Days postpartum (n=4)	55	215	138.00	87.94695

Semen from 13 different bulls was used, with the majority of inseminations (63%; 17/27) utilizing semen from 4 bulls (K, A, C and F), as shown in Table 22. Most SOV protocols used insemination with two straws of semen (n=16), but some used 3 (n=7) or 4 straws (n=4).

Table 22 - Frequency of use of each bull's semen

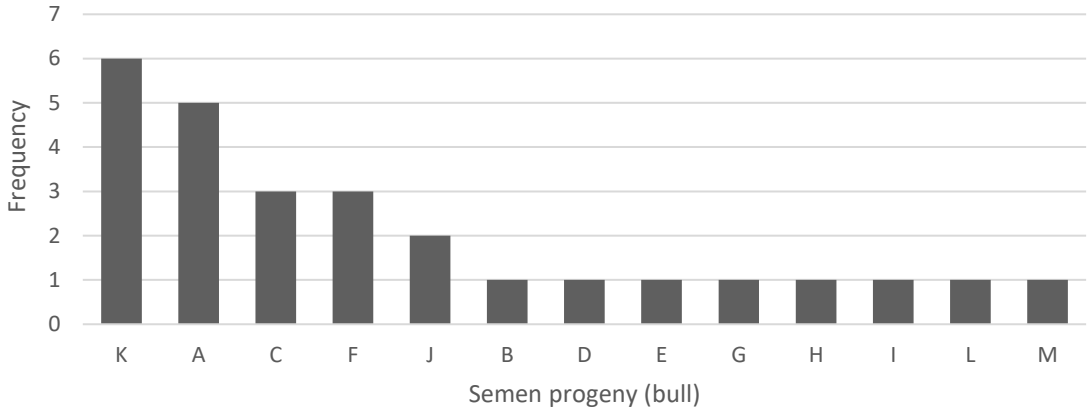


Table 23 - Descriptive statistics: ovarian response to superovulation

	Minimum	Maximum	Mean	Std. Deviation
Ova+ embryos	0	32	9.41	8.599
Viable embryos	0	29	4.81	6.516
Quality Code 1	0	24	4.48	5.733
Quality Codes 2 & 3	0	5	0.33	1.074
Degenerate embryos	0	3	0.44	0.934
Unfertilized ova	0	23	4.04	5.660

A total of 51.2% (130/254) of viable embryos was obtained (4.8 ± 6.52/flushing).

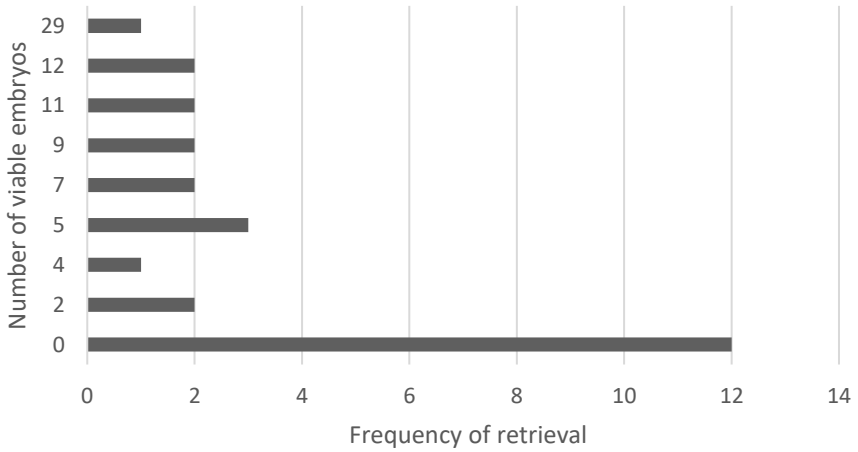


Figure 20 - Distribution of number of viable embryos retrieved

4.3. ET

Pregnancy rate (number of animals that had positive pregnancy diagnosis divided by number of embryos transferred) was 54.7% (58/106). Pregnancy rates for heifers and cows was calculated at 59.1% (52/88) and 33.3% (6/18), respectively. Differences were considered significant (p=.045).

Variables described below were grouped in 3 sections (recipient related variables, embryo related variables and procedure related variables) as the effect of a number of different variables on a successful outcome - a positive pregnancy diagnosis - was analysed. These included: age, number of calvings, days postpartum, embryo fertilization method (*in vivo* or *in vitro*), embryo stage (compact morula, early blastocyst, blastocyst and expanded blastocyst), embryo quality (quality code 1: excellent or good, code 2: fair, code 3: poor), donor-recipient synchrony, CL quality (code 1- compact CL or code 2- cavitary CL), ET procedure verification (good embryo placement, good embryo placement with blood stain, reasonable embryo placement, and reasonable embryo placement with blood stain) and the technician.

4.3.1. Recipient related variables - Age at ET, Parity, days postpartum, and recipient CL quality

Recipient ages ranged from 1.3 to 5.6 years, but most recipients 54.7% (58/106) were heifers under 2 years old, as shown in Table 24 and Figure 21.

Table 24 - Descriptive Statistics on Age at ET

	Age at ET in years n=106
Mean	2.31
Median	2.00
Minimum	1.25
Maximum	5.67
Standard Dev.	0.98

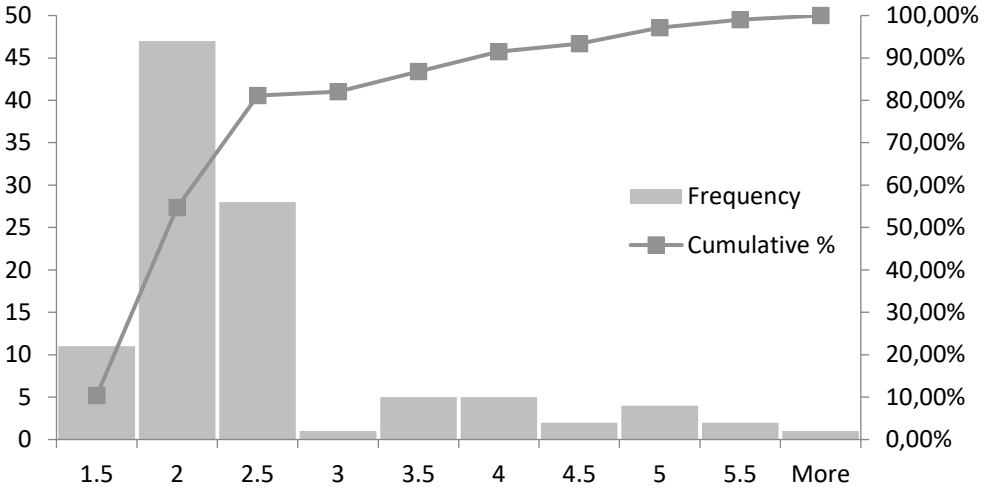


Figure 21- Age (in years) of recipient females at embryo transfer

In order to test if there is an association between age and pregnancy diagnosis, independent samples t test was used, where the null hypothesis stated that means between two groups are not different. As means were very similar, (2.5 vs 2.2) and p=.113 the null hypothesis was not rejected (Table 25).

Table 25 - Association between quantitative ET variable Age and PD: the independent samples t test results

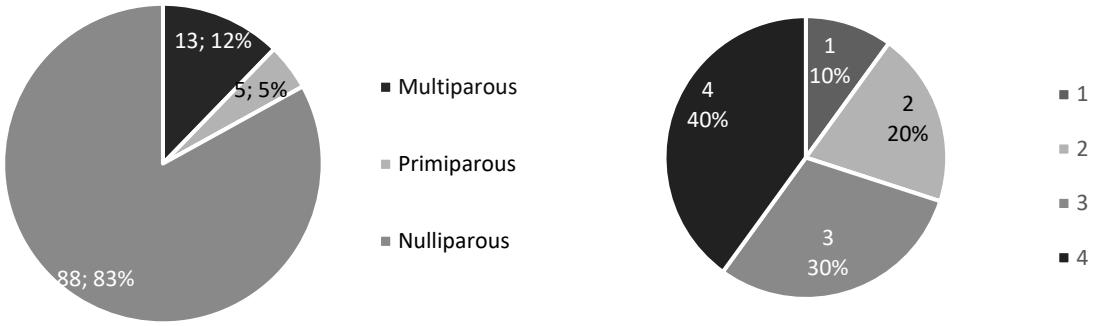
	Negative PD			Positive PD			Total	Significance
	n	Mean	Std. Deviation	n	Mean	Std. Deviation	n	
Age (recipient)	48	2.49	1.15	58	2.18	0.81	106	.113

As stated before, the majority of recipient females were heifers, but parity is further illustrated in Table 26 and Figure 22.

Table 26 - Descriptive Statistics on Parity and Postpartum interval at ET

	Parity	Postpartum Interval (days)
	n=106	n=18
Mean	0.37	149.61
Median	0.00	115.00
Minimum	0.00	66.00
Maximum	4.00	365.00
Standard Dev.	0.91	77.30

Figure 22 - Parity and number of calvings of the recipients at embryo transfer



When looking at the pregnancy rates for each parity category, it was necessary to reject the null hypothesis that stated that parity was not associated with the PD. There are significant differences between proportions, as data shows there is significantly higher pregnancy rate in nulliparous females (59% vs. 46% vs. 0%). However, it is important to safeguard that the sample of the primiparous category is very small, which could influence the results. Pregnancy rates stated in the beginning of this chapter and in the discussion chapter refer to pregnancy rates comparison between heifers and cows, however still statistically significant, they allow for the increase of the sample size by joining primiparous and multiparous females.

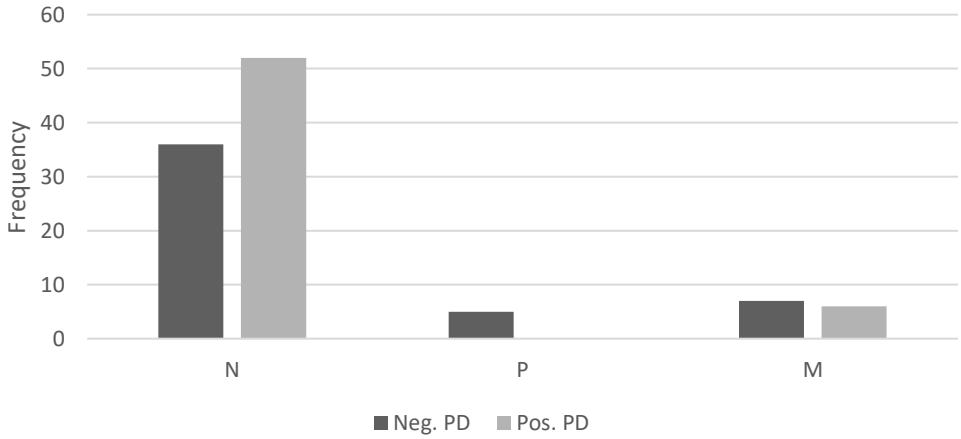


Figure 23 – Comparison of ET outcomes between parity categories

Table 27 - Association between ET variables and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Recipient Parity							.021[b]
Nulliparous	36_a	40.9 (36/88)	52_a	59.1 (52/88)	88	83.0 (88/106)	
Primiparous	5_b	100.0 (5/5)	0_b	0.0 (0/5)	5	4.7 (5/106)	
Multiparous	$7_{a,b}$	53.8 (7/13)	$6_{a,b}$	46.1 (6/13)	13	12.3 (13/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level; b- more than 20% of cells have expected counts less than 5

According to the results of the independent samples t-test, data showed the null hypothesis stating that the means between the negative and the positive PD groups were the same had to be rejected (Table 29), as there is a significant difference in the mean of days postpartum in procedures that resulted in positive PD (M=106.50, SD= 12.42) and negative PD (M=171.17, SD=87.44; t(11.863)=2.512, *p*=.028). The average postpartum period for procedures that resulted in positive PD is approximately 65 days shorter (mean difference=64.667) than the postpartum period for procedures that resulted in negative PD. This difference might have been caused by underlying issues, which prevented these females to be selected as adequate candidates for embryo transfer in the first place. It is also important to note that the values registered for this variable vary drastically between 66 and 365 days (Table 28), which is in line with what is suggested above.

Table 28 - Descriptive statistics on postpartum interval at ET

	Postpartum Interval (days)
	n=18
Mean	149.61
Median	115.00
Minimum	66.00
Maximum	365.00
Standard Dev.	77.30

Table 29 - Association between quantitative ET variables and PD: the independent samples t test results

	Negative PD			Positive PD			Total	Significance
	n	Mean	Std. Deviation	n	Mean	Std. Deviation	n	
Postpartum interval (recipient)	12	171.17	87.44	6	106.50	12.42	18	.028

The majority of transfers were performed on recipients whose CL quality was 1 (74%). Nonetheless, when assessing if the recipient CL quality affects the ET outcome, the null hypothesis stated there was no association between the two variables. However, statistical results forced the rejection of this hypothesis. There is a significantly higher proportion of positive pregnancy diagnoses in procedures where recipients had CL quality 1 (62% vs 36%), (χ^2 (1) = 5.546 p = .026, Fisher's Exact test p =.026) as shown in Table 30 and pictured in Figure 24.

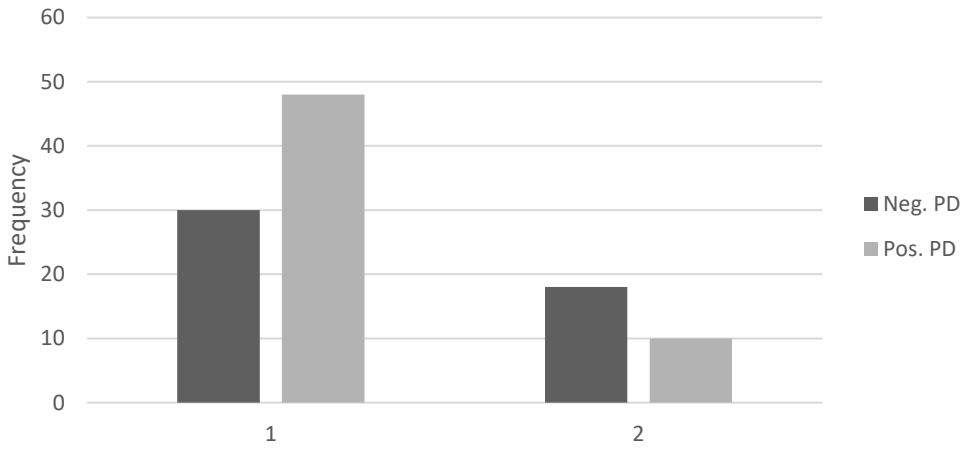


Figure 24 – Comparison of ET outcomes between Recipient CL quality categories

Table 30 - Association between ET variables – Recipient CL quality - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
CL Quality							.026
1	30 a	38.5 (30/78)	48 a	61.5 (48/78)	78	73.6 (78/106)	
2	18 b	64.3 (18/28)	10 b	35.7 (10/28)	28	26.4 (28/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level.

4.3.2. Embryo related factors – Type of fertilization Stage code, quality code, embryo age, and donor-recipient synchrony

The majority of embryos used were fertilized *in vivo* (Figure 25). Even though there could be the inclination to think that pregnancy rates were different between the two categories (39% vs 59%), they did not differ statistically (Table 31), therefore, it was possible to conclude that data shows there is no association between fertilization type and the pregnancy diagnosis (p=.103).

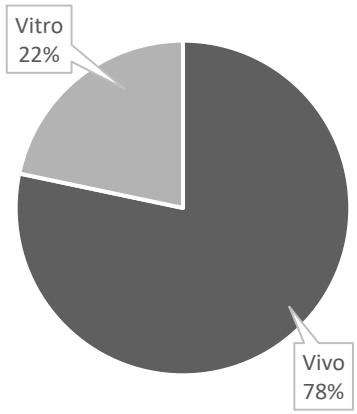


Figure 25 - Type of fertilization of embryos used ET

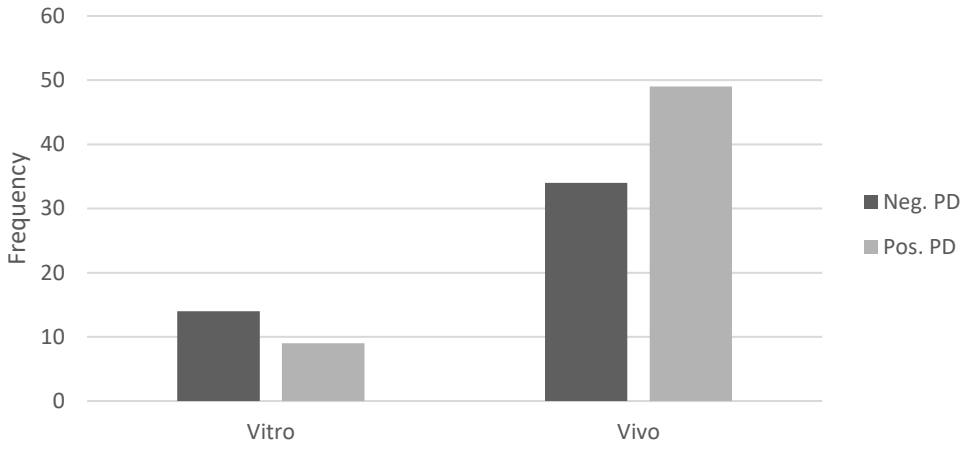


Figure 26 - Comparison of ET outcomes between embryo fertilization categories

Table 31 - Association between ET variables - Type of fertilization- and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Fertilized							.103
In Vitro	14 a	60.9 (14/23)	9 a	39.1 (9/23)	23	21.7 (23/106)	

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
In Vivo	34 a	41.0 (34/83)	49 a	59.0 (49/83)	83	78.3 (83/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level

The majority of embryos transfers were classified as compact morulas (46%) and early blastocysts (3%). Regarding the embryo stage code and its impact on the PD, it is also not possible to reject the null hypothesis that these two variables are independent (p=.715), and therefore, it is also not true that pregnancy rates differ between subcategories, as shown in the figures bellow (Figure 28, Table 32).

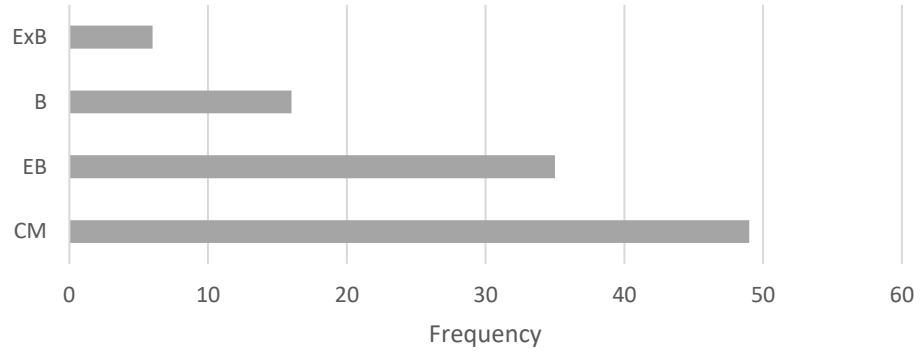


Figure 27- Stage code of transferred embryos

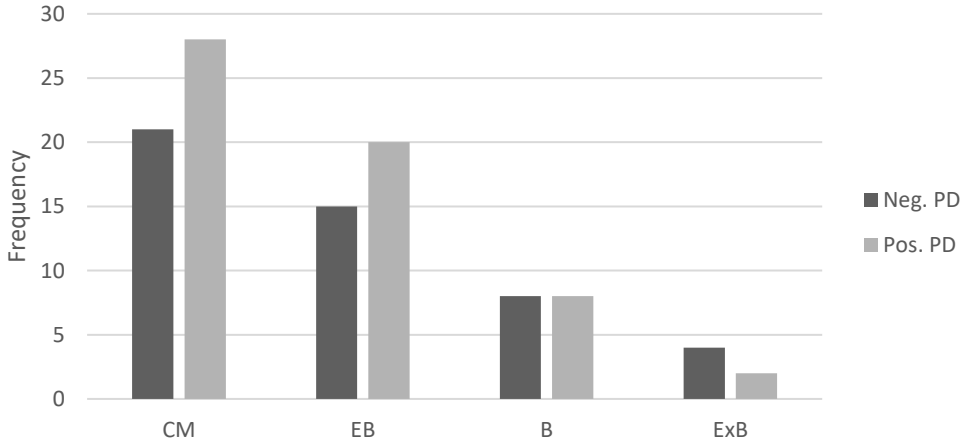


Figure 28- Comparison of ET outcomes between embryo stage categories

Table 32 - Association between ET variables - Embryo Stage code - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Embryo stage code							.715
CM	21 a	42.9 (21/49)	28 a	57.1 (29/49)	49	46.2 (49/106)	
EB	15 a	42.9 (15/35)	20 a	57.21 (20/35)	35	33.0 (35/106)	
B	8 a	50.0 (8/16)	8 a	50.0 (8/16)	16	15.1 (16/106)	
ExB	4 a	66.6 (4/6)	2 a	33.3 (2/6)	6	5.7 (6/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level; b- more than 20% of cells have expected counts less than 5

Embryo quality code was another variable that data showed not to be associated with the PD, and even if there could be the drift towards considering, the pregnancy rates different (59% - code 1, vs 33% - code 2 vs 20% - code 3). The statistical analysis proved that the difference is not statistically significant, therefore it is not possible to reject the null hypothesis that states the two variables are independent (Table 33).

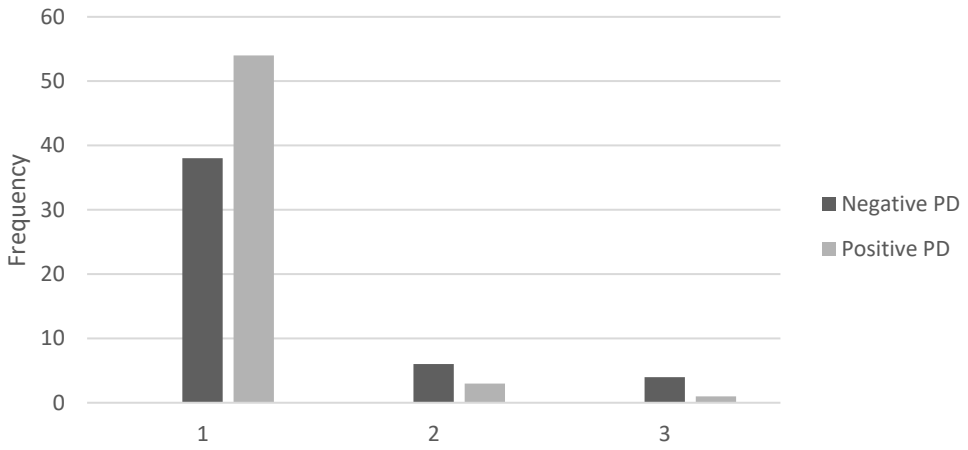


Figure 29 - Comparison of ET outcomes between embryo quality code categories

Table 33 - Association between ET variables - Embryo quality code - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Embryo quality code							.119[b]
1	38 [a]	41.3 (38/92)	54 [a]	58.7 (54/92)	92	86.8 (92/106)	
2	6 [a]	66.7 (6/9)	3 [a]	33.3 (3/9)	9	8.5 (9/106)	
3	4 [a]	80.0 (4/48)	1 [a]	20.0 (1/5)	5	4.7 (5/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level; [b]- more than 20% of cells have expected counts less than 5

However, embryonic age does seem to be associated with the PD, as there is a significantly higher proportion of positive pregnancy diagnoses in procedures where embryonic age was 7 compared to 6.5 and 7.5 days (64.6 % vs 43.8% and 36.0%, respectively) (χ^2 (3) = 6.882 p = .031, Fisher's Exact test p =.035).

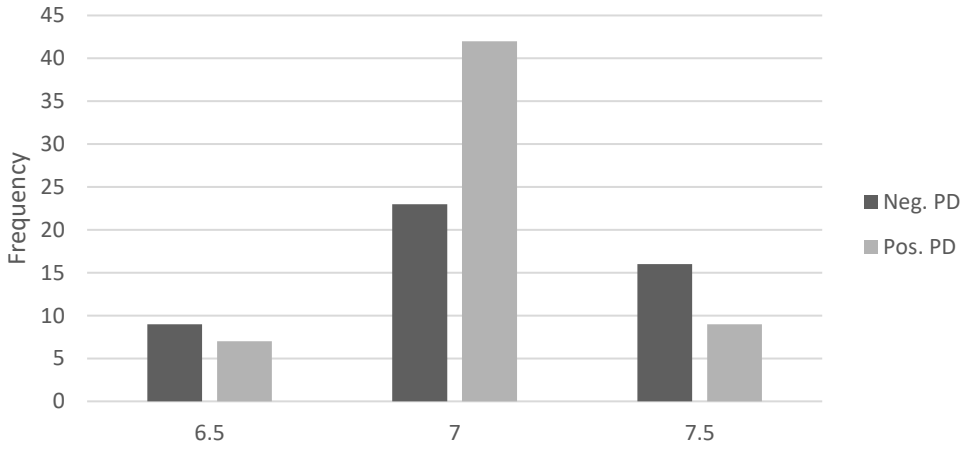


Figure 30 - Comparison of ET outcomes between embryonic age categories

Table 34 - Association between ET variables - embryonic age - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Embryonic age							.035
6.5 days	9 [a]	56.3 (9/16)	7 [a]	43.8 (7/16)	16	15.1 (16/106)	
7 days	23 [b]	35.4 (23/65)	42 [b]	64.6 (42/65)	65	61.3 (65/106)	
7.5 days	16 [a,b]	64.0 (16/25)	9 [a,b]	36.0 (9/25)	25	23.6 (25/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level.

The majority of transfers (67%) were registered as having 0 hours synchrony with the recipient female. The statistical analysis did not allow for the rejection of the null hypothesis, so it was not possible to conclude that embryo recipient-synchrony affects the desirable outcome.

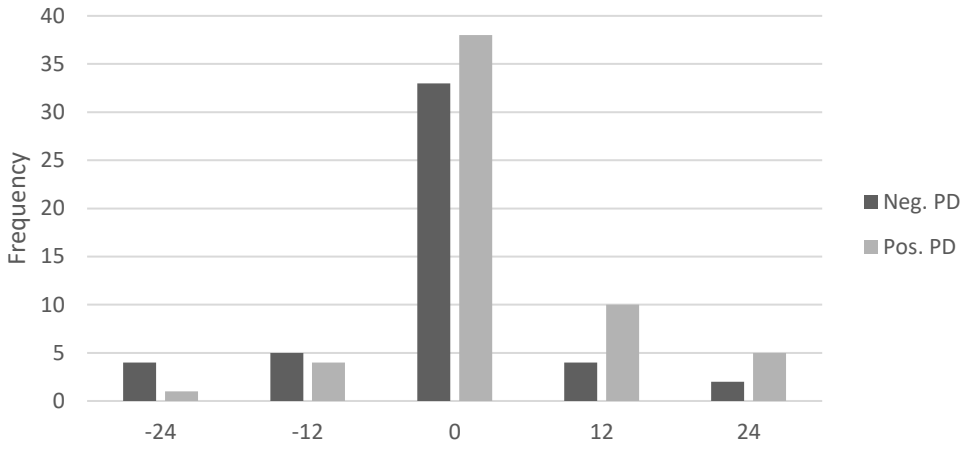


Figure 31 - Comparison of ET outcomes between donor- recipient synchrony categories

Table 35 - Association between ET variables – donor-recipient synchrony- and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Donor- Recipient Synchrony							.283[b]
-24h	4 [a]	80.0 (4/5)	1 [a]	20.0 (1/58)	5	4.7 (5/106)	
-12h	5 [a]	55.6 (5/9)	4 [a]	44.4 (4/9)	9	8.5 (9/106)	
0h	33 [a]	46.5 (33/71)	38 [a]	53.5 (38/71)	71	67.0 (71/106)	
+12h	4 [a]	28.6 (4/14)	10[a]	71.4 (10/14)	14	13.2 (14/106)	
+24h	2 [a]	28.6 (2/7)	5 [a]	71.4 (5/7)	7	6.6 (7/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level; b- more than 20% of cells have expected counts less than 5

4.3.3. Procedure related variables – Embryo transfer score and technician

The procedure was evaluated and recorded as having “good embryo placement” (n=78), “good embryo placement with blood stain” (n=17), “reasonable embryo placement” (n=5), and “reasonable embryo placement with blood stain” (n=6).

There is a significantly higher proportion of positive pregnancy diagnoses in procedures where there was no degree of trauma to the tissues GEP and REP (63% and 60%, respectively) and significantly higher negative pregnancy outcomes from procedures where embryo placement resulted in bloodshed GEP BS and REP BS (71% and 83%, respectively) (χ^2 (3) = 10.023 *p* = .013, Fisher's Exact test *p* =.013).

Figure 32 - Duration of ET procedure

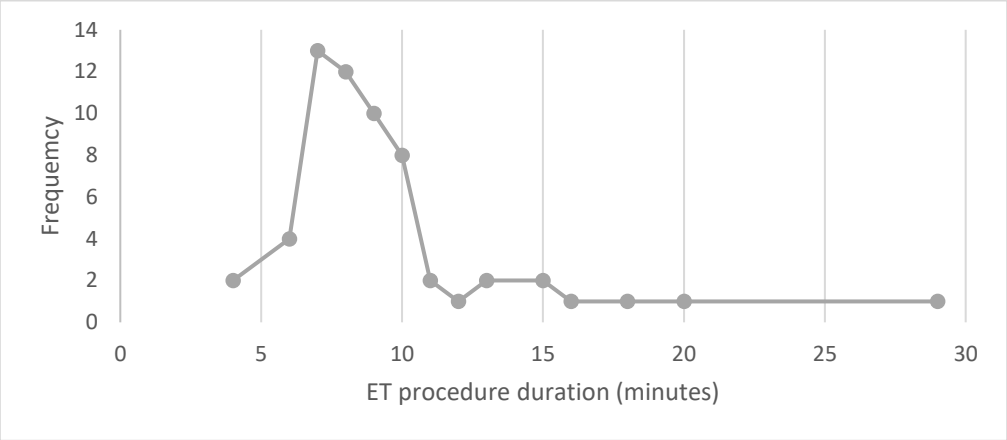
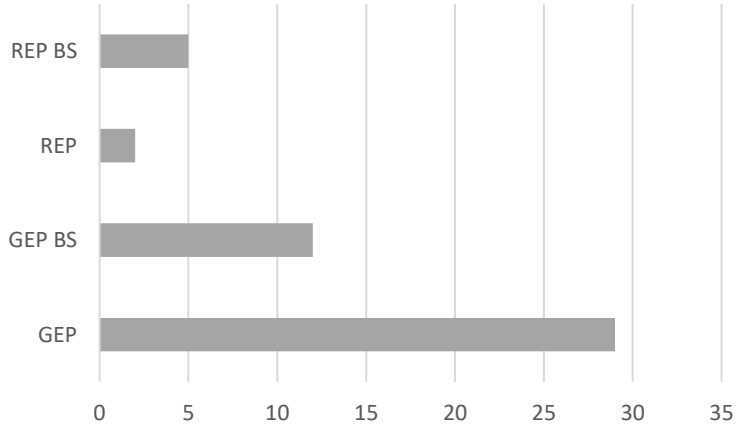


Figure 33 - ET procedure quality check



GEP: Good Embryo Placement; GEP BS: Good Embryo Placement with blood stain, REP: Reasonable embryo placement, REP BS: reasonable embryo placement with blood stain

Table 36 - Association between ET variables – ET procedure score - and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
ET procedure score							.013[b]
GEP	29[a]	37.2 (29/78)	49[a]	62.8 (49/78)	78	73.6 (78/106)	
GEP BS	12[b]	70.6 (12/17)	5[b]	29.4 (5/17)	17	16.0 (17/106)	
REP	2[a,b]	40.0 (2/5)	3[a,b]	60.0 (3/5)	5	4.7 (5/106)	
REP BS	5[b]	83.3 (5/6)	1[b]	16.7 (1/6)	6	5.7 (6/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level; b- more than 20% of cells have expected counts less than 5

Transfers were done by technicians A (n=83) and B (n=23). Pregnancy rates for technician A and B were calculated at 57.8% (48/83) and 43.5% (10/23), respectively. And although proportions shown in the Figure 34 might look like they indicate that there could be more negative PD for technician B and more positive PD for technician A, differences were not considered significant (p=.245), and the null hypothesis stating that there is no association between variables could not be rejected (Table 37).

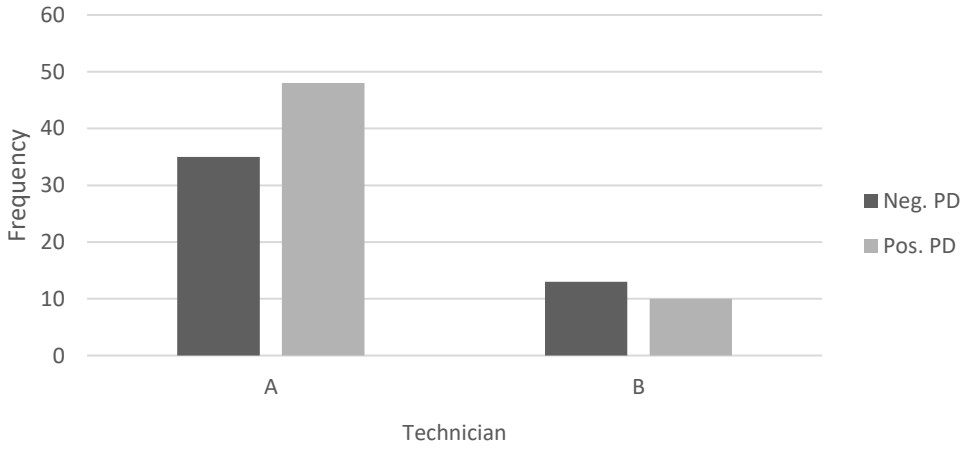


Figure 34 - Comparison of ET outcomes between technicians A and B

Table 37 - Association between ET variables – technician- and PD: the Chi-square test results

	Negative PD		Positive PD		Total		Significance
	N	%	N	%	N	%	
Technician							.245
A	35 a	42.2 (35/83)	48 a	57.8 (48/83)	83 a	78.3 (83/106)	
B	13 a	56.5 (13/23)	10 a	43.5 (10/23)	23 a0	21.7 (23/106)	

Within each column, proportions that do not share a subscript differ significantly from each other at the .05 level;

4.3.4. Logistic Regression

The likelihood ratio difference test (omnibus tests of model coefficients; p = .005) indicates that the difference between the constant-only equation and the equation with added explanatory variables is statistically significant; therefore, the equation with added explanatory variables offers an improvement over the baseline equation. According to Nagel-Kerke's coefficient of determination, 42.3% of the total variation of the dependent variable (pregnancy diagnosis) can be explained by independent variables in the current equation, and the value of the Hosmer and Lemeshow goodness of fit test is $\chi 2$ (8) = 10.071, p = .185, showing an adequate fit to the data. The independent variables did not prove to be significant predictors of a positive pregnancy diagnosis (p > .05).

Table 38 - Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	40.327	20	.005
	Block	40.327	20	.005
	Model	40.327	20	.005

Table 39 - Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	105.675	.316	.423

Table 40 - Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	10.071	7	.185

Table 41 - Variables in Equation

	B	Sig.	Exp(B)	95% C.I. for EXP(B)	
				Lower	Upper
Age	-0.056	0.928	0.945	0.277	3.229
Parity		0.885			
Primiparous	-21.458	0.999	0.000	0.000	
Multiparous	-0.852	0.621	0.427	0.015	12.465
Fertilized in vivo	-21.536	0.999	0.000	0.000	
Embryonic stage code		0.994			
EB	-0.014	0.985	0.986	0.235	4.132
B	0.129	0.896	1.138	0.165	7.867
ExB	0.315	0.818	1.370	0.093	20.083
Embryonic quality code		0.153			
2	-1.284	0.212	0.277	0.037	2.075
3	-3.525	0.070	0.029	0.001	1.330
Embryonic age		0.910			
7 days	0.451	0.664	1.571	0.204	12.069
8 days	-22.381	0.999	0.000	0.000	
Donor – Recipient Synchrony		0.789			
-12 h	0.083	0.962	1.086	0.035	33.445
0h	0.773	0.605	2.166	0.116	40.578
+12 h	0.932	0.577	2.539	0.096	67.392
> +12h	1.881	0.332	6.557	0.147	292.898
CL quality 2	-0.995	0.096	0.370	0.115	1.191
ET procedure quality		0.111			
REP BS	-0.866	0.565	0.421	0.022	8.022
REP	1.823	0.306	6.192	0.188	203.439
GEP	1.280	0.090	3.596	0.819	15.797
Technician B	0.543	0.673	1.722	0.139	21.407
Constant	20.712	0.999	988308806.449		

Categories of variables not shown were considered redundant or linearly dependent upon preceding variables and thus excluded by the statistics software.

5. Discussion

The current chapter serves the purpose of interpreting the obtained results and exploring their relevance. Evidence will be discussed following the same sequence of results, so, aspects relating to FTAI will be exposed firstly, followed by the discussion of results pertaining to MO and lastly by ET.

Overall and specific group (nulliparous, primiparous, and multiparous) pregnancy rates after FTAI reported in the current study (42.5%, 52.6% and 51.07%, respectively) are in line with the ones Fontes et al. (2020) reported in herds that had been exposed to 7-d CO-Synch+CIDR oestrus synchronization protocols followed by FTAI 60-66h after CIDR removal, between 44.4% and 65.8%. However, lower than what Bridges et al. (2008) obtained, between 56% and 67%, following the same protocol. This indicates there is space for improvement to bring pregnancy rates closer to the upper limits of the ranges reported. The answer might lie in specific situations related to the productive management of the herds or environmental aspects, and, for this reason, it is important to look at specific pregnancy rates for each FTAI program, in order to evaluate if any specific situations or factors could have led to suboptimal results, and, if possible, address those issues in the future programs. The calculated intrauterine mortality rate (5%) is in line with what is expected as abortion rates have been reported to vary between 0.5 and 10% (Mee 2020).

Age at FTAI was analysed based on the premise that the age of the oocyte donor plays a crucial role in determining the developmental competence of the oocyte, and therefore might show a relevant effect on the pregnancy rates. As stated by Armstrong (2001) several age-related abnormalities may affect oocytes, such as: a) meiotic incompetence, where oocytes cannot complete maturation and thus cannot be fertilized; b) meiotic errors that, while compatible with fertilization, result in genetic abnormalities compromising embryo viability; and c) deficiencies in cytoplasm, which can affect development stages before or after fertilization. However, the available data did not lead to a conclusion that supports this premise. This could be impacted by the fact that the herd is correctly managed, so on the one hand older cows that have a history of reproductive failure are culled, and heifers that are not yet ready to be reproduced are not included in these programs.

Contrary to the findings of this study, Bridges et al. (2012) reported that FTAI pregnancy rates in suckled beef cows after synchronization were impacted by parity ($P< 0.01$), and that breeding season pregnancy rates were greater ($P< 0.01$) for multiparous (1,730/1,913 = 90%) than for primiparous (404/520 = 78%) cows and influenced by the days postpartum, and that FTAI pregnancy rates increased ($P< 0.01$) by 4.4±0.7% for each 10-d increase in days postpartum (range 13 to 133 d) at the initiation of treatments. It is known that several factors may influence postpartum interval, including suckling, nutrition, age, dystocia, genetic

variation, stress, and disease(Perry and Cushman 2013). Previous studies have shown that cows that are less than 50 days postpartum at the beginning of the breeding season have significantly lower pregnancy rates when compared to cows that are further ahead (Fontes et al. 2020). A study by Stevenson et al. (2015) involving more than 8,500 postpartum suckled beef cows, revealed that multiparous and primiparous cows have reduced pregnancy rates when they are less than 72 days postpartum. A review of several recent studies has shown that cyclic activity increased curvilinearly from 9% at 30 days or less, to a peak of 70% at 81 to 90 days postpartum (Lamb and Mercadante 2016; Mura et al. 2019). Therefore, the resulting median number of days postpartum (81) represented in Table 10 is in line with the available evidence; however, it must be noted that the minimum value reported for the days postpartum (11 days) is drastically lower than the recommended > 50 days. We believe this number may have resulted from a technical error in the field at the time of the reproductive tract examination. On the other hand, long postpartum periods may indicate either an underlying issue or the failure to detect animals that have not bred in due time. Some of these animals might have been separated to be included in embryo retrieval protocols and therefore were not included in FTAI programs, at the right time. Clique ou toque aqui para introduzir texto.Fontes et al. (2020) stated that when they compared herds with higher pregnancy rates (>50% vs. <50%), the average number of days postpartum at the beginning of the breeding season for cows in the highest and lowest performing herds was 79 days and 64 days, respectively. Additionally, only 7% of the cows in the first group were less than 50 days postpartum at the beginning of the breeding season, whereas in the lowest group 43% of cows were less than 50 days postpartum. Moreover, the vast majority of the cows (88%) in the highest performing herds calved within the first 30 days of the calving season, compared to the less fertile herds where only 44% of cows calved within the first 30 days of the breeding season. These results reinforce the significance of having cows to calve early in the breeding season, as this also influences results during succeeding breeding seasons.

Although not observed in the current study, it could be expected that the type of device used in the synchronization protocol would have a significant impact on the outcome, as reported by van Werven et al. (2013), where a study in lactating Holstein cows found that the PRID-Delta produced more circulating P_4 compared to the CIDR, and pregnancy per AI (P/AI) after the 1st AI in cows treated with PRID-Delta was significantly higher than in cows treated with CIDR during the synchronization protocol.

Association between the procedure verification and the technician that performed the AI, and the pregnancy outcome was tested based on the premise that correct technique is essential for the success of the FTAI program; the results obtained state there is no association between the variables and that pregnancy rates between technicians are not significantly different from what was expected, since all three technicians are expertly trained.

This might indicate that, as long as the technician is effectively taught how to employ the insemination technique and makes sure to follow correct procedures every time, pregnancy rates should not be affected by this variable.

Conversely, association between the procedure score and the pregnancy outcome was experiment based on the premise that a degree of trauma to the tissues could influence the uterine environment and makes it trickier for fertilization to occur. Although this study did not find a significant difference between the pregnancy rates between each procedure verification category, it is believed that if there were a higher proportion of traumatic AIs, as opposed to only 4%, there would be a significant difference. Overall, this validates the assumption above regarding the adequate performance of the technicians and gives a clear indication that the animals were handled under the best conditions, minimizing any movements that often result in traumatic procedures.

Additionally, it has been shown that deposition of semen into the uterine body results in higher fertility than deposition into the cervix, and that uterine body and intracornual insemination ipsilateral to the side of impending ovulation yield no significant differences in the pregnancy rates, when compared (Lopez-Gatius 2000). It was not possible to verify the first premise regarding higher pregnancy rates when comparing deposition sites, so the results achieved in this study are partly in line with the applicable evidence. This difference might be explained by the fact that only 1% (5/499) of inseminations were registered as having been cervical deposition.

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The goal of superovulation in cattle is to optimize the production of high-quality embryos that have a high likelihood of resulting in successful pregnancies.(Jahnke and Youngs 2021)

Potentially more significant factors that affect the superovulatory response are intrinsic to the animal and its surroundings. These factors encompass the season of the year, as well as the nutritional status, reproductive history, age, breed, previous superstimulation, and ovarian condition of the donor at the start of treatment. Despite improvements in our comprehension of bovine reproductive physiology, the factors specific to the donor animal that impact the response to superovulation remain poorly understood. (Jahnke and Youngs 2021)Clique ou toque aqui para introduzir texto.

Although age seems to have very unimportant effects on the SOV response and number of embryos recovered, there seems to be a declining interest in producing embryos from old donors due to high pressure in genetic selection process. This ultimately makes the age of the animal that will be subjected to SOV an irrelevant issue. However, some available data points towards a tendency for heifers to produce fewer transferable embryos than cows (Mikkola et al. 2019)

The sire has been shown to be an important variable factor in successful fertilization in superovulated cattle, with data implying that semen quality not only affects fertilization success, but also the quality of the resulting embryos (Mikkola et al. 2019).

Successful embryo transfer programs are a result of the interconnection of various factors. Important aspects that impact the results range from factor affecting donors and recipients such as nutrition, oestrus cycle control, the use of a heifer or a cow, the quality of the corpus luteum of the recipient, to factors related to the embryos such as their developmental stage and quality, or their conservation state (fresh vs frozen), to factors related to the procedure of embryo transfer such as the site of embryo deposition in the uterus, the difficulty level of the transfer (and whether it resulted in some degree of injury) or how experienced the technician is (Erdem et al. 2020; Lamb et al. 2021).

In a study conducted by Rodrigues et al. 2018 , the authors reported a 52% overall pregnancy rate, which is in accordance with the pregnancy rates observed in the present study (55%). However, pregnancy rates for cows seem to be significantly lower than those for heifers (33% vs 59%, p=.045). This may indicate an underlying issue with recipient selection and identification process, as it is not expected to find significant differences between pregnancy rates due to parity differences, as demonstrated in several studies(Hasler 2001; Bényei et al. 2006). Some producers prefer to use virgin heifers, others prefer to select cows with an established fertility history, but if heifers and cows are correctly evaluated and managed - with appropriate reproductive tract assessment and adequate nutrition (Lamb et al. 2021), pregnancy rates should not differ.

As discussed above, it has been reported that age might influence the number of transferable embryos produced by donor females, as there seems to be a tendency for heifers to produce less transferable embryos than cows, however, recipient age does not seem to have an influence on ET outcome, with authors focusing more on other management factors such as nutrition and postpartum interval (Looney et al. 2006; Lamb et al. 2021). These two factors go hand in hand, as body condition score and energy are major factors regulating reproductive successes and/or failures in recipients, and postpartum intervals are affected by the nutritional plane in which females are kept in (Looney et al. 2006). It is known females should be at least at the 50 day postpartum mark (Looney et al. 2006), so the data analysed in this study is in accordance to that recommendation.

Even though no significant differences in the proportions of positive and negative PD were found between different embryo quality codes, a study (Erdem et al. 2020) that evaluated the effect of embryo quality and developmental stages on pregnancy rates in beef heifers showed significantly higher pregnancy rates with code 1 quality embryos when compared with code 2 embryos ($P< 0.05$). This same study showed that different embryonic developmental

stages had no effect on the pregnancy rate, which is in line with what is reported above in the current study. Rodrigues et al. (2018) also reported significantly higher pregnancy rates (57%) of grade 1 embryos when compared to embryos classified as grade 3 (43%) ($p < .01$). Roper et al. (2018) found that embryo stage of development, embryo quality, and whether embryos were fresh or frozen did not impact recipient pregnancy rates.

Although the present study did not find any significant difference between donor-recipient synchrony, Rodrigues et al. (2018) found an effect on pregnancy occurrence of oestrous synchrony between the donors and recipients. The results showed a higher pregnancy rate tendency ($p = .08$) in recipients that were in oestrus before the donors, with values of 47.2% for +24 h and 41.0% for -24 h synchronies. Regarding donor-recipient synchronies within the 12-hour limit, pregnancy rates in this study were 52% and 56% when the oestrus occurred with a difference of −12 and +12 h, respectively, and 64% for a −6 hr difference between the recipient vs donor.

Recipients with cavitary corpus luteum (CL quality 2) might be considered less suitable for embryo transfer based on the premise that this morphological form leads to lower P_4 levels in the blood and, therefore to lower pregnancy rates (Jaśkowski et al. 2021), given that the establishment and maintenance of a successful pregnancy involves complex interactions between the embryo, the uterine environment, and the corpus luteum. (Nogueira et al. 2012). However, these authors have reported that the type of corpus luteum does not influence the pregnancy rates of bovine embryo recipients, as P_4 concentrations are not influenced by the presence of the cavity. Contrary to the results obtained in the present study, Jaśkowski et al. (2021) reported higher progesterone levels in blood serum in heifers with cavitary CL ($p < 0.001$) and higher pregnancy rates - 52% vs 33%, compared to females with compact CL ($p < 0.05$).

As described for FTAI, the influence of the technician on the outcome was analysed based on the premise that an adequately trained professional, with correct technique performance is essential for the success of an ET procedure. The analysed data showed no statistically significant difference, which is in line with what Roper et al. (2018) reported, in their study comparing the performance of seven experienced technicians in where the technician performing the procedure did not significantly affect recipient pregnancy rates - however, the technician did influence the uterine location of transfer, transfer score, and transfer time. The uterine location in which the embryo was placed has been shown to impact pregnancy rates, with embryos transferred into the cranial part of the uterine horn demonstrating higher rates of pregnancy success compared to those transferred into the middle and caudal thirds of the uterine horn (Roper et al. 2018; Lamb et al. 2021). Transfer score also tended to influence pregnancy rates, and the time required to complete the embryo transfer procedure significantly affected pregnancy rates. This suggests an inverse correlation

between pregnancy rates and the time spent in the uterus during embryo transfer, as it implies that as the time spent in the uterus decreases, pregnancy rates tend to increase (Roper et al. 2018; Lamb et al. 2021). This can be noted in the results obtained in this study, since there was a significantly higher proportion of positive pregnancy diagnoses in procedures where embryo placement was correctly performed and significantly higher negative pregnancy outcomes from procedures where embryo placement resulted in a degree of trauma to the tissues. This can be explained because when the embryo transfer syringe is in the uterus, any recipient movement increases the risk of damage to the endometrium, and this damage can trigger the release of $PGF_{2\alpha}$, a substance that may adversely affect pregnancy success. In addition to this aspect, it is important to note that although the uterine location in which the embryo was placed was not recorded and so, not analysed in this study, this factor has been shown to impact pregnancy rates, with embryos transferred into the cranial part of the uterine horn demonstrating higher rates of pregnancy success compared to those transferred into the middle and caudal thirds (Lamb et al. 2021). This gives us a clear understanding of the utmost importance of employing precise, and swift, technique during ET.

Several other factors could have impacted the success of the ARTs used. The scope of this work was limited to the analysis of the available data, however, in the future, there could be the opportunity to integrate information regarding environmental and management factors to access their impact on the outcomes of this use.

6. Conclusion

The Aberdeen Angus breed has earned a stellar reputation worldwide, establishing itself as a leading choice for beef production. Its superior meat quality has placed it favourably in the cattle industry, catering to the increasing demand for premium beef products. Aberdeen Angus cattle are known for efficient feed conversion, making them economically viable for producers around the world. Beyond economic considerations, the breed's genetic characteristics contribute to sustainable and resilient cattle farming practices, aligning with the demanding needs of the modern livestock industry.

Artificial reproduction technologies indeed play a crucial role in modern livestock production, with an important impact on beef cattle farming. These technologies have revolutionized breeding practices, offering numerous advantages for both producers and the industry as a whole.

In this study, the results highlight the potential influence of certain variables on the outcome of the results. Acknowledging the existence of these associations provides a foundation for refining and optimizing procedures, thereby enhancing the overall efficacy and success of the implemented protocols. However, it is to acknowledge that this study is constrained by a limited amount of available data, and data was collected under field conditions. Recognizing that the success of implementing these techniques is a complex, multifactorial issue, it is important to note that even though certain variables may not have demonstrated a clear association with the outcome, they should not be overlooked or dismissed.

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