Systems approach to economic risk analysis of *Bos taurus* beef embryo transfer programs through stochastic simulation

by

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Abstract

The dynamic environments, varying production practices, and general biological uncertainty associated with bovine reproduction makes informed, strategic decision making regarding the implementation of bovine reproductive technology a great challenge for producers. One might also argue that traditionally, ET's primary focus of genetic improvement has greatly overshadowed any consideration of short to mid-term financial gain.

To accomplish the objective of creating an economic risk analysis tool for user-defined embryo transfer (ET) programs, a circumstantial, stochastic prediction model utilizing @Risk© software to generate comparable economic values as an aid in the ET decision making process has been created. More realistic than the use of means in deterministic models, distributions defining the biological uncertainty for a multitude of reproductive outcomes are estimated through extensive literature review and limited industry sources. Applying the Latin Hypercube variation of Monte Carlo simulation, a sample value from the descriptive distribution associated with each stochastic variable is included in an iteration of the simulation. Through large numbers of iterations with dynamic combinations of variables, the process culminates in a distribution of possible values for the net present value (NPV), annuity equivalent net present value (ANPV), and return on investment (ROI) associated with the model described scenario of in-vivo derived (IVD) or in-vitro produced (IVP). Finally, using the distributions of NPV, ANPV, and ROI a decision maker can assess the economic risk linked to a user-defined ET program.

To further complicate matters, cattle producers are now presented with a choice between two primary methods of ET. IVD ET describes the traditional method of ET that involves follicular stimulation and insemination of a donor female followed by the collection of fertilized embryos from the uterus. IVP commonly refers to the method of generating transferable embryos by

collecting oocytes by ovarian aspiration; in-vitro fertilization of the collected oocytes; and incubated maturation of the fertilized oocytes. Encompassed within the two methods of ET exist several different sub-techniques, principally regarding the exception or inclusion of follicular synchronization and/or stimulation before ovum pick-up (OPU) in IVP procedures. Ultimately, operators must decide whether ET programs, of any type, serve as an economically viable means to increase rate of genetic improvement or take advantage of marketing opportunities. Although several economic value predictors for ET programs already exist (Beltrame et al. 2010), the opportunity remains to create more applicable models for *Bos taurus* beef production and varying marketing avenues in the U.S. This circumstantial, stochastic simulation model can serve as an aid in the ET decision making process by generating output that allows for the financial risk and sensitivity analysis of a user-defined ET program.

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Preface

In the business world, the ability to project the potential range of investment profit or loss and mitigate risk, before making an initial investment, serves as a crucial step towards the success or failure of an enterprise. The cattle feeding segment of the beef industry has adopted similar techniques with advanced breakeven projections that utilize a combination of performance predictors and risk management practices. Conversely, a majority of the seedstock sector, particularly as it pertains to the use of embryo transfer (ET) technology in seedstock operations, seems to place more trust in intuition and optimism rather than proven methods of investment analysis.

Whereas it stands to reason that numerous, highly successful seedstock operations must implement some degree of strategic scrutiny before committing the extensive amount of both financial and labor resources into ET programs; the depth of the evaluation comes into question, especially for operations without years of experience to reinforce assumptions. The lack of financial investigation can be rationalized. Hasler (2003) stated that although the quantity seems to be declining, traditionally a significant volume of beef ET was conducted by hobby farmers funded through sources not directly related to the beef business. One also might argue that ET's primary focus of genetic improvement has greatly overshadowed any consideration of short to mid-term financial gain. In addition, dynamic environments, small sample size, poor record keeping, and the immense variability associated with bovine reproduction account for several more potentially limiting factors.

To further complicate matters, cattle producers are now presented with a choice between two primary methods of ET. In-vivo derived (IVD) ET describes the traditional method of ET that involves follicular stimulation and insemination of a donor female followed by the collection of

fertilized embryos from the uterus. In-vitro production (IVP) commonly refers to the method of generating transferable embryos by collecting oocytes by ovarian aspiration; in-vitro fertilization of the collected oocytes; and incubated maturation of the fertilized oocytes. Encompassed within the two methods of ET exist several different sub-techniques, principally regarding the exception or inclusion of follicular synchronization and/or stimulation before ovum pick-up (OPU) in IVP procedures. Ultimately, operators must decide whether or not ET programs, of any type, serve as an economically viable means to increase rate of genetic improvement or take advantage of marketing opportunities. Although several economic value predictors for ET programs already exist (Beltrame et al. 2010), the opportunity remains to create more applicable models for *Bos taurus* beef production and varying marketing avenues in the U.S.

To conduct economic risk analysis of user-defined ET programs, a circumstantial, stochastic prediction model utilizing @Risk© software to generate comparable economic values as an aid in the ET decision making process has been created. More realistic than the use of means in deterministic models, distributions defining the biological uncertainty for a multitude of reproductive outcomes are estimated through extensive literature review and limited industry sources. Applying the Latin Hypercube variation of Monte Carlo simulation, a sample value from the descriptive distribution associated with each stochastic variable is included in an iteration of the simulation. Through large numbers of iterations with dynamic combinations of variables, the process culminates in a distribution of possible values for the net present value (NPV), annuity equivalent net present value (ANPV), and return on investment (ROI) associated with the model described scenario of IVD or IVF. Finally, using the distributions of NPV, ANPV, and ROI a decision maker can assess the economic risk linked to a user-defined ET program.

Chapter 1 - Literature Review

Brief History of Bovine Embryo Transfer Techniques

In-vivo Derived Embryo Transfer

While the concept of embryo transfer (ET) has existed for centuries, even millennia, its realization, especially in commercial application, is still relatively recent (Betteridge, 2003). Walter Heape's 1890 transfer of rabbit embryos from one breed of doe to another, immediately following embryo collection represents the first recorded transfer of mammalian embryos to result in live progeny (Heape, 1891). More than 60 years later, in 1951, the first embryo transfer derived calf was born through the efforts of Elwyn Willet and his team at the University of Wisconsin (Betteridge, 2003). At the time, ET technology was still in its early, inefficient stages of application (Betteridge, 2003) Thus, much of the efforts of bovine ET pioneers, including Willet's, were redirected to artificial insemination (A.I.) because of its industry perception as a more practical application towards genetic advancement (Betteridge, 2003).

The surge in the importation of "exotic", continental European beef breeds by North American breeders during the early 1970s finally sparked the commercial application of ET as a means of propagating expensive genetics rare to the North American continent (Hasler, 2003). During this time, surgical collection and transfer of bovine embryos was the most common practice (Hasler, 2003). It was not until the mid- to late 1970s that nonsurgical collection (flushing) and transfer techniques became a commercially viable means of ET, which expanded its use from in-lab procedures to on-farm practice (Hasler, 2003). The use of pituitary hormone extracts to stimulate the ovulation of multiple follicles, allowing for multiple ovulation embryo transfer (MOET) and the development of prostaglandins to aid in the timing of ovulation also started in the

1970s (Bó and Mapletoft, 2014). Over the years, superovulation protocols have continued to improve in respect to convenience and time efficiency (Bó and Mapletoft, 2014).

Beginning in the early 1980s, the ability to successfully freeze and thaw embryos made embryo recipient management much more efficient, as embryos could be transferred at a later date if the number of available recipients did not meet or exceed the number of fresh embryos available for transfer (Hasler, 2003). ET technology cemented its place in the cattle industry with incredible growth within both the beef and dairy industries during the 1980s (Table 1) (Betteridge, 2003; Hasler, 2003). Today, North America, the U.S. in particular, remains the largest producer of in-vivo derived (IVD) embryos.

Table 1. Number of embryonic transfer (ET) calves registered by three US breed associations during selected years between 1973 and 1988.

Birth Year	Simmental (n)	Angus (n)	Holstein (n)
1973-75	1,558	0	21
1976-78	4,163	53	1,234
1979-81	7,786	1,552	13,103
1982-84	13,916	8,303	43,253
1985	4,068	4,680	20,991
1988	1,879	5,940	22,070

Adapted from Hasler(2014) (From Baker, 8th AETA Conference 1989 and compliments of Simmental, Angus, and Holstein Associations.

Both Betteridge (2003) and Hasler (2014) voice some concern over the stagnation of progress in the now mature industry of conventional IVD ET. While the annual number of IVD collections has fluctuated in recent years, there has been little to no growth. (Table 2, Table 4). Furthermore, "The mean number of transferrable embryos from reproductively normal cows has remained relatively unchanged during the past 30 years for both beef and dairy cows" (Table 3) (Hasler, 2014). Looney (1986) calculated a mean of 6.2 "good" embryos per collection from more

than 2,000 flushes of beef donors from a sampling of 14 different breeds (Proceedings of 5th AETA Conference 1986) (Hasler, 2014). In 2014, an average yield of 7.0 transferable embryos per collection from a total of 31,333 IVD collections of beef donors in the U.S. was reported to the International Embryo Transfer Society (IETS) Data Retrieval Committee (Table 5) (IETS, 2014).

Table 2. Number of in-vivo embryo collections in North America by year, from 1997-2012 (includes beef and dairy).

2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
50,527	55,981	42,238	47,638	52,855	65,520	64,711	68,633	67,684	52,921	51,735	54,837	52,701	57,735	58,934

Adapted from 2012, 2013, 2014 Statistics of Embryo Collection and Transfer in Domestic Farm Animals, George Perry- IETS Data Retrieval Committee Chair

Table 3. Average number of transferrable in-vivo embryos collected per collection in North America by year, from 1997-2012 (includes beef and dairy).

2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
5.69	5.64	6.28	5.89	6.07	5.99	6.42	6.18	6.20	6.57	6.54	6.62	6.75	6.80	6.74

Adapted from 2012, 2013, 2014 Statistics of Embryo Collection and Transfer in Domestic Farm Animals, George Perry- IETS Data Retrieval Committee Chair

Table 4. North American bovine in-vivo derived embryo activity 2009-2014.

Year	Embryo Collections	Transferrable Embryos	% global embryos	Fresh Transferred Embryos	Frozen Transferred Embryos	Total Transferred Embryos	% global transfers
2009	52,921	347,531	49.48	111,106	137,599	248,705	46.57
2010	51,735	338,540	46.23	106,400	147,271	253,671	42.95
2011	54,837	362,781	49.50	109,197	139,418	248,615	43.44
2012	52,701	355,866	50.87	100,354	134,990	235,344	46.52
2013	57,735	392,530	53.83	115,832	161,785	277,617	48.31
2014	58,934	397,306	64.66	107,700	163,646	271,346	58.41

Adapted from 2009, 2010, 2011, 2012, 2013, and 2014 IETS Statistics and Data Retrieval Committee Report

Table 5. United States dairy and beef cattle in-vivo derived embryo activity 2012-2014.

Year	Dairy Embryo Collections	Beef Embryo Collections	Dairy-Transferable Embryos	Beef-Transferable Embryos
2012	15,443	23,342	96,515	160,429
2013	16,252	28,433	100,479	201,192
2014	15,217	31,333	93,460	219,335

Adapted from 2012, 2013, and 2014 IETS Statistics and Data Retrieval Committee Report

In-vitro Produced Embryo Transfer

While the IVD ET industry may have matured over the past several decades, the later developing in-vitro produced (IVP) embryo industry has experienced substantial growth since the late 1990s and early 2000s (Table 6, Table 7)**.** The process of IVP can be broken down into 3 distinct in-vitro procedures: 1) In-vitro maturation (IVM) describes the process of maturing oocytes prior to fertilization; 2) In-vitro fertilization (IVF) is the process of sperm capacitation and

subsequent fertilization of the oocyte; and 3) In-vitro culture (IVC) describes the growth of the fertilized oocyte to blastocyst stage (Hasler, 2000). Finally, as with IVD the option exists for either fresh transfer or cryogenic freezing of the embryos produced. Several methods regarding follicular synchronization and/or stimulation exist in the pre-ovum pick-up (OPU) procedure. Potential advantages of IVF protocols, when compared with IVD ET include: the ability to aspirate during gestation; the ability to fertilize several hundred oocytes with a single straw of semen; follicular synchronization/stimulation is optional, but not required; and the potential to generate embryos in prepubertal heifers.

In 1959, the first live, IVF offspring were generated in rabbits (Hasler, 2000; Chang, 1968). The first IVF produced calf was born in 1981 (Bracket et al., 1982), and Hanada et al. (1986) describes 2 calves born from IVF embryos following culture in a rabbit oviduct; however, the first live calves generated from the full process of IVP were not produced until 1987 (Fukuda et al., 1990; Hasler, 2000). OPU, the technique of ultrasound-guided follicular aspiration for the non-surgical collection of oocytes from live donors was developed in 1988 and remains the most common procedure for oocyte collection (Pieterse et al., 1988; Hasler, 2000).

In 1990s North America, commercial uptake of IVP was slow as oocytes per OPU hovered around only 5 oocytes and effective culture media was notorious for generating abnormally large calves and increased abortion rates in recipients (Hasler, 2014). This condition is often termed Large Offspring Syndrome (LOS) (Hasler, 2014). Until the mid to late 2000s, most cattle breeders and ET practitioners within the U.S. viewed IVP as a last resort for high value donors whose fertility issues led to limited or no response to traditional IVD procedures (Hasler, 2014). By 2011, emphasis of the leading ET companies in the U.S. had shifted from problem donors to IVP production using reproductively sound donors, often utilizing OPU during early pregnancy

(Hasler, 2014). Hasler (2014) cited communication with Trans Ova stating that most donors were stimulated with follicle stimulating hormone (FSH) pre-OPU resulting in a yield of 18 to 20 oocytes per OPU followed by a blastocyst development rate of roughly 25%. While there is limited literature on the recent reduction in LOS occurrence, the state of the industry indicates that changes in culture medium have helped to alleviate some of the concern (Hasler, 2014).

In Brazil, the world leader in IVP production, different cattle types and industry conditions led to an increased rate of IVP commercialization. Successful, large scale IVP production first occurred in the late 1990s, when several reproductive companies in Brazil began specializing in the production and transfer of IVP embryos (Viana et al., 2012). Driven primarily by the motivation to find an alternate method of ET for *Bos indicus* cattle, a species with a typically poor response to the FSH superovulation protocols required with MOET, and the tendency for *Bos indicus* cattle to yield a significantly greater number of oocytes per non-FSH stimulated OPU, the Brazilian cattle industry was the first to prove the technology's widespread application (Viana et al., 2012**;** Hasler, 2014)**.**

Table 6. Number of OPU in-vitro embryos collected in North America and South America by year, from 2000- 2014 (includes beef and dairy).

Region	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
N.A.	1,741	0	20,378	27,413	2,385	29,243	134,162	137,958	17,747	20,390	43,058	48,474	74,242	112,300	206,139
S.A.	12,667	668	51,063	63,341	80,833	143,916	204,469	211,496	220,465	256,033	268,310	325,349	355,205	376,459	356,960

Adapted from 2012, 2013, 2014 Statistics of Embryo Collection and Transfer in Domestic Farm Animals, George Perry- IETS Data Retrieval Committee Chair

Table 7. Number of transferred in-vitro embryos in North America and South America by year, from 2000- 2014 (includes beef and dairy).

Region	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
N.A.	1,915	576	494	2,153	2,119	1,469	4,309	9,252	13,142	18,657	28,100	20,780	40,546	66,602	92,930
S.A.	12,527	401	48,670	63,164	80,333	129,408	196,791	195,920	220,441	256,348	269,123	323,157	335,994	304,928	251,273

Adapted from 2012, 2013, 2014 Statistics of Embryo Collection and Transfer in Domestic Farm Animals, George Perry- IETS Data Retrieval Committee Chair

Factors Influencing ET Program Success

IVD Embryo Production

The first, and perhaps most obvious, contributor to the success of any ET program is the ability to generate transferable embryos. IVD and IVP, as described in the preceding pages, are the primary methods of embryo production (excluding somatic cell nuclear transfer to create cloned embryos and the practice of embryo splitting to produce multiple embryos from a single original). Although a conglomerate of factors, including breed, age, and reproductive soundness of a donor impact the success of an ET system; several human controlled elements of the embryo production process, including hormone protocol, interval between embryo or oocyte collections, control of follicular wave dynamics, and in-lab procedures influence the ultimate success or failure of embryo production.

Beginning in the early 1970s, equine chorionic gonadotropin (eCG) was the primary means of ovarian stimulation to cause multiple follicular ovulation, followed shortly thereafter by the addition of prostaglandin ($PGF_{2\alpha}$) to aid in control of the estrous cycle (Bó and Mapletoft, 2014). By the late 1970s, it was discovered that donors maintained an endocrine profile that was both more normal and conducive to multiple ovulation through FSH stimulation rather than eCG (Bó and Mapletoft, 2014). Pituitary extracts containing FSH have a variable ratio of FSH to luteinizing hormone (LH) concentration unless they are purified (Bó and Mapletoft, 2014). Chupin et al., 1984 reported that lower levels of LH improved response to ovarian FSH stimulation. Looney et al. (1988) found that exogenous stimulation utilizing DNA recombinant FSH containing no LH caused high superovulatory response suggesting that exogenous LH is not beneficial and perhaps detrimental to superovulation (Bó and Mapletoft, 2014). Today, a typical superovulation schedule may include twice daily intramuscular injection of some form of FSH for 4 to 5 days with $PGF_{2\alpha}$

treatment to cause luteolysis given at 48 to 72 hours following the start of FSH treatment (Bó and Mapletoft, 2014). Often, a progestin insert is included to prevent donors from coming into estrus ahead of schedule (Bó and Mapletoft, 2014). Estrus should occur 36 to 48 hours following $PGF_{2\alpha}$ injection with insemination occurring at both 12 and 24 hours following the onset of estrus (Bó and Mapletoft, 2014). Ovarian follicular waves play another important role in superovulation. Traditionally, gonadotropin treatment for follicular stimulation has been initiated between 8 and 12 days following estrus, which typically coincides with the timing of emergence for the second follicular wave of a 2-wave cycle; however, studies have reported that initiation of ovarian stimulation treatment that is not timed exactly with the day of follicular wave emergence has decreased effect (Bó and Mapletoft, 2014). Most superovulation treatments now incorporate a means of controlling follicular wave emergence through exogenous control (Bó and Mapletoft, 2014).

There are several exogenous methods of follicular wave control. In countries where estradiol is available for use, a treatment combining estradiol with progesterone serves as the most common means to cause regression of the current follicular wave and emergence of the next wave roughly 4 days later, at which time gonadotropin treatment starts (Bó and Mapletoft, 2014). A method more common in the U.S. is dominant follicle removal (DFR) by physical ablation using ultrasound guided follicle aspiration. If a skilled technician or ultrasound equipment are not available, several variations of gonadotropin releasing hormone (GnRH) protocols have proven effective in stimulating the emergence of a new follicular wave (Bó and Mapletoft, 2014). With both DFR and GnRH protocols a new follicular wave usually emerges 1 to 2 days following ablation or ovulation of the dominant follicle (Bó and Mapletoft, 2014).

While the traditional protocols call for donor females to have a minimum of 2 estrous cycles between superovulation treatments creating an interval of roughly 60-70 days between embryo collections, recent studies indicate that comparable results can be attained through protocols with a superovulation interval of 30-40 days (Hasler, 2010; Bó and Mapletoft, 2014). Bó and Mapletoft (2014) reported no difference in embryo production from donor superovulation intervals ranging from 28-30 days to intervals 90 days or greater.

As mentioned before, while many of the preceding adaptions to superovulation protocols can help increase embryo production per unit of time and improve convenience by reducing the need for estrus detection and allowing for more on-farm protocol administration, the number of embryos collected per superovulation has remained relatively unchanged (Bó and Mapletoft, 2014). Hasler (2003) states, "I believe that the current success level of superovulation represents a significant obstacle to the future growth of the ET industry. As long as mean embryo production remains at less than 6, with a range of (0 to >60), with 20% of donors producing 0 embryos, superovulation will remain an expensive, inefficient procedure." Bó and Mapletoft (2014) shared a similar view, "…Thus, a high degree of unpredictability in superovulatory response still exists more than 35 years later, creating problems which affect the efficiency and profitability of commercial embryo transfer."

Influence of Semen Quality on IVD Embryo Production

Semen quality also impacts embryo production, with the substitution of sex-sorted semen for conventional, unsorted semen typically having a negative effect. In single ovulating heifers, the fertility of low dose, sex-sorted semen tends to be 60%-90% of conventional, unsorted semen (Schenk et al., 2006). Although there is not a definite answer to the cause of this concern, it may be caused by added stress applied to spermatozoa when undergoing the process of sorting X- and

Y-chromosome bearing sperm (Schenk et al., 2006) and the lower dose. Following insemination, Mitchell et al. (1985) found that >90% of inseminate was no longer capable of fertilization 12 hours after insemination and that >99% was no longer capable of fertilization after 24 hours within the female bovine genital tract. Furthermore, it has been demonstrated that increasing the number of inseminated sperm increases number of accessory sperm (sperm found in the zona pellucida of the oocyte but not responsible for fertilization) and fertilization rate (Nadir et al., 1993**;** Hawk, 1988).

In a study of MOET in Angus cows and heifers comparing non-sexed versus sex-sorted, freeze-thawed semen the mean number of transferable embryos was significantly reduced when using sex-sorted semen (Schenk et al., 2006). The mean transferrable embryo yield of non-sexed semen was 8.7 embryos per flush, while the mean transferrable embryos per flush for sex-sorted semen at a dose of 10×10^6 and 2×10^6 sperm per inseminate was 4.1 and 3.3 transferrable embryos, respectively (Schenk et al., 2006). The numeric improvement in embryo production for the higher dose of sex-sorted semen was not significant (Schenk et al., 2006). Hayakawa et al. (2009) demonstrated that MOET of Holstein heifers using sex-sorted, freeze-thawed semen generated a numeric reduction in the total number of transferrable embryos and fertilization rate in one experiment and a numeric reduction in the number of transferrable grade 1 embryos in another experiment.

IVP Embryo Production

To increase embryo production per OPU from IVP, more viable oocytes must be aspirated and/or a greater percentage of cultured oocytes must develop to the blastocyst stage. While roughly 80% of naturally occurring, single ovulating oocytes develop into embryos following in-vivo fertilization and roughly 60% of oocytes following superovulation develop into embryos, the range

of embryo development percentage for OPU-IVP embryos tends to range from 10% to 40%, depending on oocyte and semen quality (Merton et al., 2003; Pontes et al., 2010; Morotti et al., 2014)**.** Another point to consider is the number of embryos generated over a specific time span. Three factors can be readily manipulated to influence the number of oocytes collected per OPU or in a set time span from a specific donor: OPU interval, synchronization of follicular wave emergence, and ovarian stimulation protocol.

The goal of OPU is to perform the aspiration at a time in the estrous cycle that the current follicular wave has as many developmentally competent, retrievable oocytes as possible. Successful OPU retrieves all follicles ≥2-3 mm in diameter. Synchronization of follicular wave emergence has been shown to improve both the number of oocytes collected per OPU and the percentage of oocytes that reach blastocyst stage (Antonio de Carvalho Fernandes et al., 2014). One way to manipulate the synchronization of follicular waves is by managing the time interval between OPU sessions. When considering methods without ovarian stimulation, several different approaches to OPU interval are common in the industry today, depending on the production goals and restrictions of a program.

One option is a twice weekly OPU schedule in which donors are aspirated at 3-4 day intervals. This protocol allows for OPU to be performed before the differentiation of a dominant follicle at day 5 or 6 and the subsequent atresia of the remaining follicles in the cohort (Merton et al., 2003**;** Chaubal et al., 2006). Several studies have shown that larger follicles undergoing prematuration, and even slightly atretic follicles, whose oocyte ultrastructure resembles that of oocytes during prematuration, have a greater developmental competence than smaller follicles (Lonergan et al., 1994; Merton et al., 2003**;** Machatkova et al., 2004). With a twice weekly OPU schedule, follicles will have a diameter <8 mm with reduced developmental competency when

compared to follicles approaching preovulatory stage with a diameter >13mm. (Merton et al., 2003**;** Hagemann et al.,1999**).** The labor required for the frequent OPU, in-lab procedures, and potential recipient synchronization make man hours an important element to consider with this schedule.

Extending the interval between OPU to one week allows for the development of a dominant follicle and regression of some subordinate follicles (Chaubal et al., 2006). Although there is some discrepancy within the literature as to the differences between once-weekly and twice-weekly OPU as it pertains to a per session basis on the number of oocytes retrieved (Table 8), blastocyst rate, and number of embryos produced; Chaubal et al. (2006) and Ding et al.(2008) reported no difference between the mean number of oocytes retrieved and blastocysts produced. When using a once-weekly interval the number of embryos in a set time span tends to be reduced when compared to twice-weekly OPU (Chaubal et al., 2006); however, again, availability of labor and recipients may dictate that this interval is the most efficient option for a specific operation.

Further lengthening the OPU interval to 2 weeks, without hormonal interference or follicular ablation, sets OPU during the 2nd follicular wave (Ding et al., 2008). Given that the second follicular wave emerges somewhere between 7 to 11 days after ovulation, there will be variation between donors regarding the exact physiological stage of the follicular wave when OPU is performed with the follicular wave somewhere between 3 and 7 days post-emergence**.** Ding et al. (2008) reported no difference in mean number of recovered oocytes per OPU when comparing OPU twice-weekly, every 5 days, once-weekly, every 10 days, and once in 2 weeks; although numerically, the mean for twice-weekly OPU was lower than the mean of the other intervals.

The final approach to non-stimulated OPU is the application of a random OPU schedule. While variation in the retrieval of oocytes (Table 9) and production of embryos is likely because

of the inconsistency of follicular wave status between donors, added flexibility, convenience, and a potential improvement in labor efficiency make this a feasible option in some scenarios. Merton et al. (2003) reported that in the breeding herd at Holland Genetics, in The Netherlands, 41 pregnant heifers produced an average of 14 oocytes with random OPU and no DFR preceding OPU. Oocyte yield fell to 9 oocytes per OPU over the next 3 sessions once a 3-4 day interval was established. Cows within the same herd showed no difference in oocyte yield between random OPU and OPU sessions with a 3-4 day interval. The random approach may fit best into a program that is aiming for sheer quantity of embryos from a large group of donors with little concern for the embryo production of a specific donor.

Table 8. Mean number of viable oocytes per non-stimulated OPU session**.**

Mean number of viable oocytes	Number of OPU sessions	Interval (days)	Study
3.9	96	3-4	De Roover et al., 1997 (abstract)
7	24	3-4	Guyador Joly et al., 1997, (abstract)
6.7	169	3	Hanenberg et al., 1997 (abstract)
7.2	516	3	Hanenberg et al., 1997 (abstract)
9.3	192	3	Hanenberg et al., 1997 (abstract)
5.6	162	4	Hanenberg et al., 1997 (abstract)
6.6	502	4	Hanenberg et al., 1997 (abstract)
8	182	4	Hanenberg et al., 1997 (abstract)
9.1	48	7	Hanenberg et al., 1997 (abstract)
7.8	75	3-4	De Ruigh et al., 2000
5.9	75	3-4	De Ruigh et al., 2000
7	236	3-4	Wagtendonk-de Leeuw et al., 2000
8.6	1753	3-4	Wagtendonk-de Leeuw et al., 2000
8.4	446	3-4	Wagtendonk-de Leeuw et al., 2000

(*cont.)*

Table 8 (*cont.*). Mean number of viable oocytes per non-stimulated OPU session.

Mean number of viable oocytes	Number of OPU sessions	Interval (days)	Study
7.6	4308	3-4	Wagtendonk-de Leeuw et al., 2000
7.8	2015	3-4	Wagtendonk-de Leeuw et al., 2000
3.8	60	3-4	Chaubal et al., 2006
4.1	1396	3-4	De Roover et al., 2008
4.96	24	3-4	Ding et al., 2008
6.19	36	14	Ding et al., 2008

Another potential approach to an IVP program is ovarian FSH stimulation with the timing of stimulation based upon follicular wave emergence. The complement of wave synchronization with FSH stimulation improves the number of oocytes (Table 10) and subsequent number of embryos generated when compared to IVP procedures without stimulation and with or without follicular wave synchronization (Antonio de Carvalho Fernandes et al., 2014). Although there are various protocols depending on practitioner and producer preference, a typical protocol may begin with the initiation of a follicular wave by either DFR through follicular ablation or hormonal control using either a GnRH or estradiol-progesterone based technique, depending on availability of estradiol, followed 2 to 3 days later by twice daily treatment of FSH for 2 consecutive days (De Roover et al., 2008). A 48-hour coasting period between the final FSH treatment and OPU has been shown to improve blastocyst development rates of cumulous oocyte complexes (COCs) (Sirard et al., 1999). While the control of follicular wave emergence allows for the interval between OPUs to vary, a typical interval is 14 days (Hasler, 2014; De Roover et al., 2008). De Roover et al. (2008) reported that stimulated OPU done at 14 day intervals, using DFR, generates

significantly more oocytes per session and embryos per session, while significantly increasing blastocyst development percentage when compared to twice weekly non-stimulated OPU.

Table 9. Number of viable oocytes per OPU session performed at random.

Mean number of viable oocytes	Number of OPU sessions	Interval (days)	Study
14.0	41	First OPU, no interval, no DFR	Merton et al., 2003
8.0	1,138	Random, Minimum 15 d interval	Pontes et al., 2010
5.4	44	First OPU, no interval, no Pre-OPU DFR	Antonio de Carvalho Fernandez Fernandez et al., 2014
10.04	925	First OPU, no interval, no DFR	Stevenson Sputnik, 2014

Table 10. Number of viable oocytes collected per OPU session with pre-OPU follicular wave synchronization and FSH stimulation.

Mean number of viable oocytes	Number of OPU sessions	Study
13	12	Guyador Joly et al., 1997 (abstract)
14.8	20	Lacaze et al., 1997 (abstract)
13.2	30	De Ruigh et al., 2000
11.8	640	De Roover et al., 2008
11.9	42	Antonio de CarvalhoFernandez et al., 2014
10.4	32	Barceló-Fimbres et al., 2015
9	32	Barceló-Fimbres et al., 2015
8.6	24	Barceló-Fimbres et al. 2015
11	78	Barceló-Fimbres et al. 2015

Influence of Semen Quality on Blastocyst Rate

Semen quality also influences the blastocyst rate of cultured oocytes. Everett et al. (1978) demonstrated a significant difference in the semen characteristics of ejaculate volume, sperm concentration per ml, percent motile sperm, and total sperm per ejaculate when compared across sires, months of the year, and days between ejaculates. While semen quality is subject to variability even in conventional, non-sorted semen, the greatest concern for semen quality comes with sex-sorted semen; whether it is sorted before freezing or reverse-sorted (Palma et al., 2008; Morotti et al., 2014). Although Zhang et al. (2003) found no significant difference in blastocyst rate between sex-sorted sperm and unsorted sperm following IVF, Palma et al. 2008, demonstrated that when compared to the control non-sexed sperm, sex-sorted sperm from 4 out of 5 sires differed significantly in blastocyst rate of IVF embryos. In Palma et al. (2008), the control unsorted sperm was not from the same sires as the sex-sorted sperm. Xu et al. (2006) found significant bull to bull variation in the fertility of sex-sorted sperm based on blastocyst rate. It was also demonstrated that when individualized concentration of heparin treatment was applied to sex-sorted sperm there was no significant difference compared to the unsorted sperm from the same sire, in 3 out of 4 bulls; however, the fertility of sex-sorted sperm was numerically lower than unsorted sperm for each sire. Considering freeze-thawed reverse sorted semen, Morotti et al. (2014) not only reported blastocyst rates ranging from 15% to 48% in comparison of 11 different sires, but, in a separate analysis, also demonstrated a significant reduction in blastocyst rate for reverse-sorted semen when compared to sex-sorted, freeze-thawed semen from the same bull.

Semen quality also impacts the efficiency of semen use in IVF. In Palma et al. (2008), when both non-sexed and sexed semen had a concentration of 10×10^6 spermatozoa per straw, 35-120 oocytes could be fertilized with 1 straw of sexed-semen, while 210-320 oocytes could be

fertilized with non-sorted semen, differing significantly. In situations where a primary goal for the use of IVP is to generate as many embryos as possible from expensive or rare semen, semen efficiency plays a significant role in success or failure of the program.

Bos taurus vs. *Bos indicus*

Breed of cattle also greatly influences the success of an ET program, which helps explain the differing dynamics between the South American, especially Brazilian, and North American embryo transfer industries. The distinct differences between the ovarian characteristics and folliculogenesis of *Bos taurus* and *Bos indicus*, particularly Nelore, females have been well documented over the years, even if those differences are not particularly well understood (Pontes et al., 2009). *Bos indicus* females tend to respond poorly to the exogenous stimulation of superovulation protocols of traditional IVD ET (Hasler, 2014). On the other hand, *Bos indicus* females tend to have 3 follicular waves per estrous cycle and a population of more small follicles (<5 mm), compared to *Bos taurus* cattle that usually have 2 waves (Pontes et al., 2009). Antonio de Carvalho Fernandes et al. (2014) reported that *Bos indicus* cattle produced more embryos per OPU, but follicular wave synchronization could increase oocyte and embryo production in both subspecies. Follicular stimulation by exogenous FSH was shown to improve oocyte and embryo production only in *Bos taurus* cattle (Antonio de Carvalho Fernandes et al., 2014). Pontes et al. (2011) reported a mean of 23 viable oocytes per non-stimulated OPU using Nelore donors, with one donor generating 128 viable oocytes. When considering Holstein donors, Pontes et al. (2010) reported a mean of 8 viable oocytes per non-stimulated OPU.

Pregnancy

The value of live, marketable, or genetically superior calves determines the ultimate worth of an embryo transfer program. Thus, successful gestation of transferred embryos is vital. Like

most biological events, a multitude of factors contribute to the success or failure of pregnancy following embryo transfer, including origin of embryos, embryo quality, nutritional and production status of the recipient, donor-recipient synchrony, and skill of the transfer technician, just to name a few.

Following embryo transfer and rectal palpation at 50 to 60 days, Hasler et al. (1995) found a significant difference between the pregnancy rate of fresh versus frozen grade 1, day 7, IVD embryos; whereas Chagas e Silva et al. (2002) found no significant difference. Also in Hasler et al. (1995), a significant improvement was demonstrated in pregnancy rate with the transfer of fresh over frozen grade 1, day 7, IVF embryos. On the other hand, Markette et al. (1998) found no difference in pregnancy rate between fresh and vitrified IVF embryos at 30 or 60 days after estrus; although the sample size was small with only 14 embryos transferred fresh and 33 transferred after thawing.

Traditionally, the survival of IVP embryos following cryopreservation has been shown to improve with the implementation of vitrification over slow-freezing (Nedambale et al., 2004), however, vitrification has not always been readily adopted because of the slower thawing procedures that are not conducive to on-farm thawing (Caamaño et al., 2015). Recently, methods have been reported to both improve the survival of IVP embryos (Bruyère et al., 2012) and develop a more convenient method of warming vitrified IVP embryos (Caamaño et al., 2015). Furthermore, recent industry reports indicate an increased use of slow-freezing and direct transfer of IVP embryos, suggesting more acceptable pregnancy rates following transfer.

Cumulatively, over all embryo grades and fresh or frozen embryos, a significant difference has been shown between pregnancy rate following the transfer of all IVD embryos and all IVF embryos (Table 11) (Hasler et al., 1995; Farin and Farin et al., 1995). Similarly, Pontes et al. (2009)

reported a significant reduction in the pregnancy rate of 910 IVP transfers when compared to 289 MOET transfers at both 30 and 60 days post-estrus. In contrast, Wagtendonk-de Leeuw (2000) reported no statistical difference between MOET and IVP embryos at 73 days post-estrus (Table 18). The data describing pregnancy differences between embryos of the same grade indicate that there are non-visual differences between embryos. The retrospective study Hasler et al. (1995), also shows a significant improvement in the pregnancy rate of day 7 IVF embryos when compared to day 8 embryos.

Donor-Recipient Factors Influencing Pregnancy

In regards to *Bos taurus* cattle breeds, Hasler (2001) reported no difference in recipient pregnancy based upon donor breed (Table 12). When considering recipient type a significant difference in the pregnancy rate of dairy cows resulted when compared to dairy heifers, beef heifers, and beef cows (Hasler, 2001). It has been well documented that as the milk production of dairy cows has increased, fertility has decreased, while the fertility of dairy heifers has remained relatively constant (Hasler, 2001). In contrast, Hasler et al. (1987) showed no significant difference between post-transfer pregnancy rate of lactating dairy cows, non-lactating dairy cows, and beef cows (Table 13). Age of recipient also contributes to pregnancy rate. A significant improvement in pregnancy rate was found in recipients 3-14 years of age compared to virgin heifers, first-calf heifers, and recipients >15 years of age (Hasler et al., 1987).

Table 11. Pregnancy rate following transfer of fresh and frozen IVD and IVF embryos (pregnancy determined 50-60 days post-transfer).

Embryo	Age (Days)	No. Transfers	Grade 1 (% preg)	Grade 2 (% preg)	Grade 3 (% preg)	All Grades (% preg)
IVF-Fresh	7	1,884	59[a]	45[a]	----	56[a]
IVF-Fresh	8	362	48[b]	30[b]	----	43[b]
IVF-Fresh	9	22	41	----	----	41
IVF-Frozen	7	67	42[b]	----	----	42[b]
IVF-Frozen	8	30	20[e]	----	----	20[e]
IVD-Fresh	7	320	76[c]	65[c]	54	66[c]
IVD-Frozen	7	325	67[d]	----	----	67[c]

Adapted from Hasler et al. (1995). [a,b,c,d,e] Values within a column with different superscripts differ significantly ($P<0.01$, c vs d: $P<0.05$)

Table 12. The effect of embryo breed and recipient breed and parity on pregnancy rates of fresh and frozen-thawed IVD embryos.

Donors	**No. Transfers (Fresh)**	**% Pregnant (Fresh)**	**No. Transfers (Frozen)**	**% Pregnant (Frozen)**
Dairy	7,457	68.5	4,038	58.8
Beef	1,566	67.3	1,259	57.2
Recipients				
Dairy Heifers	6,612	70.5[a]	3,477	60.9[a]
Dairy Cows	844	52.8[b]	518	47.1[b]
Beef Heifers	267	65.9[a]	252	60.0[a]
Beef Cows	835	68.6[a]	461	58.6[a]

Adapted from Hasler et al. (2001). [a,b] Values in columns without common superscripts differ significantly ($P<0.001$) (chi-square analysis)

Table 13. The effect of breed, fertility, lactational status and age of donor on the pregnancy rate of recipients following transfer of IVD embryos.

Donor	No. Transfers	No. Pregnant	Percent Pregnant
Dairy Cows-Lactating	3,809	2,786	73
Dairy Cows-Non-lactating	1,698	1,235	73
Beef cows	1,311	941	72
Age			
Virgin heifers	69	47	68[ab]
First-calf heifers	87	60	69[ab]
3-6 years	2,406	1,755	73[a]
7-10 years	2,347	1,715	73[a]
11-14 years	780	560	72[a]
>15 years	98	54	55[b]
Fertility Status			
Normal	5,126	3,730	73
Infertile	1,264	889	70

Adapted from Hasler et al. (1987). [a, b] Age groups with different superscripts differ in pregnancy rate ($p<.005$).

Estrous synchrony between donor and recipient may also impact the establishment of pregnancy following embryo transfer. Hasler et al. (1987) demonstrates a significant improvement in pregnancy rate between IVD embryo recipients that are in estrus synchrony with the donor or plus (recipient estrus prior to donor) up to 36 hours when compared to recipients that are minus (recipient estrus after donor) 24 hours or more. A significant difference in pregnancy rate was also reported in Hasler (2001) for recipients both plus 1 day or minus 1 day compared to recipients in synchrony with the donor.

The ability to reuse recipients that do not become pregnant after 1 embryo transfer also influences management decisions and economics. In a study analyzing a large sample of recipient transfers, it was demonstrated that failing to become pregnant 1 or 2 times prior did not affect pregnancy rate for recipients receiving an embryo for a second or third time (Table 14) (Hasler et al., 1987). Nelson et al. (1980**)** and Looney et al. (2006) (Table 15) found contrasting results; whereas, Remsen et al. (1982) found similar results.

Table 14. The effect of the number of previous unsuccessful transfers on pregnancy rate of transferred embryos.

No. of times used as recipient	No. transfers	No. Pregnant	% Pregnant[a]
1	5,196	3,771	73
2	1,351	977	72
3	83	56	67
4	4	2	50

Adapted from Hasler et al. (1987). [a] No differences (P>.05)

Table 15. Pregnancy rates of beef recipients after three consecutive embryo transfers followed by natural mating (Ovagenix, Bryan, TX).

No. consecutive ET services	No. recipients	No. pregnant (%)
1	753	489 (65) a
2[d]	420	222 (53) b
3[e]	193	79 (41) c
Natural mating [f]	253	209 (83)

Values with unlike letters (a-c) within column differ P< 0.01; analyzed by Pearson's Chi-square test.

[d] Recipients not pregnant to first transfer were resynchronized for the second transfer.

[e] Recipients not pregnant to second transfer were resynchronized for the third transfer.

[f] After three consecutive non-successful ET, recipients were exposed to bulls for 60 days.

Adapted from Looney et al. (2006)

Obviously, for a recipient to have the opportunity to become pregnant, she must receive an embryo. For a recipient to receive an embryo, she must be deemed to have a corpus luteum (CL) of high enough quality and progesterone producing potential to maintain pregnancy, while being in suitable estrous synchrony with the donor. With recipient management and synchronization cost comprising a considerable expense, the percentage of recipients deemed eligible to receive an embryo following estrous synchronization greatly contributes to the cost effectiveness of an ET program. When comparing pregnant to non-pregnant recipients, Spell et al. (2001) found no statistical difference in mean CL diameter, luteal volume, or plasma progesterone level. This suggests that specific CL characteristics do little to affect pregnancy status; whereas, the most influential factors on recipient quality are simply the occurrence of estrus, ovulation, and the presence of a CL (Spell et al., 2001).

Many different protocols exist for recipient estrous synchronization. Originally, the treatment of recipients with PGF to lyse a PGF receptive CL served as the primary means of estrous synchronization. Since then, several protocols utilizing progesterone with estradiol and/or GnRH

in conjunction with progesterone releasing controlled internal drug release (CIDR) inserts and PGF have been developed to control both follicular wave emergence and luteal regression (Looney et al., 2006). With these newer protocols, fixed-time ET without estrus observation, has become possible. Looney at al. (2006) discovered that a greater percentage of transferred recipients became pregnant following a single PGF injection and observed estrus when compared to an estradiol-progesterone-PGF-CIDR protocol, however, because the percentage of recipients receiving an embryo was greater when using the latter protocol, the total pregnancy rate was greater for the estradiol-progesterone-PGF-CIDR protocol (Table 16) (Table 17).

Table 16. Embryo transfer service and pregnancy rate of recipients with either a PGF single injection or CIDR + P4/E2 + PGF + E2 (Ovagenix, Bryan, TX).

	No. synchronized	**Mean days to estrus**	**Detected in estrus (%)**	**ET service rate**	**ET service pregnancy rate (%)**
25 mg PGF	1,390	3.6	50	93 a	63 a
7 d CIDR + P4/E2 + PGF + E2	753	2.1	97	83 b	53 b

Values with unlike letters (a, b) within column differ $P < 0.01$; analyzed by Pearson's Chi-square test.

[a] Defined as the percentage of total potential recipients to which an embryo was transferred.

[b] Defines as the percentage of recipients to which an embryo was transferred that were subsequently confirmed pregnant.

Adapted from Looney et al. (2006) and Looney (2017).

Table 17. Pregnancy rate to ET by duration of CIDR (5,6,7, or 8 days) exposure in recipient cows (Ovagenix, Bryan, TX).

Duration of CIDR	No. synchronized	No. rejected (%)	No. transferred	No. ET pregnant (%)
5	123	18 (15.1)	105	43 (41)
6	240	27 (11.3)	213	102 (48)
7	1,533	182 (12.0)	1,350	743 (55)
8	380	44 (11.8)	336	178 (53)
Total	2,276	272 (11.9)	2,004 (88%)	1,066 (46.8)

[a] Defined as the percentage of recipients to which an embryo was transferred that were subsequently confirmed pregnant.
Adapted from Looney et al. (2006)

Pregnancy Loss

Embryonic Mortality

When there is potential to reuse recipients or to increase the probability of any female being pregnant at the end of the breeding season, not only is pregnancy status important, but the timing of pregnancy failure and whether there is still time in the breeding season for another mating opportunity also plays a key role in success or failure. Innskeep and Dailey (2005) reported that following successful fertilization of an oocyte, embryonic mortality (pregnancy loss up to d 42 of gestation) accounts for 57% of pregnancy failures in cattle, presumably referring to A.I. or natural service. As summarized by Innskeep et al. (2004), late embryonic loss, d 27 to d 42 of gestation, occurred in 2% to 6% of pregnancies in dairy heifers and beef cattle following A.I. or natural service. This agrees with the values found in Lamb (2002) and Beal et al. (1992), although Stevenson et al. (2003) was numerically higher at 10.5% pregnancy loss between roughly d 29-33 and d 54-61 of gestation (Table 19).

One could logically surmise that because embryos are transferred after development to blastocyst stage and morphological evaluation for normality, that embryonic mortality would be less than or equal to that of natural service; however, as with most reproductive technologies, the literature suggests that reproductive success is lower than that of natural service, assuming healthy breeding animals. Following the transfer of 673 good/excellent IVD embryos, Markette et al. (1985) reported that by d 46.5 post-estrus, pregnancy loss had occurred in 14.6% of recipients that were pregnant at d 24. In some cases, embryonic mortality is even greater. Heyman (1985) found an embryonic mortality rate of 32% between d 24 and d 60 after 28 pregnancies were initiated following IVD embryo transfer. In the same study, no significant difference was found in the embryonic mortality rate of fresh versus frozen IVD embryos between d 21 and d 90 post-estrus, which agrees with Chagas e Silva et al. (2002). Chagas e Silva et al. (2002) also reported a significant decrease in late embryonic (d 21 to d 60) mortality after the transfer of IVD embryos into dairy heifers, when compared to dairy cows. Based on work by Markette et al. (1985) and King et al. (1985), Markette et al. (1985) estimated that following the surgical transfer of 1000 IVD embryos, 63 pregnancies would fail between d 24 and d 60 post-estrus, resulting in an 8.6% late embryo mortality rate.

Throughout the industry, concern has also been raised about the survivability of IVP embryos. Farin and Farin (1995) reported that through d 215 post-transfer the probability of embryonic survival was similar between grade 1 IVD, grade 2 IVD and grade 1 IVP embryos. For grade 2 IVP embryos, however, there was a substantial decrease in probability of survival, with only 1 of 9 recipients pregnant 14 days after transfer. Wagtendonk-de Leeuw et al. (2000) found that transferred IVP co-culture and IVP synthetic oviduct fluid (SOF) cultured embryos had an

embryonic loss of 30% and 18.5%, respectively, between d 24 and d 52; while transferred MOET embryos underwent an embryonic loss of 23% over the same period of gestation (Table 18).

Table 18. Return pattern of recipients after transfer of an MOET, IVP co-culture, or IVP SOF embryos at the Holland Genetics recipient herd.

Parameter	MOET (n=465)	IVP co-culture (n=157)	IVP SOF (n=101)
Total Return (%)	253 (54.4)[a]	85 (51.5)[a]	47 (46.1)[a]
% return 0-31 d[1]	80.6[a]	68.2[a]	80.9[a]
11-18 d[2]	10.8[a]	19.0[b]	13.2[a]
19-23 d[2]	72.0	50.0	73.7
24- 31 d[2]	17.2	31.0	13.2
% return 32-52 d[1]	13.1	20.0	10.6
% return 53-73 d[1]	5.1	8.3	8.5
% return> 73 d	1.2	3.5	0.0

[1] Calculated as percentage of total return. No significant difference in distribution of returns over the 4 classes (0-31 d, 32-52 d, 53- 73 d, and > 73 d) among the 3 groups. Difference in distribution of return over 2 classes (0-31 d and > 31 d) among the 3 groups is statistically significant (P=0.05; Chi- square analysis).
[2] Calculated as percentage of return 0-31 d. Difference in distribution of return over the 3 classes is statistically significant (P<0.05; Chi-square analysis) among the 3 groups.
[ab] data with different superscripts in the same row are significantly different (P<0.05; Chi-square analysis)
Adapted from Wagtendonk-de Leeuw et al. (2000)

Fetal Loss

Reaching the fetal stage of development does not guarantee a successful pregnancy. Bellows et al. (1979) reported that of 10,595 natural service pregnancies determined at the conclusion of the breeding season, 2.8% resulted in fetal pregnancy loss. Following the transfer of IVD embryos, King et al. (1985) reported that out of 1776 2-month pregnancies 5.29% had aborted by month 7 of gestation. Chagas e Silva et al. (2002) found no significant difference in fetal mortality for fresh versus frozen IVD embryos, with a fetal mortality rate of 5.90% and 7%, respectively.

In one study, Wagtendonk-de Leeuw et al. (2000) concluded no difference between the abortion rate of AI (1.3% of 5,353 pregnancies) and MOET (1.1% of 2,242 pregnancies), while both differed significantly from IVP co-culture embryos (2.6% of 1,452 pregnancies) (Table 20). In a separate study, there was no difference between the abortion rate of AI (0.5%), IVP co-culture (0%) and IVP SOF (1.3%) (Wagtendonk-de Leeuw et al., 2000). Accounting for sexed IVF embryos, Xu et al. (2006) found no difference in fetal abortion rate between non-sexed IVF, sexed IVF, and IVD embryos. As is the case for most traits in cattle production, environment and management practices have great influence on the scale and variability of a specific trait, such as pregnancy loss.

Table 19. Late embryonic and fetal losses in lactating beef cows and primigravid beef heifers (A.I. or natural service).

Number of Pregnancies	Days of gestation at 1st diagnosis	Days of gestation at 2nd diagnosis	Interval (days)	Pregnancy Loss %	Pregnancy loss (% per day)	Reference
Lactating Cows						
138	25	45	20	6.5	0.33	Beal et al. (1992)
223	29-33	54-61	~26	10.8	0.42	Stevenson et al. (2003)
Primigravid						
149	30	60	30	4.0	0.13	Lamb (2002)
271	35	75	40	4.1	0.10	Lamb (2002)
105	30	90	60	4.8	0.07	Lamb (2002)
Overall: 525	30-35	60-90	30-60	4.2 (4.0- 4.8)	0.09 (0.07-0.13)	

Adapted from Santos et al. (2004)

Table 20. Percentage abortions, caesarian sections (C-sections), male calves, and calves with a congenital malformation in Study 1 and 2a.

	Study 1			Study 2		
Parameter	**AI**	**MOET**	**IVP co-cul**	**AI**	**IVP co-cul**	**IVP SOF**
% Abortions	1.3 (5,353)[2a]	1.1 (2,242)[a]	2.6 (1,452)[b]	0.5 (1,764)[a]	0 (110)[a]	1.3 (152)[a]
% Congenital malformation	0.8 (5,353)[a]	1.5 (1,089)[b]	3.7 (1,129)[c]	0.6 (1,764)[a]	3.7 (81)[b]	1.0 (97)[ab]
% C-sections	1.5 (3,313)[a]	8.4 (1,107)[b]	11.2 (1,179)[c]	0.7 (1,764)[a]	3.8 (80)[b]	8.3 (96)[b]
% Male	49.8 (5,353)[a]	53.7 (2,194)[b]	52.9 (1,415)[b]	52.8 (1,764)[a]	56.1 (110)[a]	54.9 (152)[a]

[ab] data with a different superscript in the same row within each study are significantly different ($P<0.05$; Chi-square analysis). [1]co-cul: co-culture; [2] numbers between brackets.
Adapted from Wagtendonk-de Leeuw et al. (2000)

Calf Loss

Of course, pregnancy survival to term does not ensure calf survival to its marketing endpoint. Bellows et al. (1979) reported that of 10,300 calving cows who conceived via natural service, 8% of calves died in the perinatal period and 2.9% died between the perinatal period and weaning. King et al. (1985) found that neonatal calf loss, birth weight, and calving assistance were similar between IVD ET calves and non-IVD ET calves. Wagtendonk-de Leeuw et al. (2000) found that when compared to AI calves, perinatal mortality was not affected by MOET; although calving ease was significantly reduced and gestation length was significantly increased.

Since its commercial application , much concern has been raised regarding the survival of IVP calves. Wagtendonk de Leeuw et al. (2000) reported that IVP calves tended to have higher perinatal mortality when compared to AI calves and significantly higher perinatal mortality when

compared to MOET calves. Similar results were found in Kruip and den Daas (1997). Significant increases in birth weight, gestation length and calving difficulty of IVP calves when compared to both AI and MOET calves were also found by Wagtendonk de Leeuw et al.(2000) (Table 21).

Table 21. LSM and SE of different parameters per type of calf (number of observations) produced by the statistical model of 282 OPU procedures by Holland Genetics at 2 different locations.

Parameter	AI (n)	MOET (n)	IVP co-culture (n)
Birth weight (kg)	42.7 ± 0.2^{a} (4,878)	43.4 ± 0.3^{a} (1,058)	47.1 ± 0.3^{b} (1,049)
Gestation length (d)	281.2 ± 0.2^{a} (4,946)	282.1 ± 0.2^{b} (2,139)	283.6 ± 0.2^{c} (1,358)
Perinatal mortality (%)	5.3 ± 0.4^{ab} (4,949)	4.6 ± 0.7^{a} (2,180)	7.5 ± 0.7^{b} (1,374)
Ease of calving[1]	2.4 ± 0.04^{a} (4,861)	2.7 ± 0.07^{b} (991)	3.2 ± 0.05^{c} (971)

[1] ease of calving was scored in 6 different classes (1= easy, to 6= very difficult).
[a,b,c] data with a different superscript in the same row are significantly different ($P<0.05$)
Adapted from Wagtendonk de Leeuw et al. (2000)

Abnormal Offspring Syndrome

Much of the concern regarding the survival of an IVP embryo from time of transfer through the neonatal period has been attributed to Abnormal Offspring Syndrome (AOS). AOS can be split into four categories. Type I AOS is associated with embryonic loss before day 42 of gestation; Type II describes fetal loss up to roughly 280 days of gestation; Type III is defined as neonatal mortality; and Type IV describes a surviving calf that exhibits a congenital defect that may or may not be anatomically or physiologically observable (Farin et al., 2015). The first indication of abnormality from IVP embryos was the occurrence of exceptionally large offspring at birth (Farin et al., 2015). Thus, AOS was originally termed, and is often still referred to, as Large Offspring Syndrome (LOS) (Farin et al., 2015); however, as noted previously, AOS is associated with much more than just increased birthweight. Conversely, as it relates to postnatal growth rate, origin of embryo and birthweight had no impact on performance (Wilson et al., 1995**;** McEvoy et al., 1998).

AOS is thought to be rooted in changes in epigenetic regulation because of differences between the maternal environment and that of maturation, fertilization, and culture media (Farin et al., 2015). Much of the blame for AOS has been placed upon the inclusion of serum into culture medium and somatic cell co-culture (Hill, 2014). In some cases, specific medium formulation has been found to play a role in the prevalence of AOS (Wagtendonk-de Leeuw et al., 2000), while in others it has not (Hasler, 2000).

Wagtendonk-de Leeuw et al. (2000) showed a statistical increase in caesarian sections (C-sections), birthweight, and calving difficulty in calves derived from IVP using co-culture and serum when compared to either calves from IVP using Synthetic Oviduct Fluid (SOF) or MOET (Table 22) (Table 23). When comparing TCM 199-BRL co-culture medium with serum to Ménézo's B2 (B2) co-culture with or without serum for the first 72 h of culture, Hasler (2000) found no difference in pregnancy loss, calving ease, or congenital defects. While the scope of adoption of IVP in recent years indicates a reduction in AOS concerns, there exists little to no literature on the current industry prevalence of AOS (Hasler, 2014).

Table 22. Percentage of abortions, perinatal mortality, caesarian sections (C-sections), stillborn, male calves and calves with a congenital malformation from embryos transferred into the recipient herd of Holland Genetics.

Parameter	MOET (n=34)	IVP co-culture (n=32)	IVP SOF (n=33)
% Abortions	5.9[a]	3.1[a]	6.0[a]
% Perinatal mortality	2.9[a]	3.1[a]	3.0[a]
% Congenital malformation	0[a]	3.1[a]	0[a]
% C- sections	2.9[a]	25.0[b]	0[a]

[a,b] data with the same superscript in the same row are not significantly different (P>0.05; chi-square analysis).
Adapted from Wagtendonk-de Leeuw et al. (2000)

Table 23. LSM and SE of different parameters per type of calf (number of observations) from embryos transferred into the recipient herd of Holland Genetics.

Parameter	MOET (n)	IVP co-culture (n)	IVP SOF (n)
Birth weight (kg)	41.3 ± 1.0 (30)[a]	46.4 ± 1.0 (30)[b]	42.3 ± 1.0 (30)[a]
Gestation length (d)	279.9 ± 1.3 (30)[a]	279.2 ± 1.3 (30)[a]	279.6 ± 1.2 (30)[a]
Ease of calving	2.9 ± 0.18 (29)[a]	4.0 ± 0.20 (22)[b]	3.4 ± 0.17 (30)[c]

[1] ease of calving was scored in 6 different classes.
[a,b,c] data with a different superscript in the same row are significantly different (P<0.05).
Adapted from Wagtendonk-de Leeuw et al. (2000)

Natural Service

If an operation wishes to calve out its recipients and capture added value from those that did not become pregnant via embryo transfer or experience embryonic mortality after the establishment of pregnancy, a producer may choose to expose the recipient herd to a natural service sire. Assuming a healthy, fertile bull, the fertilization rate of a natural service sire should fall

between 90-100%; however, for reasons described in the preceding pages, approximately 70% of fertilizations will result in a live calf (Lamb, 1999). Typically, the pregnancy rate at the end of the breeding season is much higher than what the percentage of surviving fertilizations indicates because cows usually have multiple opportunities to conceive within a given breeding season. In a 14-year summary of 12,827 cows, Bellows et al. (1979) reported a natural service pregnancy rate of 82.6%, following the breeding season, with the number of calves weaned per cow exposed at 71%. Other literature reports a post-breeding season pregnancy rate ranging from 59.3% to 98.8% (Lamb et al., 2008). Of course, cow type, natural environment, and management play a pivotal role in all reproductive outcomes.

Chapter 2 - Embryo Program Business Model

Just as a multitude of strategies and techniques exist as a means of embryo production and subsequent transfer, the business model of ET also offers an array of options. Whether an entity owns only donors, only recipients, both, or neither, represents the first of many flexible management decisions. Marketing also comes into question. Is the end-product a saleable embryo, a pregnant recipient, a weaned calf, or a developed yearling? An operation should design a business model that best combines both feasibility and profitability.

The ownership option involving the least number of business entities is to own both donors and recipients. While this may require a greater amount of feed, labor, and land resources available to the operation in question, it also allows for the most direct control over cattle management and health.

Another common practice is the implementation of a cooperator herd. Typically, in this scenario, a contract is agreed upon between the owner of the donor females/embryos and the owner of the recipients. The embryos produced by the donors of one operation are transferred into recipients of another operation. Oftentimes, the agreement includes details pertaining to calf health, data collection, and overall herd management. In many cases, the cooperator herd will sell the resulting progeny back to the owner of the donor females/embryos at weaning or sometime thereafter for an agreed upon premium over market price that is typically set as either dollars per cwt over market price or a flat bonus over the market value of the progeny in question. As always, there are distinct variations in the specific cooperator agreements and to try to account for them all is beyond the scope of this paper.

Many enterprises within the ET industry specialize in the commercial creation of pregnant recipients. These custom recipient operations might also be involved in commercial embryo

production or they may simply own and manage a recipient herd with the owners of embryos sending the custom recipient operator embryos that are set to be transferred. Typically, an agreement between the owner of embryos and the commercial recipient operation is made before the transfer of embryos to ensure that the embryo owner is obliged to purchase either the subsequent pregnant recipient or weaned calf from the custom recipient operator. A specific day of gestation is usually set as the day upon which pregnant recipients qualify to be purchased by the embryo owner. Weaned calf agreements may follow closely with the cooperator agreements that were previously described.

Many of the standard marketing options for an ET program have already been touched on, but they can be described further. If an operation owns a highly valuable donor, it may choose to sell an MOET flush or IVP session on that female. Often, included in the transaction is a guarantee of number of embryos or subsequent pregnancies. Taking it one step down the production chain, if a program has an over-abundance of embryos or wishes to capitalize on the embryos of a highly valuable mating, it may choose to sell frozen or fresh embryos before they are transferred. Another option is to sell pregnant recipients in the commercial recipient scenario as described previously, or the owner of the donor and/or embryos may also market pregnant recipients. If an operation chooses to calve out pregnant recipients, calves are often sold either around the time of weaning or developed until the time of a production sale.

Economics

In the business world, the ability to project the potential range of investment profit or loss and mitigate risk before making an initial investment serves as a crucial step towards the success or failure of an enterprise. The cattle feeding segment of the beef industry has adopted similar techniques with advanced breakeven projections that utilize a combination of performance

predictors and risk management practices. Conversely, a majority of the seedstock sector, particularly as it pertains to the use of embryo transfer (ET) technology in seedstock operations, seems to place more trust in intuition and optimism rather than proven methods of investment analysis.

Whereas it stands to reason that numerous, highly successful seedstock operations must implement some degree of strategic scrutiny before committing the extensive amount of both financial and labor resources into ET programs, the depth of the evaluation comes into question, especially for operations without years of experience to reinforce assumptions. The lack of financial investigation can be rationalized. Hasler (2003) stated that although the quantity seems to be declining, traditionally a significant volume of beef ET was conducted by hobby farmers, funded through sources not directly related to the beef business. One also might argue that ET's primary focus of genetic improvement has greatly overshadowed any consideration of short to mid-term financial gain. In addition, dynamic environments, small sample size, poor record keeping, and the immense variability associated with bovine reproduction account for several more potentially limiting factors.

Profitability of an ET program depends on the marketability of an ET program's end-product (embryos, pregnant recipients, progeny, etc.) and the resource expense required to produce marketable animals or embryos. The previous descriptions of the variability in the outcomes of embryo production, pregnancy rate, and other biological factors indicate a great deal of uncertainty in the number of marketable animals or embryos that can be generated per flush or aspiration or over a specific time-period. The financial risk associated with such biological uncertainty requires adequate understanding for an ET program to establish realistic expectations rather than emotional decision making based on optimistic hope or over-exaggerated pessimism.

Ultimately, operators must decide whether ET programs of any type serve as an economically viable means to increase rate of genetic improvement or take advantage of marketing opportunities. To date, few tools have been created to establish the realistic expectations required for sound decision making, especially when comparing MOET and IVP of *Bos taurus* cattle in a temperate climate.

Stochastic Modeling

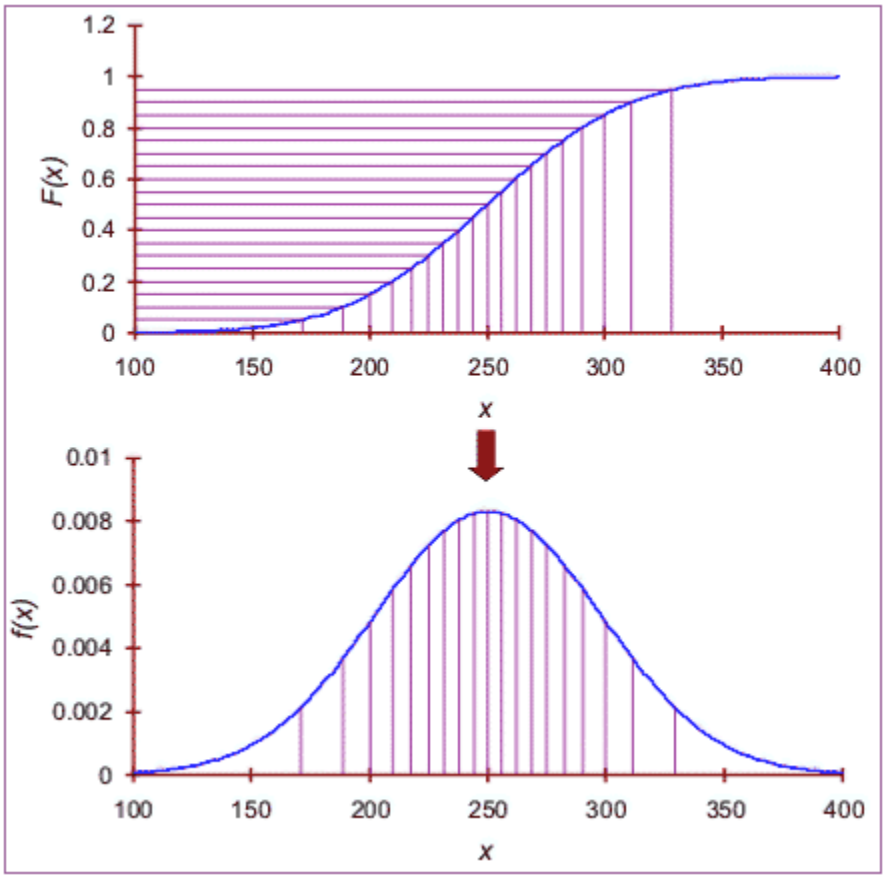
Latin Hypercube Sampling

One method applicable in accounting for the previously described biological uncertainties is the use of stochastic modeling and simulation. In contrast to deterministic modeling that uses fixed input variables, typically a mean value, to conduct an analysis and thus, generates outcomes that are almost nonexistent in the natural world, stochastic modeling utilizes a sampling of possible variable values from a defined probability distribution. Based upon the distribution, a likelihood of occurrence is tied to each variable value.

Simulation strategies have been developed to appropriately sample stochastic variables from a defined distribution. Applying the Latin Hypercube Sampling (LHS) variation of Monte Carlo simulation (Iman and Shortencarier, 1984), a sample value from the descriptive distribution associated with each stochastic variable is included in an iteration of the simulation. Through large numbers of iterations with dynamic combinations of variables, the process culminates in a distribution of possible outcome values. Unlike simple random sampling, LHS prevents clustering by accounting for the probability of drawing a value from a set range of values. This is accomplished by segmenting the probability density so that the area under the curve is the same for each segment (Figure 1).

Consider *k* variables, with the probability density of each variable split into *n* segments. Applying LHS, one value for variable X_1 is randomly selected from each of *n* segments. These *n* values are then randomly paired with *n* values from X_2. The X_1, X_2 pairs are then combined randomly with X_3 values and so on, for all *k* variables in question. The number of intervals within the probability or cumulative density function is equal to the number of iterations to be run, so that at the conclusion of the simulation a single value has been sampled from each interval (Epix Analytics). Iman and Shortencarier (1984) explain the intricacies in further detail.

Figure 1. Graphical depiction of LHS methodology.



From epixanalytics.com
Identical data is represented by each graph.
F(*x*) represents the fraction of values numerically less than or equal to the corresponding value on the horizontal axis, cumulative density function (cdf).
f(*x*) represents the probability of selecting a value along the horizontal axis and under the curve, probability density function (pdf).
The area between each pair of line segments and under the curve is equal.

Application of Stochastic Modeling

Beltrame et al. (2010) applied stochastic Monte Carlo simulation to an economic analysis of MOET and IVP in Brazil, presumably based upon Nelore production statistics. The goal of the simulation was to analyze the economic impact of recipient management and synchronization protocols in both MOET and IVP production systems. By allowing economic and production parameters to be altered, Beltrame et al. (2010) conducted a sensitivity analysis to determine the influence of different variables. Based on the simulation model's output of NPV from the marketing of pregnant recipients, it was determined that optimizing the number of recipients per donor was the most effective means of reducing cost per pregnancy. Of further interest, Beltrame et al. (2010) found that in this *Bos indicus* based model, the IVP program produced more pregnancies than MOET in all evaluated scenarios. Fixed-time embryo transfer (FTET) also eliminated the need for estrus detection and reduced the number of unused recipients, which lowered the cost per pregnancy (Beltrame et al., 2010).

Investment Analysis

Business enterprises have several capital budgeting/investment analysis techniques available for use. One of the most common and the most conceptually correct (Briggeman, 2014) is net present value (NPV), with the equation: $\text{NPV}=\sum_{n=1}^{N}\frac{ANCF_n}{(1+i)^n}+\frac{RESID_N}{(1+i)^N}-\text{INV}$; where *N*=life of investment; *i*= discount/interest rate; *ANCF*= annual net cash flows; *RESID*= residual value; and *INV*= original investment cost. The basis of NPV encompasses the idea that future cash flow is discounted to today's value at the opportunity cost of capital, relative to the initial investment outlook to represent the economic profit of the proposed investment (Briggeman, 2014).

An NPV greater than zero represents positive economic profit; whereas, a negative NPV signifies economic loss. Comparison between investments using NPV is only possible if the investments in question have identical time horizons. For potential investments with different investment lives, NPV can be adjusted to a measure of annual economic profit. Annuity equivalent NPV is represented by the equation: ANPV=NPV$\left[\frac{i}{1-(1+i)^{-N}}\right]$; where i= discount/interest rate; and N= investment life in years.

Financial feasibility must be assessed by analyzing the ratio of projected cash flows to debt payments due for a specific time frame (Briggeman, 2014).

Risk Analysis

At its most fundamental understanding, risk is a subjective measure where an identical situation may represent different levels of risk for different individuals. Thus, the nature of risk makes it difficult to quantify in a numerical sense. Levy (2006) offers a definition of financial risk: "A risky position is a situation in which there is more than one financial outcome, say x_1, x_2, …, x_n and, for at least one value x_i, $0 < p(x_i) < 1$, where p denotes a probability of x_i, occurring. Note that if there is one value such that $0 < p(x_i) < 1$, there must be at least one more observation, x_j, with $0 < p(x_j) < 1$. The total probability must be equal to 1; $\sum p_i = 1$. By this definition, the future value of a risky asset may have more than one value x_i, with $0 < p(x_i) < 1$." Furthermore, noting the difference between risk and uncertainty, Knight (1921) defines risk "as a pair of values $(x, p(x))$ (with at least one value x_i for which $(0 < p(x_i) < 1)$) such that both x and $p(x)$ are known. Uncertainty is a pair $(x, p(x))$ such that the possible value of x are known but $p(x)$ is unknown," (Levy, 2006). Therefore, because the likelihood of a specific outcome in most biological traits, including most bovine reproduction measures, is merely an estimate, biological uncertainty, rather

than biological risk, represents the primary concern of most beef production systems. That said, it is still acceptable to use the terms risk and uncertainty interchangeably (Levy, 2006).

Ample effort has been applied to overcoming the inherent challenge of ranking investment or financial scenarios based on risk level. One method of accounting for risk within an NPV calculation is to adjust the risk-free rate, typically represented by the return on treasury bills, by incorporating the standard deviation of a business's normal risk and the standard deviation of a project's expected return (Briggeman, 2014). Levy (2006) reviews several other existing methods for the numerical ranking of financial risk. Domar and Musgrave Risk Indexes, Roy's Safety First Rule, Baumol's Risk Measure, Value at Risk, and Minimax Regret, all mathematical attempts at creating the most appropriate technique for quantifying risk, are detailed in Levy (2006). Perhaps, the most useful means of providing the end-user with the information needed to make an individualized risk assessment is through the interpretation of mean and variance and/or the evaluation of a distribution of probability density.

Synopsis

Since its inception as a commercial application, ET has had a profound impact on the cattle industry by creating a means to propagate the genetics of elite females. More recently, through sexed-semen technology, ET has allowed for progressive producers to respond to market signals by predetermining the sex of resulting progeny. While the adaptation of technology serves as a crucial mode of industry advancement and improvement, financial feasibility and risk must be assessed when developing a strategy for implementation. The potential inefficiencies and biological uncertainties associated with ET make such financial risk assessment a challenging prospect. Producers are not only faced with the decision of whether to apply ET to a breeding program, but on what scale; whether to rely on MOET or IVP; use unsorted or sex-sorted semen;

whether to utilize superovulation and/or estrous synchronization; and if or how to market the resulting product of an ET program. To accomplish the objectives of conducting risk and sensitivity analysis, along with the potential for optimization, a circumstantial, stochastic prediction model utilizing @Risk© software to generate comparable economic values as an aid in the ET decision making process has been created.

Chapter 3 - Materials and Methods

To accomplish the objective of creating an economic risk analysis tool for user-defined ET programs, a circumstantial, stochastic prediction model utilizing @Risk© software to generate comparable economic values as an aid in the ET decision making process has been created. User-defined, deterministic parameters are accompanied by stochastic variables of economic importance to generate a flexible model. More realistic than the use of means in deterministic models, distributions defining the biological uncertainty for a multitude of reproductive outcomes are estimated through extensive literature review and limited industry sources. Applying the Latin Hypercube Sampling (LHS) variation of Monte Carlo simulation, a sample value from the descriptive distribution associated with each stochastic variable is included in an iteration of the simulation. Through large numbers of iterations with dynamic combinations of variables, the process culminates in a distribution of possible values for the annuity equivalent net present value (ANPV) associated with the model described scenario of IVD or IVP. Finally, using the probability distribution of ANPV, a decision maker can assess the economic risk linked to a user defined ET program. The model can be applied as a tool to compare different user-defined parameters and ET production methods or protocols, to answer the question of if and/or how a producer should carry out an ET program.

The outcome of an ET program is an accumulation of a multitude of steps in the production process. Tied to these steps are decisions that need to be made. First, a producer must decide how to generate embryos. Will MOET or IVP be used? Will a follicular stimulation and/or follicular synchronization protocol be implemented? Next, a decision must be made between the use of conventional, unsorted semen or semen sorted for sex. Once healthy embryos have been generated it must be determined if those embryos are to be transferred into recipients as fresh embryos or

cryogenically frozen and transferred later. If the decision is made to transfer embryos fresh, a population of recipients must be in estrous synchrony with the donors for the embryos to be successfully implanted. As described in the literature review, each combination of decisions may have an alternate impact on production success, depending on management, cattle type, and luck.

Once embryos are transferred to recipients, pregnancy is not a guarantee. In some cases, the embryo never properly implants into the uterus or is not recognized by the recipient and she comes into estrus again, with the timing of her normal estrous cycle. In other situations, the pregnancy may be lost or aborted later in the gestation period. For recipients that either return to estrus early enough in the breeding season, there is opportunity to either receive another embryo or be naturally serviced by a clean-up bull. The establishment of pregnant recipients also presents a marketing opportunity for many operations. If the pregnant recipient is retained through full gestation, the parturition process brings a multitude of other risk factors into play. Frequency of dystocia, death in the perinatal period, or other health concerns can be influenced by whether the calf is a result of MOET, IVP, or natural service.

A live calf at birth must then survive to weaning. At that time, a marketing plan must be executed. Will all calves be marketed at weaning or only the natural service and cull calves? A producer may wish to add value to calves by developing them and selling them later.

Throughout this entire process, ownership or contracting decisions must be made. While it is common for one operation to own the entire production process from donors to recipients to the resulting progeny; other operations may send embryos to a separate operation and buy back the subsequent pregnant recipients or establish a contract where ET calves are purchased after being weaned.

The process of ET allows for immense flexibility accompanied by a great deal of variability in results. An operation should strive to make the most appropriate combination of process decisions to fit their desired outcome.

Model

The model allows for the comparison and analysis of the production and economic factors of eight primary ET protocols.

1. MOET: Unsorted Semen
2. MOET: Sex-Sorted Semen
3. IVP: No Ovarian Stimulation (NS), Random OPU Interval, Unsorted Semen
4. IVP: No Ovarian Stimulation (NS), 3-4 d or 14 d OPU Interval, Unsorted Semen
5. IVP: Follicular Synchronization and Ovarian Stimulation (SS), Unsorted Semen
6. IVP: NS, Random OPU Interval, Sex-Sorted Semen
7. IVP: NS, 3-4 d or 14 d OPU Interval, Sex-Sorted Semen
8. IVP: SS, Sex-Sorted Semen

In all sections of the model, unless otherwise noted, the number of embryos, recipients, pregnancies, calves, etc. are determined using a binomial distribution with *n* number of trials and success probability, *p*. For each iteration of the simulation, probability, *p*, is sampled per LHS from the distribution around the mean for the trait in question.

It is assumed that all donors, recipients, and bulls are healthy and fertile with all females having a 21 d estrous cycle. Also, assume all purchases occur on d 1 of the fiscal year.

Figure 2 depicts the production system of MOET IVD ET (protocols 1 and 2); while Figure 3 depicts the IVP ET production system (protocols 3 through 8). All the economically relevant probability distributions described in the "Distributions of Biological Uncertainty" section illustrate the potential range of possibilities when transitioning (arrows in Figure 2 and Figure 3) from one stage of production (rectangles in Figure 2 and Figure 3) to another. The results of each stage of production serve as inputs for the subsequent transition to the next stage of production.

Figure 2. MOET IVD ET production system

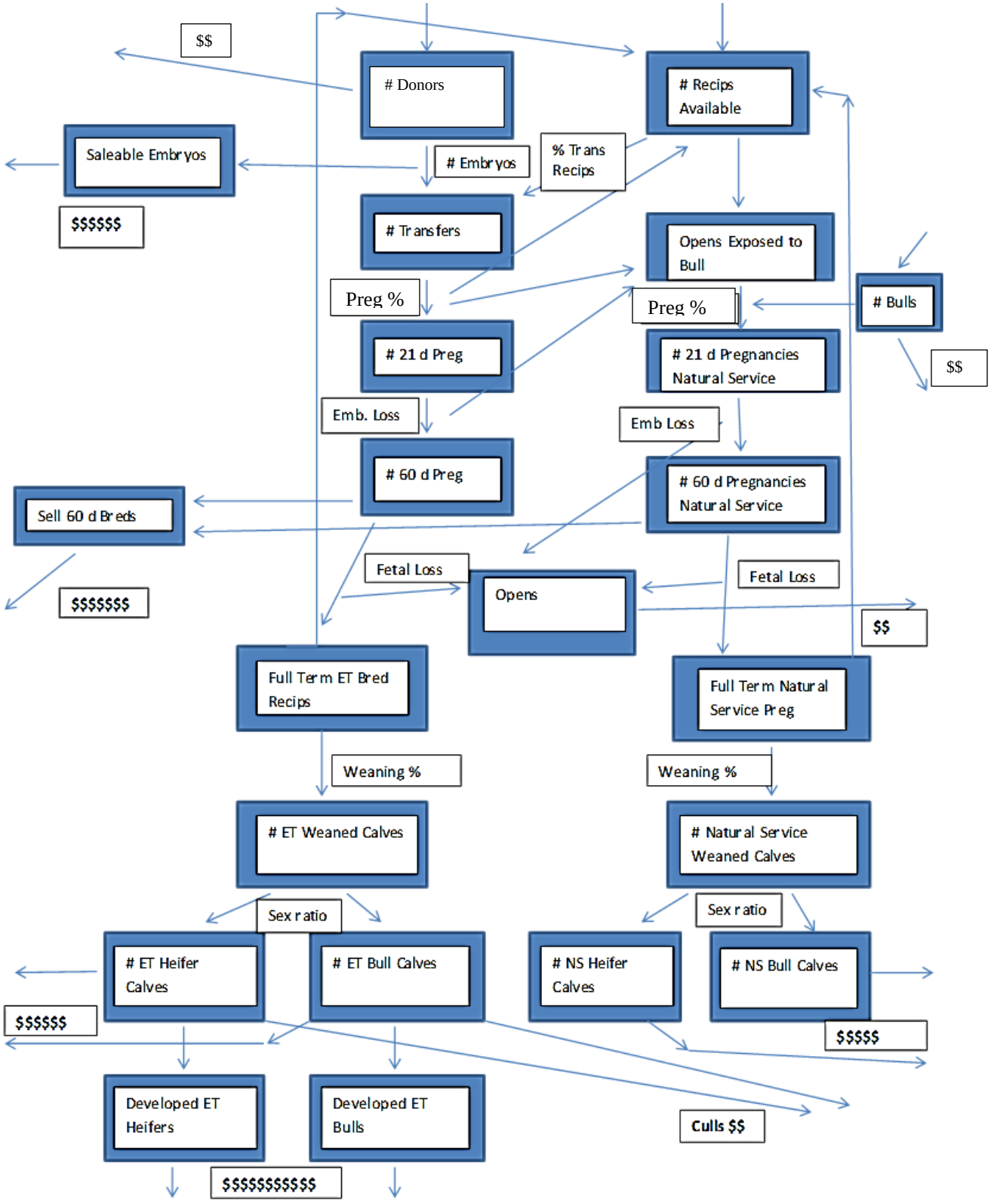
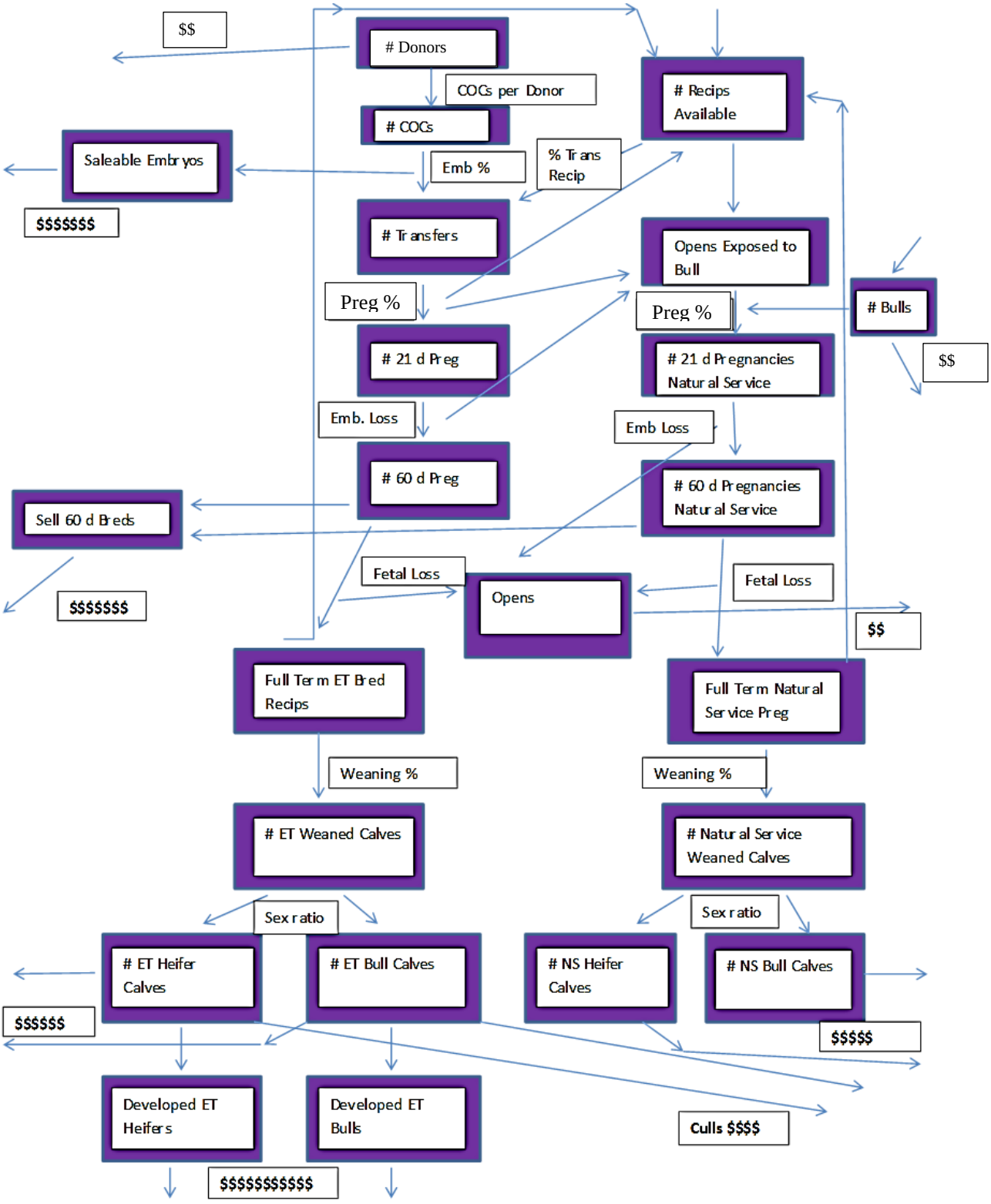


Figure 3. IVP ET production system



MOET: Unsorted Semen Embryo Production

Using the deterministic variable for the number of donors in the ET program as a starting point, the number of donors collected following each round of superovulation is calculated using a binomial distribution with n number of donors in the ET program and probability, *p*, of a donor showing signs of estrus and being subsequently inseminated and flushed. The probability, *p*, of a donor showing signs of estrus equals 1 minus the probability of donors not showing estrus after superovulation; this is sampled stochastically per LHS from the probability distribution of the mean rate of donors not showing signs of estrus following superovulation. The number of donor superovulations is entered as a user-defined deterministic variable. The number of embryos collected per flush is sampled stochastically per LHS using the negative binomial distribution describing the number of embryos retrieved per collection.

A user-defined, deterministic variable with a minimum of 30 d, determines the time interval between flushes. To maintain a structured time frame for calving season within the model, the number of MOET flushes is limited to 3.

The number of embryos transferred is dependent on the number of embryos available and the number of synchronized recipients deemed qualified to receive an embryo. It is assumed that a round of fresh transfers accompanies every round of embryo collections. For the model in question, if there is an overabundance of embryos compared to recipients, the left-over embryos are frozen and transferred later, in the case that there are more available recipients than embryos in a later transfer round or in a specific frozen-thawed transfer session after all rounds of embryo collections have taken place. It is assumed that if unused embryos remain at the end of all transfer rounds, they are marketed as frozen embryos.

The user-defined variable for the number of recipients purchased at the start of the ET program sets the size of the recipient herd. All recipients are purchased as open, fertile females without a calf at side. The number of recipients synchronized is determined using a sampled value from the distribution for the percentage of synchronized recipients qualified for transfer to estimate the number of synchronized recipients required to match the number of available embryos. Ultimately, the number of recipients deemed qualified for transfer is computed using a binomial distribution with probability, p, of a recipient being qualified for transfer drawn per LHS from the aforementioned distribution.

Embryo transfer pregnancy rate at 21 d (d 14 after transfer) following transfer of IVD embryos is split into fresh ET pregnancy rate and frozen-thawed ET pregnancy rate. After the establishment of a 21 d pregnancy, there is opportunity for pregnancy loss. The distribution for the mean of pregnancy loss is separated into a distribution for pregnancy loss between d 21 and d 60 of gestation and a distribution for pregnancy loss between d 60 and term.

Assumed within the model, the earliest a recipient can return to estrus following a synchronized estrus, regardless of whether she cycled or received an embryo or not, is 21 d post-synchronized estrus. Any recipient that is not pregnant at d 21 reenters the pool of available recipients, depending on whether the ET program has concluded for the breeding season in question. Recipients that experience pregnancy loss between d 21 and d 60 are eligible for exposure to a natural service sire, depending on the timeframe of the ET program; the time interval between transfer rounds; the transfer round that the recipients in question received transfer; and the length of bull exposure. All recipients, unless selling as a bred recipient with a confirmed 60 d pregnancy, are exposed to a natural service sire for a user-defined length of time after all transfer rounds have been completed. All recipients that experience pregnancy loss between d 60 of gestation and term

are considered open at the end of the breeding season and are not eligible for natural service. Any recipient that aborts a natural service pregnancy, regardless of the period of gestation, is considered open. For all recipients, it is assumed that pregnancy is determined by cyclicity or rectal palpation/ultrasound at d 60 of gestation. Final pregnancy and open totals are a result of binomial distributions using probabilities sampled from the pregnancy establishment and pregnancy loss distribution as characterized in the "Distributions of Biological Uncertainties" section.

MOET Sexed Semen Embryo Production

All methods and calculations for simulating the production of sexed, IVD embryos are identical to those used for the simulated production of unsorted, IVD embryos, except for the probability distribution of the number of embryos generated per flush. Sexed embryos are generated through the use of sex-sorted semen.

IVP Embryo Production

Most of the structure of the simulation model for IVP embryo production is identical to the production of MOET embryos. The major difference in the model comes with two steps specific to IVP. The first distinct step is OPU by follicular aspiration. According to the user-defined number of donors and number of OPUs per donor, the number of viable oocytes collected per OPU is sampled per LHS from the probability distribution describing the number of viable oocytes collected per OPU. Next, the blastocyst development rate matching the relevant stimulation protocol and semen type is applied to the viable oocytes.

The product of the number of viable oocytes and blastocyst development rate represents the number of transferrable embryos. Following transfer, distributions for the 21 d pregnancy rate; pregnancy loss between d 21 and d 60; and pregnancy loss between d 60 and term are applied for

either fresh IVP ET or frozen IVP ET, depending on embryo type. The remaining model organization remains constant for the differing types of embryo production.

Revenues and Expenses

While the model may or may not generate different values for the following sections, depending on the scenario and appropriate distribution to be sampled per LHS, the basic structure of the simulation model is identical for all types of embryo production.

Cattle Maintenance Expenses- Bred Recipient

The costs in this section are applied to the total program expense when marketing bred recipients. According to user-defined inputs, the average of individual purchase cost, annual health program cost, and annual feed cost are compiled to calculate a total expense for donors and bulls. Unless specified otherwise, donors and bulls are considered to be owned for a full fiscal year, as it is possible and rather likely that their useful life spans more than one iteration of a bred recipient marketing program. Average individual purchase cost and health program cost are summed across the herd to generate a total recipient expense. Feed costs are allocated based on the length of time, in months, that purchased recipients are owned before marketing. It is assumed that for the marketing of owned or purchased recipients the full time of ownership occurs in one fiscal year.

Cattle Expenses- Weaned Calf

As in the scenario above, the cost of purchase, feed, and health program are totaled for donors and bulls. To accurately portray different feed costs for cows that calve at different times of feed and forage availability, the annual feed cost for recipient females is split between feed cost for the length of the calving season before available grazing (see "IVD Unsexed Weaned Calf Feed Costs- Pre-Grazing Season Calving") and the rest of the fiscal year. Annual feed costs are only applied to open females for the length of the fiscal year that they are still in the herd. In year one

of the program, purchase costs of recipients are totaled with feed and health program costs. For subsequent years of the ET program, recipient replacement cost (the cost of replacing open recipients to fit the recipient herd size defined by the user) is combined with recipient maintenance costs to generate a total recipient expense.

Weaned Calf Feed Costs- Pre-Grazing Season Calving

While calves born at the beginning of calving season tend to be heavier at weaning (assuming all calves are weaned on the same day, as in this model), there is also a potential trade-off in the cost of required nutrients for early calving cows, depending on the relationship between calving season and the availability of forage and/or cost of feed. By incorporating user-defined inputs for ration cost, expected cow dry matter intake (DMI) for the third trimester of gestation, expected cow DMI postpartum, and the length of the calving season (in days) before the grazing season, the cow feed costs associated with calving at different times within the calving season can be estimated. The number of bred recipients from each respective round of ET and cycle of natural service dictate the dispersion of calving throughout the calving season. Thus, the number of third trimester and postpartum days before the grazing season can be determined based on when conception occurred during the breeding season. The resulting total pre-grazing season, calving season cost is built into the annual recipient feed cost that is used in the "IVD Unsexed Production Cattle Expenses- Weaned Calf" section.

Donor Protocol Cost

The number of doses of exogenous reproductive hormones and the cost per dose, as user-defined, are combined over the total number of superovulation protocols in one ET breeding season. Total semen cost, based on cost per dose and doses required is also accounted for, along with the total embryo collection cost based on number of procedures and cost per procedure. If

there is an overabundance of embryos compared to recipients, freezing costs are also accounted for. Furthermore, costs from non-vet labor hours required for superovulation and embryo collection are described in this section of the model.

Recipient Protocol Cost

With the user-defined costs of exogenous reproductive hormones, embryo transfer, pregnancy determination, determination of sex of pregnancy, and non-vet labor combined with the amount required of each respective resource, the total cost of recipient protocols can be applied.

Weaned Calf Preconditioning Cost

Several user-defined costs go into the estimation of weaned calf preconditioning costs. They include: Daily Backgrounding Cost per Head; Vaccine Cost per Head; and Treatment Cost per Head. The total number of head that go through the preconditioning program prior to marketing is determined from the simulated number of calves that survive to weaning. Total Backgrounding Head Days is calculated by multiplying the number of weaned calves by the user-defined preconditioning days. It is assumed that post-weaning mortality is zero.

Bull/Heifer Development Cost

Development Expense is determined by coupling the Vaccine Cost per Head; Treatment Cost per Head; Miscellaneous Development Cost per Bull (breeding soundness exam (BSE), ultrasound, registration, etc.); Miscellaneous Heifer Development Cost per Heifer (Brucellosis vaccination, reproductive tract score, registration, etc.); Daily Bull Development Cost; and Daily Heifer Development Cost with the number of bulls and heifers undergoing development and the duration (days) of development.

It is assumed that all natural service sired calves are commercial; thus, all natural service sired calves are marketed after preconditioning according to weight and the feeder calf pricing

slide within the model. The user-defined cull rate determines the number of ET culls with respect to a simulation based on *n* (number of ET bulls and ET heifers) number of Bernoulli trials. Within the model, all cull calves are marketed as preconditioned feeder calves. The expense associated with the preconditioning of naturally sired and cull calves is determined in the same manner as described in the Weaned Calf Preconditioning Cost Section.

Total Expenses- Owned Donors

Subject to the scenario in question, expenses from the sections previously described are compiled for all scenarios in which the ET program under consideration owns the donor females used in the ET program. The specific costs included in the total program expense depend on embryo production strategy; ownership of recipients; and marketing strategy.

Total Expenses- Custom Recipient

Again, according to the specifics of a given scenario, expenses from the cost calculating sections previously described are combined for all scenarios in which the ET program does not own any donor females, but does own and manage a recipient herd. The particular expenses that are incorporated into the total depend upon the embryo production or purchase strategy and the marketing scheme.

Embryo Revenue

If embryo production out-paces the availability of recipients, excess embryos are frozen and marketed at a user-defined price per embryo. An individual price is assigned unsexed embryos, bull embryos, and heifer embryos. The number of excess embryos, as calculated by the appropriate embryo production page, is multiplied by the individual price for the appropriate embryo type. This dollar value is incorporated into the total revenue of the ET program.

Bred Recipient Revenue

It is assumed that all bred recipients are marketed after d 60 of gestation at the expected value, as defined by the model user. An expected individual market value is assigned to a pregnant recipient carrying an embryo of unknown sex; a pregnant recipient carrying a bull embryo; a pregnant recipient carrying a heifer embryo; a pregnant female carrying a naturally sired calf; and an open female. The number of recipients of each pregnancy type is drawn from the appropriate embryo production page. Market uncertainty is not accounted for regarding revenue from the sale of bred or open females. Sale price is fixed. A binomial distribution within the model determines the number of ET pregnancies of each sex for each iteration of the simulation. For ET production using unsexed semen, an extra veterinary expense is applied for determination of the sex of pregnancy. Multiplying the number of females of each pregnancy type by their associated value and summing the total values yields a revenue value that is included in the total revenue of an ET program that markets pregnant recipients.

Weaned Calf Revenue

The market value per weaned calf is determined by the feeder calf slide (prices adjustable per current market) and a user-defined premium for ET bull calves and another user-defined premium for ET heifer calves. The price slide is based on the current market price of feeder steers. Heifer calves are discounted to 92% of the price per pound of steer calves (Schulz et al., 2009). It is also possible to base an ET calf premium on dollars/lb. All calves are weaned on the same day. Thus, to account for differences in weaning weight, ET rounds and natural service cycles are split according to expected calving date to form calving groups. Weaning weights are determined by the user-defined growth expectations of calves and the anticipated calf age (in days) at weaning. Growth expectations in terms of average daily gain (ADG) (pounds per day) are deterministic

variables. All weaned calves undergo a preconditioning period of a user-defined length, in days. Calf weight following preconditioning is a product of the number of days of preconditioning and the expected calf performance, as defined by the model user.

The number of calves weaned within each calving group is calculated from the number of females carrying a pregnancy to term within each calving group and the percentage of calves that survive to weaning. The number of calves that survive to weaning is based on a binomial distribution with average probability of survival, p, and number of pregnancies, n, maintained to term. For unsorted semen, a binomial distribution also determines calf sex with the probability, p, of bull calves at 0.5 and n equal to the number of pregnancies maintained to term.

By combining the applicable calf sex and weight with its associated price per pound and premium for ET calves, the individual calf value is determined. Summing the values associated with each calving group and combining that figure with the total value of open recipients yields a revenue figure that is incorporated into the total revenue of an ET program selling calves after weaning and a preconditioning period.

ET Bull/Heifer Development Revenue

ET Bull and ET Heifer Development Revenue accounts for ET programs that develop ET calves beyond preconditioning and sell them in a production sale or similar marketing strategy. All naturally sired calves are sold after preconditioning, in the same manner as described in the Weaned Calf Revenue section. A user-defined cull rate sorts off the appropriate percentage of ET calves of each sex to be sold after preconditioning. The weight associated with the cull calves in question is the average of the entire group of ET calves at the end of the preconditioning phase. All culling of ET calves is done at the conclusion of preconditioning. The number of ET calves of

each sex is determined using a binomial distribution, in the same manner as described in the previous section.

The distribution of the average price per ET bull and the distribution of the average price per ET heifer can be an estimated input based on expectations or can be constructed using past sale data. A random value, sampled per LHS, drawn from the price distribution for ET bulls and the price distribution for ET heifers is multiplied by the number of ET bulls and ET heifers, respectively, to generate a total ET calf value for each iteration. The value of ET bulls, ET heifers, cull ET bulls, cull ET heifers, natural service sired calves, and open recipients are summed to produce a revenue figure that is included in the total revenue for an ET program selling developed bulls and heifers.

Total Revenue

In this spreadsheet, revenue streams are combined for the scenario in question depending on marketing strategy. Potential marketing schemes include sale of embryos; sale of bred recipients; sale of weaned/preconditioned calves; and sale of developed ET bulls and developed ET heifers. If the operation in question owns the donor females, the revenue from the sale of any excess embryos is always combined with revenue from the sale of live animals. Within this model, an operation may only market live animals by one method within a set scenario, except for the sale of naturally sired calves and cull ET calves immediately following preconditioning in a developed ET bull/heifer marketing strategy.

Annual Cash Flow

Total expenses and total revenues are calculated on an annual basis. Excluding initial investment expense, the total expenses and total revenues for a given scenario are combined to yield an annual cash flow figure. Regarding the sale of ET progeny, whether sold after

preconditioning or development, it is assumed that revenue occurs in the same fiscal year as the birth of said calf. The final annual cash flow figure can then be used in Net Present Value (NPV) calculations.

Net Present Value/ Annuity Equivalent Net Present Value/ Return on Investment

NPV, with the equation: NPV=$\sum_{n=1}^{N} \frac{ANCF_n}{(1+i)^n} + \frac{RESID_N}{(1+i)^N}$-INV; where N=life of investment; i= discount/interest rate; ANCF= annual net cash flows; RESID= residual value; and INV= original investment cost is used to put ET program profitability into economic terms. N and i are user-defined variables; while RESID and INV are calculated by multiplying the number of donors, recipients, and bulls by their associated, user-defined residual value per head and initial value per head, respectively. ANCF values are derived from the annual cash flow section previously described.

Comparison between investments using NPV is only possible if the investments in question have identical time horizons. For potential investments with different investment lives, NPV is adjusted to a measure of annual economic profit. Annuity equivalent NPV is represented by the equation: ANPV=NPV$\left[\frac{i}{1-(1+i)^{-N}}\right]$; where i= discount/interest rate; and N= investment life in years. The outcome of the model results in a probability distribution for ANPV and NPV based on the simulation iterations of the model.

Return on investment (ROI) is calculated as: $ROI = \frac{R-E}{E}$; where *R*= total revenue over the life of the investment; and *E*= total expense over the life of the investment. A probability distribution of ROI, constructed using the ROI for each iteration of the simulation, is a result of running the model.

Within @Risk ©, model outcome can be analyzed by designating specific cells as outputs. The corresponding output values of each iteration of the simulation for that particular cell can be

displayed using different types of graphs, including probability density, relative frequency, discrete probability, cumulative ascending, and cumulative descending. Furthermore, the raw iterative values can also be viewed as part of the statistical output. Any cell within the model that is calculated using input variable values is eligible to be viewed as an output cell.

Assumptions

While many assumptions have already been mentioned in previous discussion, the following list contains all assumptions pertinent to the model.

General Model Assumptions

- No correlation between traits/measurements
- All recipients enter the system as purchased opens
- All calves weaned same day
- If calf lives to weaning, it lives through development

Reproductive Model Assumptions

- Healthy donors, recipients, and bulls
- 21 d estrous cycles
- ET on d 7 following the onset of estrus
- Recipients synchronized within 24 h of donor
- Normally cycling donors and recipients
- ET program is seasonal, not continuous
- MOET IVD is limited to 3 flushes/breeding season

Embryo Production Model Assumptions

- Recipients that return to estrus on d 21 reenter available recipient population, depending on ET round and time interval between flush/OPU.

- ET recipients that experience pregnancy loss between 21 d and 60 d of pregnancy are eligible for natural service, depending on interval between transfers and length of bull turnout.
- ET bred recipients that experience pregnancy loss between d 60 and term are not eligible for natural service.
- Natural service bred recipients that experience pregnancy loss at any point after d 21 of gestation are not eligible for another natural service conception.

Revenue Model Assumptions

- Bred recipients are sold carrying a minimum 60 d pregnancy with no calf at side.
- Calf development revenue occurs in same fiscal year that calves are born.

Expense Model Assumptions

- Expenses not included:
    - Overhead or whole ranch costs
    - Facilities
    - Random vet costs (pulling calves, emergencies, etc)
    - Labor when not applied to ET program
    - Equipment Expense
    - Taxes

By applying the methodology and calculations as described in the preceding pages, ET program scenarios are simulated to compare economic profit potential and analyze contribution factors. If a user feels that past production records for a certain operation or specific donor female better describe the reality of a variable than the stochastically sampled distributions, deterministic override options are available within the model interface.

Distributions of Biological Uncertainties

The primary challenge in creating this model is to account for the variability in the value of input factors found in the ET process that cannot be accurately described by one deterministic value, such as a mean. Furthermore, this model attempts to tie the entire ET production process together through a system of calculations that draw upon the outcome of the prior step in the production process.

To determine the expected number of pregnant recipients resulting from a fresh transfer situation, one must first predict the number of transferable embryos produced by the donor(s) female(s) and what percentage of recipients will respond to synchronization and be deemed eligible to receive an embryo. Then the number of pregnant recipients is dependent upon the pregnancy rate of the recipients that received an embryo. Many factors out of the direct control of management have a powerful influence on the number of transferable embryos produced by the donor, the percentage of synchronized recipients that are eligible to receive an embryo, and the pregnancy rate of recipients. Much can be attributed to simple chance. Thus, even under what may seem to be identical conditions the number of transferable embryos produced by a donor can vary greatly from one occasion to the next. The same can be said for the percentage of synchronized recipients deemed eligible for transfer and the pregnancy rate of said recipients.

To account for this variability one needs to create a probability distribution that estimates the probability of an outcome, such as the number of transferable embryos generated per flush. Then whatever value is drawn from the distribution of transferable embryos would be input into the calculation for the number of recipients needed. The number of embryos transferred would then be combined with a pregnancy rate drawn from the distribution of possible pregnancy rates to predict the number of pregnant recipients.

@Risk© is an Excel© add-in that allows for probability distributions to be built into an Excel© workbook and values drawn from said distributions through the simulation of an Excel© based model. @Risk© software is quite robust with extensive mathematical and statistical capability incorporated into the program. Furthermore, there are a great number of options available to the user to define how a simulation will be run, how many times it will be run, and what output a user wishes to see. Big picture methods of @Risk© application that are pertinent to this model are described in this thesis. For in-depth study of @Risk© details, refer to the user manual.

The following sections describe the methodology behind the construction of distributions representing the variation of biologically uncertain variables deemed relevant to ET programs. It may be argued that in an ideal situation all data used to base distributions upon would be collected from contemporary groups in a homogenous environment; however, unless an environmental effect is quantified, data from a wide array of sources, years, and management practices more realistically describes true industry-wide variation (Long et al., 1975). The data used to estimate the following variable distributions is derived from extensive literature review and a couple of samples of industry reported data.

Percentage of Donors Showing No Signs of Estrus Following Superovulation

Hasler et al. (1983) reported that 5.5% of 856 MOET Holstein donors failed to show signs of estrus following superovulation, and thus were not considered eligible for insemination or embryo collection. Hasler et al. (2010) found that 6% of 332 MOET Red Angus donors also failed to show signs of estrus following superovulation. By recreating the binomial data from these two studies and assigning 67 of 1188 donors a binary code of "1" and 1121 of 1188 donors a binary code of "0", it was found that a mean of 5.6% of MOET donors, with a standard error of the mean

(SEM) of 0.0067, did not show signs of estrus following superovulation. A normal distribution of the mean, 0.056, and SEM, 0.0067, with a truncation point at 0, is built into the MOET embryo production model to account for potential donors that do not undergo embryo collection. Furthermore, to appropriately account for different number of donors (*n*) within the population of potential donors, a binomial distribution is in embedded into the model. The parameters of the binomial distribution are sample size *n* and probability of success, represented by the equation: 1-percentage of donors not undergoing embryo collection (sampled according to LHS from the normal distribution of the mean described above).

An override option for the percentage of superovulated donors not undergoing embryo collection is also built into the model. All stochastic variables have an override option that allows the user to input a deterministic variable. This feature can be used for sensitivity analysis or if a user feels that a deterministic variable better represents the expected outcome than the default distribution that is derived from industry-wide data. The distributions of stochastic variables can also be modified, although not as easily. The model equations within Excel© appear as follows:

Percentage of Donors Showing No Signs of Estrus Following Superovulation

=RiskNormal(mean, standard error of mean or standard deviation depending on how distribution is to be used, additional parameters ex) truncation minimum and maximum limits)

=RiskNormal(0.056,0.007,RiskTruncate(0,))

Number of Donors Undergoing Embryo Collection in Given Scenario

=IF(B10>=1,IF(B15=1,RiskBinomial(G10,1-B17),RiskBinomial(G10,1-B13)))

G10= Number of superovulation per donor
B15= Override option (1= Yes, Otherwise No)
B10= number, *n*, of donors receiving superovulation
B17= Override value for percentage of donors showing no signs of estrus
B13= Sample from normal distribution of the mean percentage of donors showing no signs of estrus (Figure 4)

.

Table 24. Percentage of donors showing no signs of estrus following superovulation.

% Donors showing no signs of estrus following superovulation	n	Breed	Source
5.5	856	Holstein	Hasler et al., 1983
6.0	332	Red Angus	Hasler, 2010

Table 25. Distribution parameters for the percentage of donors showing no signs of estrus following superovulation.

Weighted Mean % Donors Showing No Signs of Estrus	Weighted SEM (%)
5.6	0.7

Figure 4. Estimated probability distribution of the true mean for the percentage of donors showing no signs of estrus following superovulation.

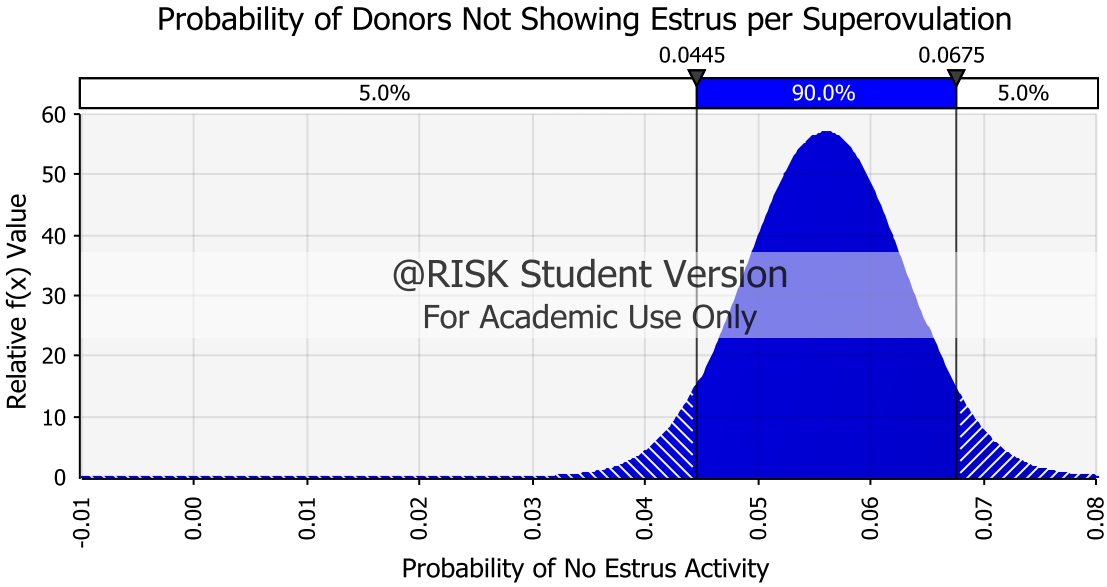


Table 26. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of donors showing no signs of estrus following superovulation.

5th Percentile	Mode	95th Percentile
0.044	0.056	0.067

Number of Embryos Collected per Flush Following Superovulation and Insemination Using Unsorted Semen

Woolliams et al. (1995) explains that a negative binomial distribution appropriately models the distribution of the number of embryos collected per donor flushed because the shape of a negative binomial distribution properly accounts for the probability that a flushed donor generates zero transferrable embryos. When considering independent Bernoulli trials, a negative binomial can be described as the probability of *k* number of failures (or successes) occurring in sequence before *r* successes (or failures) occur (Stat Trek, 2012). Reversing logic and considering the collection of each transferrable embryo a "failure", the data reported in Table 28 suggests that there is a 12% probability of 1 "success" (no transferrable embryo) before the occurrence of 1 "failure" (transferrable embryo).

The mean number of *k* failures (transferrable embryos) before *r* successes (no transferable embryos) can be calculated by the following formula:

$\mu_k = r*Q/P$

μ_k= the mean number of failures (transferrable embryos) before successes (no transferable embryos)

r= number of successes

Q= probability of failure

P= probability of success.

The negative binomial distribution can be constructed using the geometric probability formula:

$g(x; P) = P * Q^{x-1}$

x= number of independent Bernoulli trials

P= Probability of success of each trial

Q= Probability of failure

Keep in mind the logic of success and failure have been reversed for the situation of embryo collection.

Using the formula for the mean of a negative binomial as described previously, a mean of 7.33 embryos are collected before a collection attempt results in zero embryos. This can be interpreted as a mean of 7.33 embryos per flush, which was determined to reasonably describe the population mean, when compared to Table 30. The standard deviation of the negative binomial, 7.8, also followed closely with Table 30. Applying the geometric formula for a negative binomial, the distribution in Figure 5 was created using @Risk© software and concluded to sufficiently described the number of transferrable embryos generated per flush, following MOET.

Table 27. Percentage of flushed MOET donors producing zero embryos following insemination with unsorted semen.

% Flushed Donors Producing Zero Embryos	n	Breed	Source
12.5	312	Red Angus	Hasler, 2010
12.0	108	Holstein	Peippo et al., 2009
9.9	71	Holstein	Peippo et al., 2009

Table 28. Weighted mean percentage of flushes donors producing zero embryos following insemination with unsorted semen. From Table 27.

Weighted Mean % Flushed Donors Producing Zero Embryos
12.0

Table 29. Number of transferable embryos recovered per MOET flush following insemination with unsorted semen.

Transferable Embryos per Flush	SD	n	Donor Type	Source
9.8	na	172	Beef	Stroud and Hasler, 2006
4.5	na	63	Beef	Stroud and Hasler, 2006
7.0	na	31,333	Beef	IETS 2014
7.8	8.0	35	Holsteins	Hasler et al., 1983
7.5	6.6	35	Holsteins	Hasler et al., 1983
8.3	7.5	35	Holsteins	Hasler et al., 1983
7.3	7.1	35	Holsteins	Hasler et al., 1983
6.1	7.7	35	Holsteins	Hasler et al., 1983
6.6	6.6	1,073	Beef	Bo et al., 2002
6.3	10.5	307	Beef	Bo et al., 2002
4.0	4.3	29	Beef	Hasler, 2010
6.1	7.0	29	Beef	Hasler, 2010
5.0	4.9	29	Beef	Hasler, 2010

Table 30. Parameters for the number of embryos recovered per MOET flush following insemination with unsorted semen. From Table 29.

Weighted Mean Embryos per Flush	Weighted SD
6.99	7.32

Figure 5. Estimated probability distribution of the number of transferable embryos collected per MOET flush following insemination with unsorted semen.

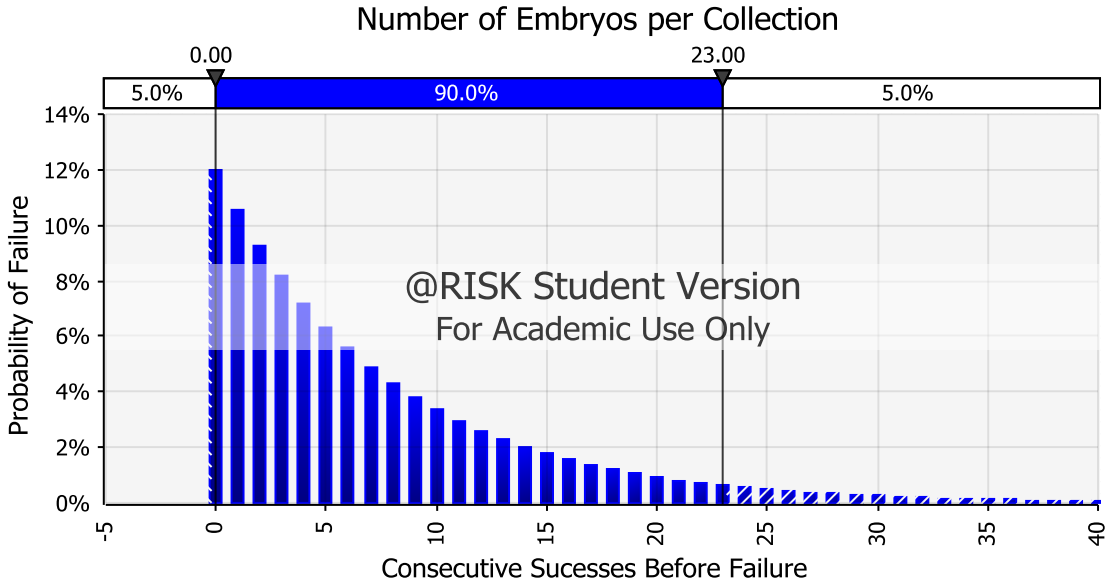


Table 31. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the number of transferable embryos collected per MOET flush following insemination with unsorted semen.

5th Percentile	Mode	95th Percentile
0	0	23

Number of Embryos Collected per Flush Following Superovulation and Insemination Using Sex-Sorted Semen

Using Table 32 and Table 34, the same logic and methodology is applied to MOET embryo production using sex-sorted semen, as previously described for MOET embryo production with unsorted semen. Because the probability of "success" (no embryo collected) before a set number of "failures" (collection of an embryo) serves as the input into the geometric equation within @Risk© the combination of Table 33 and Table 35 could not be exactly replicated in the @Risk© distribution, Figure 6. The parameters of 3.72 mean embryos per flush and standard deviation of

4.23 are deemed reasonably close to Table 35, following the input of 0.21 as the probability of "success" before one "failure".

Table 32. Percentage of flushed MOET donors producing zero embryos following insemination with sex-sorted semen.

% Flushed Donors Producing Zero Embryos	n	Source
19.0	42	Peippo et al., 2009
41.2	17	Peippo et al., 2009

Table 33. Weighted mean percentage of flushed MOET donors producing zero embryos following insemination with sex-sorted semen. From Table 32.

Weighted Mean % Flushed Donors Producing Zero Embryos
25.4

Table 34. Number of transferable embryos recovered per MOET flush following insemination with sex-sorted semen.

Transferable Embryos/Flush	SD	n	Source
4.1	1.8	30	Schenk et al., 2006
3.3	1.6	30	Schenk et al., 2006
3.2	3.1	42	Peippo et al., 2009
2.7	2.1	17	Peippo et al., 2009
6.0	4.9	5	Hayakawa et al., 2009
2.5		11	Hayakawa et al., 2009
2.4		13	Hayakawa et al., 2009
5.6		15	Hayakawa et al., 2009
3.4		11	Hayakawa et al., 2009
1.0		7	Hayakawa et al., 2009
5.2		35	Hayakawa et al., 2009
5.0		7	Hayakawa et al., 2009

Table 35. Parameters for the number of embryos recovered per MOET flush following insemination with sex-sorted semen. From Table 34.

Weighted Mean Embryos per Flush	Weighted SD
3.74	2.37

Figure 6. Estimated probability distribution of the number of transferable embryos collected per MOET flush following insemination with sex-sorted semen.

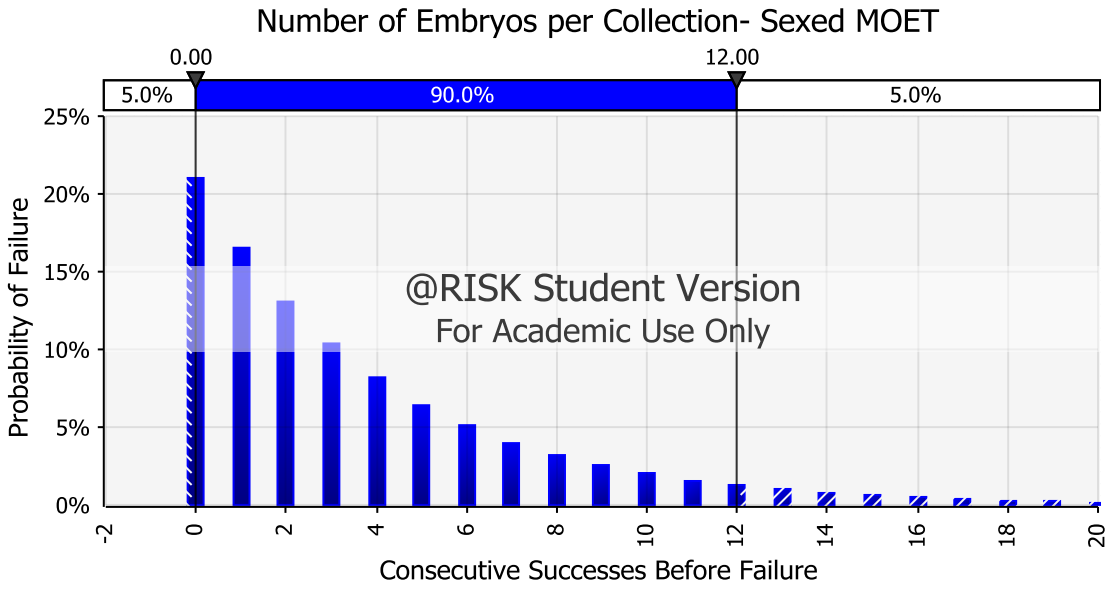


Table 36. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the number of transferable embryos collected per MOET flush following insemination with sex-sorted semen.

5th Percentile	Mode	95th Percentile
0	0	12

Number of Viable Oocytes Collected per Non-Synchronized, Non-Stimulated (NS) OPU with 3-4 d or 14 d Interval

It was decided to combine the number of viable oocytes collected per NS at 3-4 d intervals and a 14 d interval into one distribution. As explained previously, Ding (2008) reported no difference in mean number of recovered oocytes when comparing OPU twice-weekly, every 5 days, once-weekly, every 10 days, and once in 2 weeks. This conclusion seems sensible when employing the logic that the second follicular wave emerges somewhere between 7 to 11 days after ovulation, setting the physiological stage of the follicular wave somewhere between 3 and 7 days post-emergence when OPU is performed at a 14 d interval. A weighted mean, 7.26, and a weighted

standard deviation (SD), 2.71, for viable oocyte production per OPU was calculated using the data reported in Table 37.

Attempting to apply the most realistic shape to the distribution of viable oocyte production per OPU, the Akaike Information Criterion (AIC) within the "Distribution Fitting" tool of @Risk© was applied to the sample means from Table 37. An AIC score compares the ability of different model distributions to closely approximate reality (Mazerolle, 2004). Meaningless on its own, a lower AIC score is more favorable than a higher score (Mazerolle, 2004). To portray the value of models of a small sample size more accurately, the second order AIC (AIC_c) should be employed (Mazerolle, 2004). Fortunately, the AIC_c typically generates an identical score to the original AIC equation when considering models of large sample size, as well (Mazerolle, 2004).

$AICc = AIC + 2k(k+1)/(n-k-1)$

$AIC = -2(\text{log-likelihood}) + 2k$

n= sample size

k= number of estimated parameters

Taking into consideration the AIC score, discussion within the literature, and production knowledge, a lognormal distribution with a mean of 7.26 and SD of 2.71 was deemed the most accurate representation for the number of oocytes generated per NS OPU performed at 3-4 d and 14 d intervals (Figure 7).

Table 37. Number of viable oocytes collected per OPU session with twice weekly or 14 d intervals. no ovarian FSH stimulation.

Mean # Viable Oocytes Collected Per OPU	SD	n	Interval	Source
3.8	15.5	60	Twice-Weekly	Chaubal et al., 2006
3.9		96	Twice-Weekly	De Roover et al., 1997 abstract
4.1	3.1	1,396	Twice-Weekly	De Roover et al., 2008
7.8		75	Twice-Weekly	De Ruigh et al., 2000 abstract
5.9		75	Twice-Weekly	De Ruigh et al., 2000 abstract
6.7	2.0	169	3 d Interval	Hanenberg et al., 1997 abstract
7.2	1.9	516	3 d Interval	Hanenberg et al., 1997 abstract
9.3	3.7	192	3 d Interval	Hanenberg et al., 1997 abstract
5.6	1.8	162	4 d Interval	Hanenberg et al., 1997 abstract
6.6	1.9	502	4 d Interval	Hanenberg et al., 1997 abstract
8	2.9	182	4 d Interval	Hanenberg et al., 1997 abstract
7	4.4	24	3-4 d Interval	Guyador Joly et al., 1997 abstract

(*cont.)*

Table 37 (*cont.*). Number of viable oocytes collected per OPU session with twice weekly or 14 d intervals. no ovarian FSH stimulation.

Mean # Viable Oocytes Collected Per OPU	SD	n	Interval	Source
7	2.6	236	3-4 d Interval	Wagtendonk-de Leeuw et al., 2000
8.6	2.5	1,753	3-4 d Interval	Wagtendonk-de Leeuw et al., 2000
8.4	2.5	446	3-4 d Interval	Wagtendonk-de Leeuw et al., 2000
7.6		4,308	3-4 d Interval	Wagtendonk-de Leeuw et al., 2000
7.8		2,015	3-4 d Interval	Wagtendonk-de Leeuw et al., 2000
4.96		24	3-4 d Interval	Ding et al., 2008
6.19		36	14 d Interval	Ding et al., 2008

Table 38. Parameters for the number of viable oocytes collected per OPU session with twice weekly or 14 d intervals. no ovarian FSH stimulation. From Table 37.

Weighted Mean Viable Oocytes per OPU	Weighted SD
7.26	2.71

Figure 7. Estimated probability distribution of the number of viable oocytes recovered per OPU session with twice weekly or 14 d intervals. No ovarian FSH stimulation.

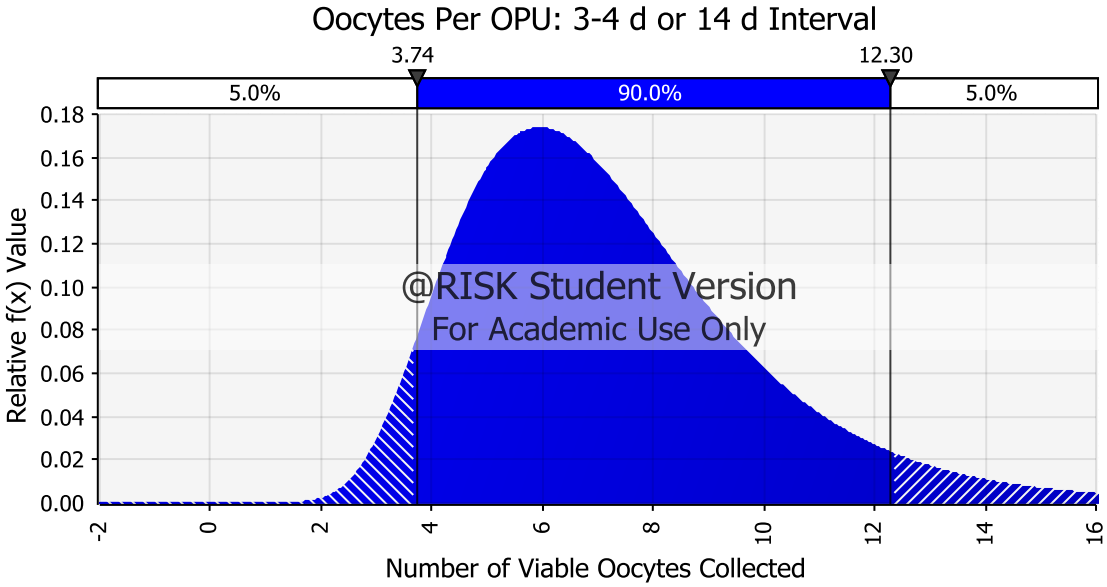


Table 39. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the number of viable oocytes recovered per OPU session with twice weekly or 14 d intervals. No ovarian FSH stimulation.

5th Percentile	Mode	95th Percentile
3.75	5.97	12.28

Number of Viable Oocytes Collected per NS OPU with Random Interval

The same strategy as described for the number of viable oocytes collected per NS OPU with 3-4 d or 14 d intervals is applied to generate an appropriate distribution for the number of viable oocytes collected per NS OPU with a random interval. The expectation of increased variability in oocyte production because of within herd differences in stage of follicular waves necessitates a separate model describing a random OPU schedule. Based upon the data from Table 40, a lognormal distribution with mean 8.94 and SD 6.73 is used to describe the distribution of NS OPU production following a random OPU schedule (Figure 8).

Table 40. Number of viable oocytes collected per OPU session with a random interval. No ovarian FSH stimulation.

Mean Number of Oocytes Collected per OPU with Random Interval	SD	n	Interval	Source
14.0		41	First OPU, no Interval	Merton et al., 2003
5.4	1.4	44	First OPU, no Interval	Antonio de Carvalho Fernandez et al., 2014
8.0	7.6	1,138	Minimum 15 d interval	Pontes et al., 2010
10.0	5.9	925	First OPU, no Interval	Stevenson Sputnik, 2014

Table 41. Parameters for the number of viable oocytes collected per OPU session with a random interval. No ovarian FSH stimulation. From Table 40.

Weighted Mean Viable Oocytes per OPU	Weighted SD
8.94	6.73

Figure 8. Estimated probability distribution of the number of viable oocytes per OPU session with a random interval. No ovarian FSH stimulation.

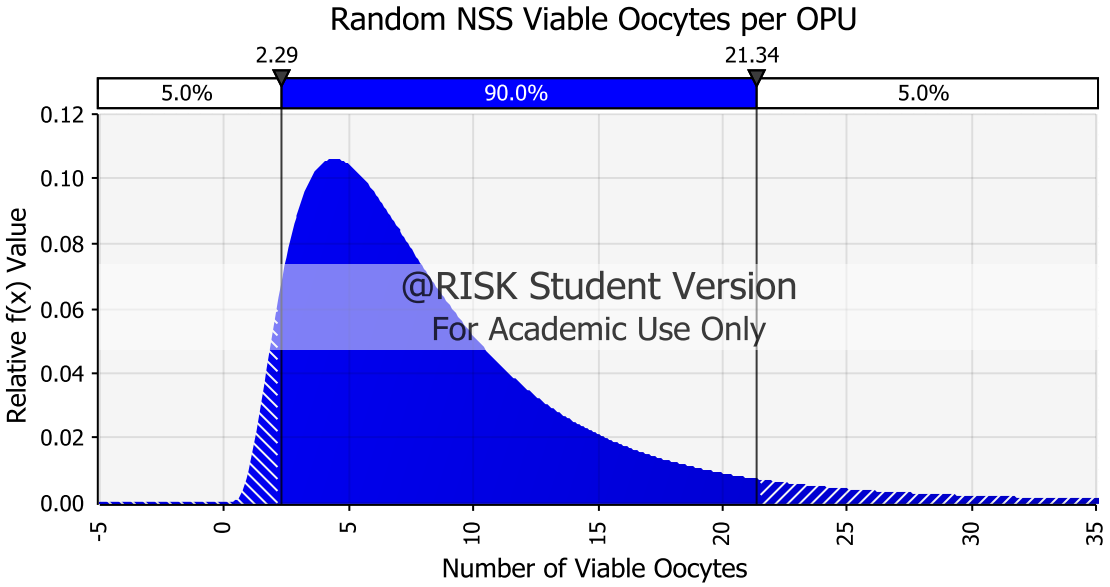


Table 42. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the number of viable oocytes per OPU session with a random interval. No ovarian FSH stimulation.

5th Percentile	Mode	95th Percentile
2.36	4.56	21.45

Number of Viable Oocytes Collected per Follicular Wave Synchronized, Ovarian Stimulated (SS) OPU

The final variation of OPU protocol is modeled assuming follicular wave synchronization and ovarian stimulation (SS). Following the same method of model determination used for the previously described OPU protocols, a lognormal distribution with a mean of 11.63 and SD of 8.20, calculated from Table 43, is used to represent the distribution of the number of viable oocytes collected following follicular wave synchronization and ovarian stimulation (Figure 9).

Table 43. Number of viable oocytes collected per OPU session following follicular wave synchronization and FSH stimulation.

Mean Number of Viable Oocytes Collected from SS OPU	SD	n	Source
11.9	7.1	42	Antonio de Carvalho Fernandez et al., 2014
10.4	7.34	32	Barceló-Fimbres et al., 2015
9.0	7.4	32	Barceló-Fimbres et al., 2015
8.6	9.8	24	Barceló-Fimbres et al., 2015
11	9.7	78	Barceló-Fimbres et al., 2015
11.8	8.2	640	DeRoover et al., 2008
13.2		30	De Ruigh et al., 2000 **(**abstract)
14.8		20	Lacaze et al., 1997 (abstract)
13.0	3.1	12	Guyador Joly et al., 1997 (abstract)

Table 44. Parameters for the number of viable oocytes collected per OPU session following follicular wave synchronization and FSH stimulation. From Table 43.

Weighted Mean Viable Oocytes per OPU	Weighted SD
11.63	8.20

Figure 9. Estimated probability distribution of the number of viable oocytes recovered per OPU session following follicular wave synchronization and FSH stimulation.

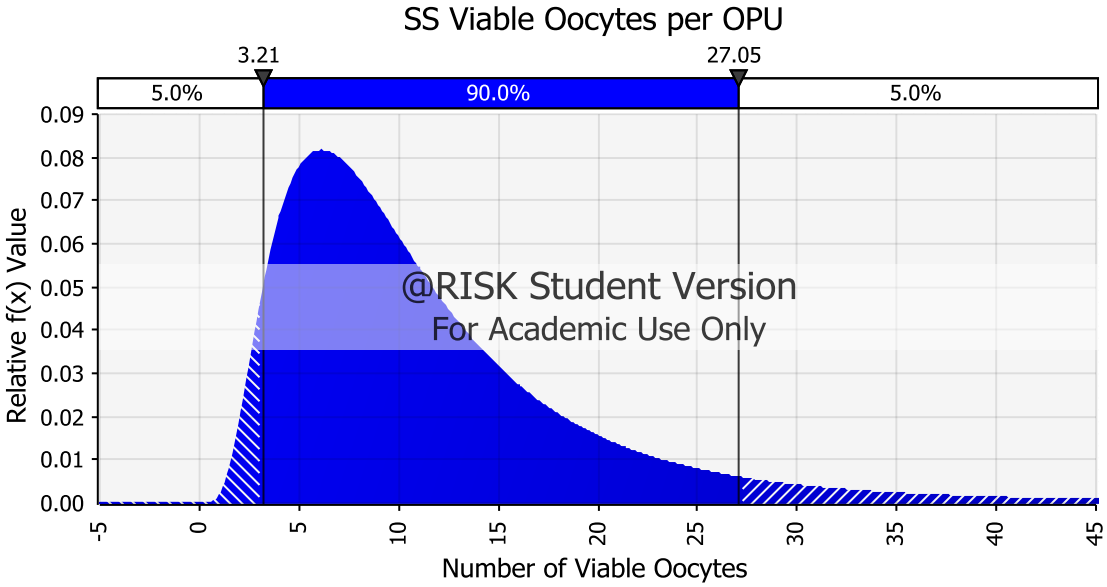


Table 45. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the number of viable oocytes recovered per OPU session following follicular wave synchronization and FSH stimulation.

5th Percentile	Mode	95th Percentile
3.26	6.24	27.12

Percentage of Incubated Oocytes Developed into Blastocysts Following Fertilization Using Semen Unsorted for Sex, Following No Synchronization or Stimulation of OPU Donors

C. Fernandes et al. (2014) demonstrated a significant difference between the blastocyst rate of viable oocytes from donors that received pre-OPU synchronization or synchronization and exogenous FSH stimulation when compared to non-synchronized, non-stimulated donors. Furthermore, Palma et al. (2008) demonstrated that when compared to the control non-sexed sperm, sex-sorted sperm from 4 out of 5 sires differed significantly in blastocyst rate of IVF embryos. Xu et al. (2006) also found significant bull to bull variation in the fertility of sex- sorted

sperm based on blastocyst rate. Thus, blastocyst rate is split into four different distributions depending on the combination of sex-sorted or unsorted semen and the synchronization/stimulation protocol of donors.

Using Table 46 for reference, a normal distribution of blastocyst rate with a mean of 0.26 and SD across sample means of 0.09 is used to characterize the mean blastocyst rate of viable oocytes aspirated from donors receiving no follicular synchronization or stimulation and fertilization from unsorted semen. This distribution is truncated at 0 and 1. As already described for other variables, the technique of implementing a binomial distribution into the equation to predict the number of embryos resulting from oocyte fertilization and incubation is utilized.

Table 46. Percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors.

Mean Blastocyst Rate (%)	n	Source
19.5	238	Antonio de Carvalho Fernandez et al., 2014
24.1	921	Barceló-Fimbres et al., 2015
39.1	233	Barceló-Fimbres et al., 2015
26.5	654	Barceló-Fimbres et al., 2015
41.1	136	Barceló-Fimbres et al., 2015
14.1	273	Barceló-Fimbres et al., 2015
24.8	273	Barceló-Fimbres et al., 2015
44.8	284	Barceló-Fimbres et al., 2015
26.5	522	Xu et al., 2006
20.7	518	Xu et al., 2006
22.9	305	Zhang et al., 2003
24.4	499	Zhang et al., 2003
19.4	501	Zhang et al. 2003
33.6	330	Palma et al., 2008

Table 47. Parameters for the percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors. From Table 46.

Weighted Mean Blastocyst Rate (%)	SD Across Sample Means (%)
26.0	9.0

Figure 10. Estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors.

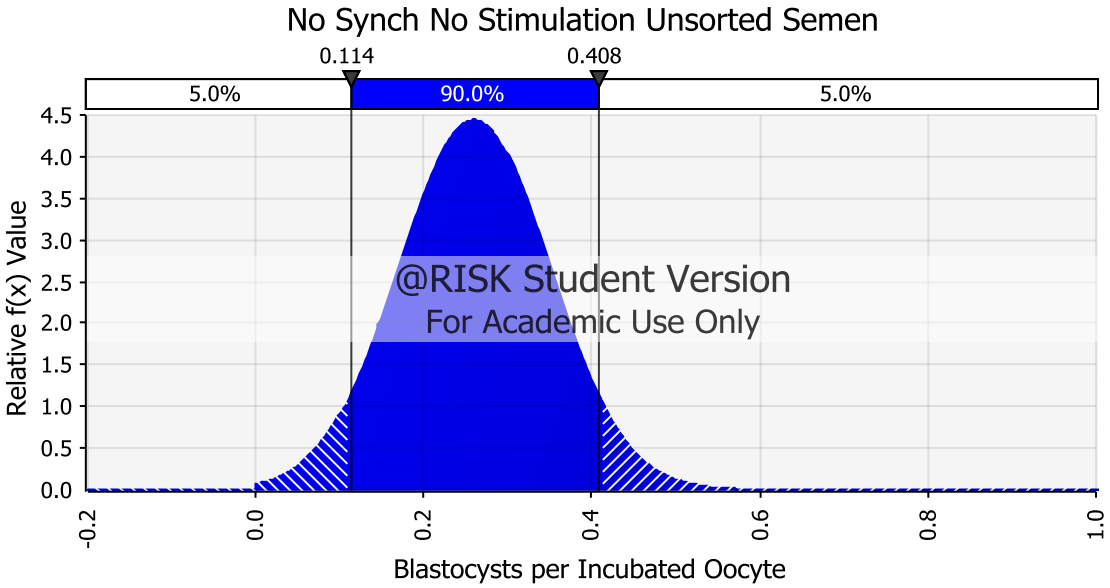


Table 48. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors.

5th Percentile	Mode	95th Percentile
0.11	0.26	0.41

Percentage of Incubated Oocytes Developed into Blastocysts Following Fertilization Using Unsorted Semen, Following Ovarian Stimulation Accompanied by Synchronization or Only Follicular Synchronization of OPU Donors

Applying the data from Table 49, a normal distribution of blastocyst rate with a mean of 0.37 and SD across sample means of 0.08 is used to characterize the mean blastocyst rate of viable oocytes fertilized with unsorted semen and aspirated from donors receiving ovarian stimulation and follicular synchronization or only synchronization. The literature demonstrated minimal difference in the percentage of incubated oocytes that developed into blastocysts when comparing

the two protocols. This distribution is truncated at 0 and 1. Again, a binomial distribution is built into the equation to predict the number of embryos resulting from oocyte fertilization and incubation.

Table 49. Percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. Follicular wave synchronization accompanied by ovarian stimulation or only follicular wave synchronization of OPU donors.

Mean Blastocyst Rate (%)	n	Source
29.6	294	Antonio de Carvalho Fernandez et al., 2014
38.7	208	Ramos et al., 2010
44.9	264	Ramos et al., 2010
44.2	252	Ramos et al., 2010
24.5	500	Antonio de Carvalho Fernandez et al., 2010
33.4	333	Barceló-Fimbres et al., 2015
42.1	855	Barceló-Fimbres et al., 2015

Table 50. Parameters for the percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. Follicular wave synchronization accompanied by ovarian stimulation or only follicular wave synchronization of OPU donors. From Table 49.

Weighted Mean Blastocyst Rate (%)	SD Across Sample Means (%)
37.0	8.0

Figure 11. Estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. Follicular wave synchronization accompanied by ovarian stimulation or only follicular wave synchronization of OPU donors.

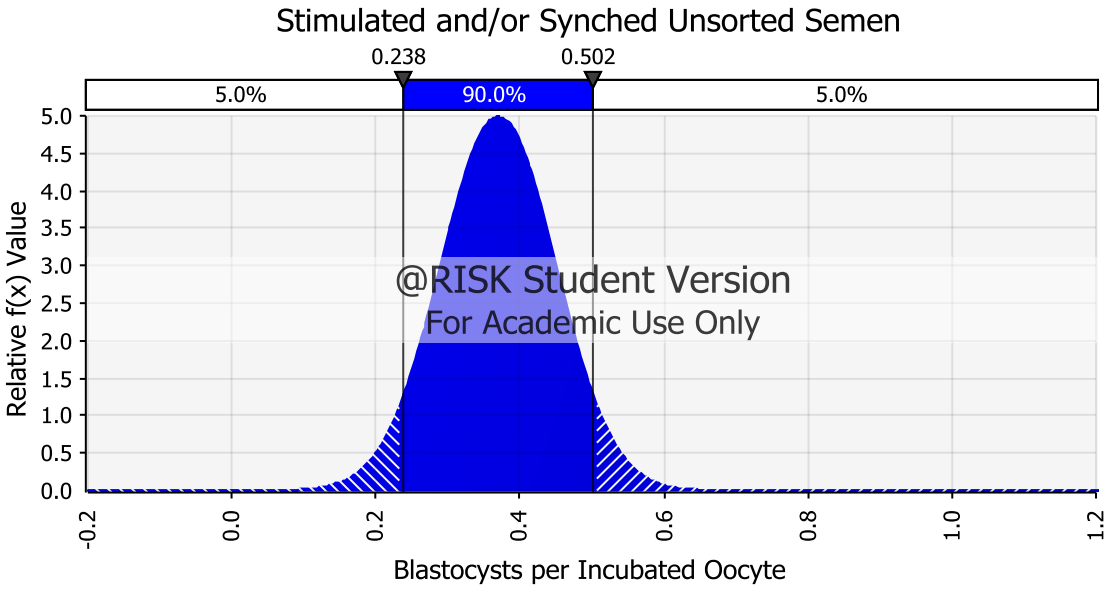


Table 51. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with unsorted semen. follicular wave synchronization accompanied by ovarian stimulation or only follicular wave synchronization of OPU donors.

5th Percentile	Mode	95th Percentile
0.24	0.37	0.50

Percentage of Incubated Oocytes Developed into Blastocysts Following Fertilization Using Sex-Sorted Semen, Following No Synchronization or Stimulation of OPU Donors

Referencing Table 52, a normal distribution of blastocyst rate with a mean of 0.23 and SD across sample means of 0.14 represented the mean blastocyst rate of viable oocytes aspirated from donors receiving no follicular synchronization or stimulation and fertilization from sex-sorted

semen. This distribution is truncated at 0 and 1. To predict the number of embryos resulting from oocyte fertilization and incubation, a binomial distribution is embedded into the equation.

Table 52. Percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors.

Sexed Semen Embryo Rate (%)	n	Source
22.1	727	Xu et al., 2006
2.0	640	Xu et al., 2006
0.7	720	Xu et al., 2006
1.2	600	Xu et al., 2006
23.5	1191	Xu et al., 2006
25.3	1207	Xu et al., 2006
20.7	1433	Xu et al., 2006
23.7	1288	Xu et al., 2006
20.3	1364	Zhang et al., 2003
15.7	1232	Palma et al., 2008
27.2	9278	Stevenson Sputnik, 2014
50.0	300	Morotti et al. 2014
35.0	194	Morotti et al. 2014
41.0	330	Morotti et al. 2014

Table 53. Parameters for the percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors. From Table 52.

Weighted Mean Blastocyst Rate (%)	SD Across Sample Means (%)
23.0	14.0

Figure 12. Estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors.

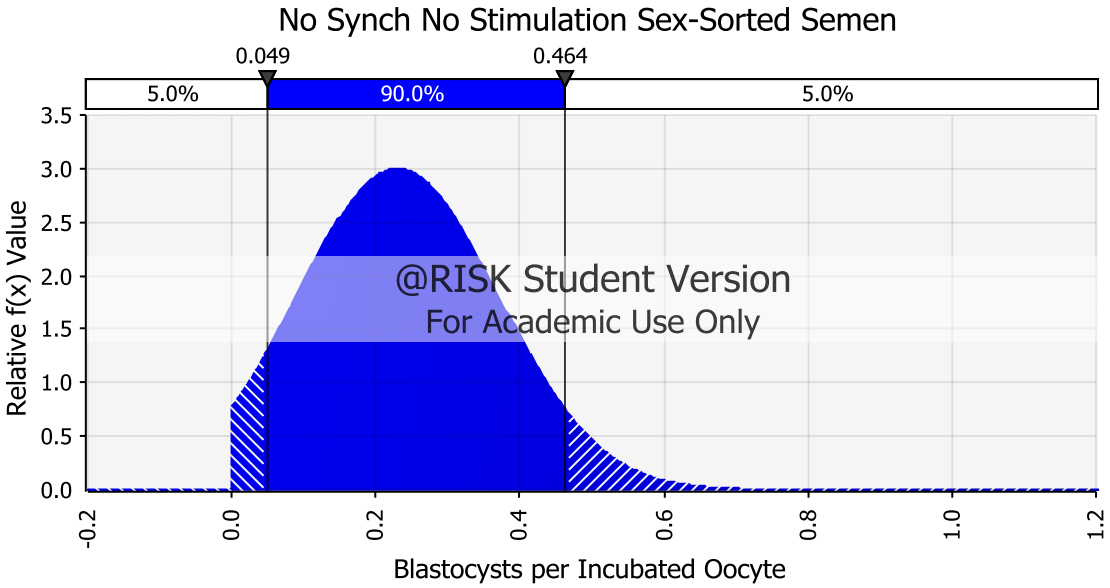


Table 54. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. No follicular wave synchronization or ovarian stimulation of OPU donors.

5th Percentile	Mode	95th Percentile
0.05	0.23	0.46

Percentage of Incubated Oocytes Developed into Blastocysts Following Fertilization Using Sex-Sorted Semen following Ovarian Stimulation and Synchronization or Only Follicular Synchronization of OPU Donors

No literature was found reporting the blastocyst rate of oocytes that are retrieved from donors that underwent exogenous FSH stimulation and/or follicular synchronization and fertilized with sex-sorted semen. Thus, the mean and SD across sample means of the blastocyst rate for oocytes fertilized with sex-sorted semen and aspirated from donors that received no synchronization or stimulation is adjusted.

Using Table 47 and Table 50 it was discovered that for unsorted semen the application of ovarian stimulation and/or follicular synchronization increased the mean blastocyst rate by 42% and the SD across sample means was decreased by 13%. The mean and SD across sample means are adjusted accordingly to give the values in Table 55; these are then applied as the parameters of a normal distribution to define the mean blastocyst rate of oocytes fertilized with sex-sorted semen and derived from donors that underwent follicular synchronization and ovarian stimulation or only follicular synchronization. A binomial distribution with probability (p) drawn from the previously described distribution per LHS and number of oocytes (n) as obtained from the model, is included in the equation used to calculate the number of embryos generated from n oocytes.

Table 55. Parameters for the percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. Follicular wave synchronization accompanied by ovarian stimulation or follicular wave synchronization alone of OPU donors.

Weighted Mean Blastocyst Rate (%)	SD Across Sample Means (%)
33.0	13.0

Figure 13. Estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. Follicular wave synchronization accompanied by ovarian stimulation or follicular wave synchronization alone of OPU donors.

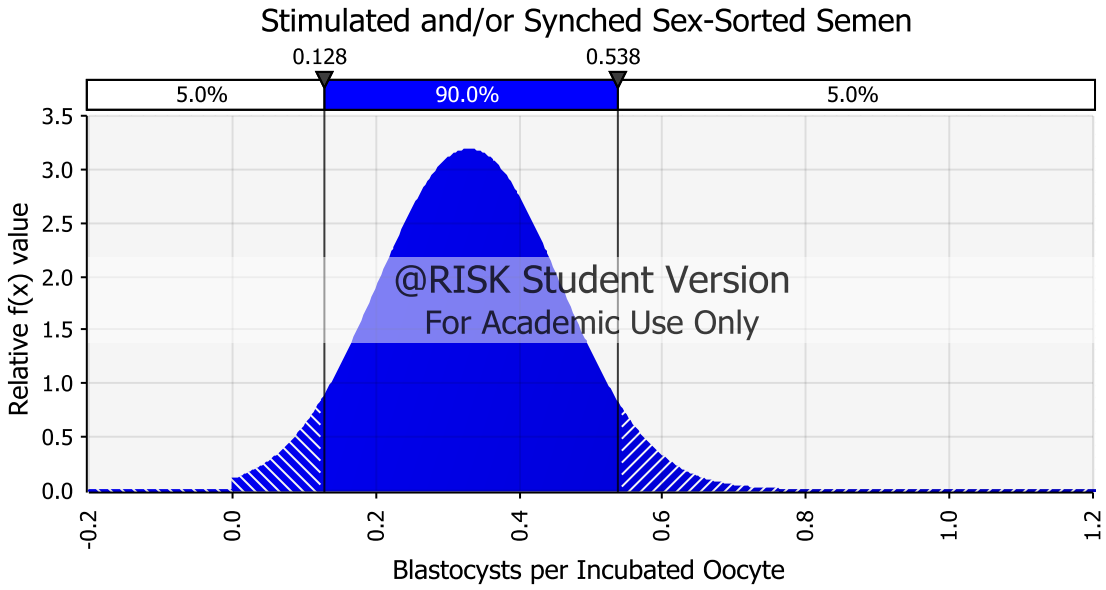


Table 56. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of cultured oocytes developing into blastocysts following fertilization with sex-sorted semen. Follicular wave synchronization accompanied by ovarian stimulation or follicular wave synchronization alone of OPU donors.

5th Percentile	Mode	95th Percentile
0.13	0.33	0.54

Percentage of Synchronized Recipients Receiving Embryo

Following the assumption that all synchronized recipients are palpated for the presence of a corpus luteum (CL) of sufficient quality to receive an inter-uterine embryo transplant, the model for the distribution of the percentage of synchronized recipients eligible to receive an embryo is based upon Table 58. From the data, a mean of 85% of synchronized recipients are deemed eligible of receiving an embryo. The SD across sample means was calculated as 0.09. The resulting normal distribution is truncated at 1 (Figure 14).

Similar to the approach previously explained, a binomial distribution to account for variation in the herd size of potential recipients is embedded into the formula, predicting the number of synchronized recipients eligible to receive an embryo. The parameters of n number of synchronized recipients (a number drawn from the model based on a combination of the predicted number of recipients required and the number of recipients available) and probability, p, of success defined the binomial distribution. The probability, p, of success is sampled from the distribution of synchronized recipients eligible for an embryo per the LHS sampling method.

Table 57. Percentage of estrous synchronized recipients qualified to receive an embryo.

% of Synchronized Recipients Qualified for Embryo	n (number synchronized)	Recipient Type	Source
93.0	76	Crossbred Beef Cows in Canada	Small et al., 2007
86.0	76	Crossbred Beef Cows in Canada	Small et al., 2007
91.0	78	Crossbred Beef Cows in Canada	Small et al., 2007
88.0	74	Crossbred Beef Cows in Canada	Small et al., 2007
59.0	763	Angus	Spell et al., 2001
73.0	408	Beef Heifers	Looney et al., 2006
94.0	1238	Beef Cows	Looney et al., 2006
93.0	1390	Ovagenix (TX) recips	Looney et al., 2006
83.0	753	Ovagenix (TX) recips	Looney et al., 2006
85.0	123	Ovagenix (TX) recips	Looney et al., 2006
89.0	240	Ovagenix (TX) recips	Looney et al., 2006
88.0	1533	Ovagenix (TX) recips	Looney et al., 2006

(*cont.)*

Table 57 (*cont.*). Percentage of estrous synchronized recipients qualified to receive an embryo.

% of Synchronized Recipients Qualified for Embryo	n (number synchronized)	Recipient Type	Source
88.0	380	Ovagenix (TX) recips	Looney et al., 2006
80.0	477	Beef Heifers	Stroud and Hasler, 2006
83.0	376	Beef Cows	Stroud and Hasler, 2006

Table 58. Parameters for the percentage of estrous synchronized recipients qualified to receive an embryo.

Weighted Mean % Recipients with Transferrable CL	SD Across Sample Means
85.0	9.0

Figure 14. Estimated probability distribution of the true mean for the percentage of estrous synchronized recipients qualified to receive an embryo.

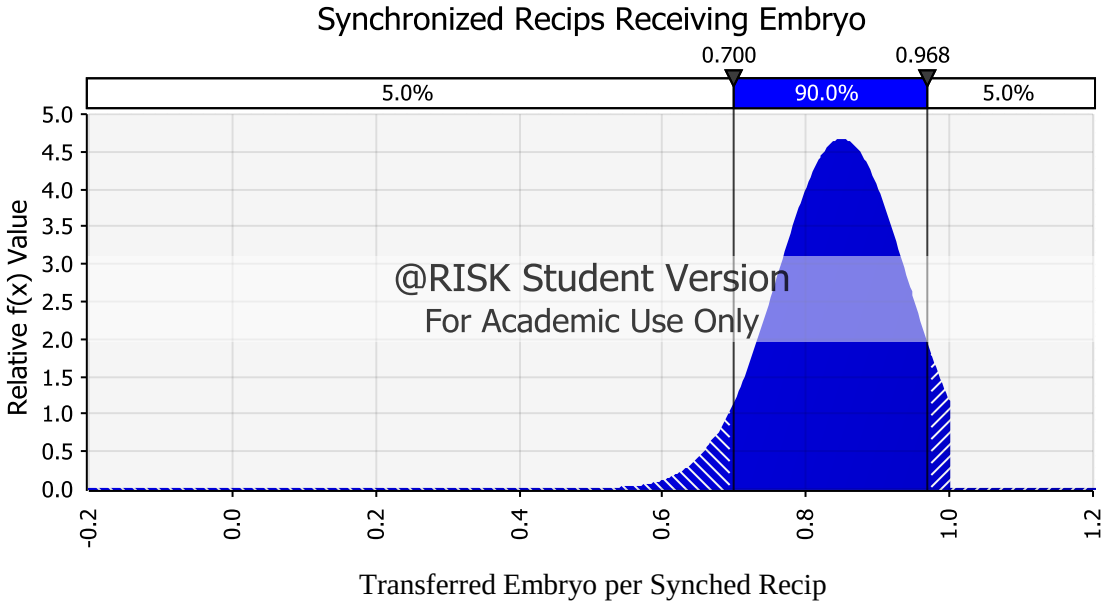


Table 59. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of estrous synchronized recipients qualified to receive an embryo.

5th Percentile	Mode	95th Percentile
0.70	0.85	0.97

21 d Pregnancy Rate Following Transfer of Fresh IVD Embryos

It is often important to consider when a recipient returns to estrus, if she does not carry the pregnancy full term, so that management has the option to transfer another embryo into the same recipient to attempt establishment of a successful pregnancy. For this reason, recipient pregnancy is partitioned into the number of recipients pregnant at d 21 of gestation (14 d post-transfer), d 60 of gestation, and term with pregnancy loss accounted for within each interval.

When considering data from the literature to estimate the pregnancy rate of fresh transferred IVD embryos, only data from transfers using *Bos taurus* recipients, excluding lactating dairy cows, is considered. An exception is made for Chagas et al. (2002) because the study reported

no significant difference in recipient pregnancy rate between lactating Holstein Friesian cows and heifers.

To account for the lack of published literature meeting the previously described requirements for recipient pregnancy rate at 21 d gestation (Table 60), data measuring pregnancy of recipients at 60 d gestation (Table 61) is adjusted to a 21 d pregnancy rate. The mean 60 d pregnancy rate from Table 61 is back-calculated using the mean embryonic loss between ~21 d and ~60 d of gestation found in the literature (Table 80)**.** This adjusted mean is then weighted, according to sample size, with the 21 d pregnancy rate data from Table 60. Furthermore, each reported 60 d pregnancy rate figure is back-calculated to a 21 d pregnancy rate by pairing one 60 d pregnancy rate figure with the sample mean of each individual ~21 d to ~60 d embryonic loss figure found in the literature (Table 79)**.** The standard deviation between all the adjusted 21 d pregnancy rates and the actual 21 d pregnancy rates from Table 60 is used to explain potential error from the true population mean of 21 d recipient pregnancy rate. The adjusted mean, 0.78, and adjusted SD between sample means, 0.09, are used as parameters for the normal distribution model of 21 d pregnancy rate of fresh IVD embryos, which is truncated at 0 and 1 (Figure 15)**.**

As explained in preceding pages, a binomial distribution with n number of recipients receiving an embryo with probability, p, of pregnancy at 21 d of gestation is inserted into the formula to calculate the number of pregnant recipients at 21 d post-ovulation.

Table 60. Percentage of recipients receiving a fresh IVD embryo that were pregnant at ~21-~30 d of gestation (14-23 d post-transfer).

~21-30 d Pregnancy Rate (%)	n	Recipient Type	Source
69.5	165	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
63.0	242	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
82.8	122	Angus	Spell et al., 2001

21 d pregnancy rate determined by return to estrus.
30 d pregnancy rate determined by ultrasound.

Table 61. Percentage of recipients receiving a fresh IVD embryo that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
50.0	165	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
58.7	242	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al.,2002
71.3	7,652	Holstein Heifers	Hasler et al., 1987
53.1	192	Chinese Yellow/Holstein	Xu et al., 2006
67.0	599	Holstein Heifers	Hasler, 2000
79.9	1,485	Holstein Heifers	Hasler, 2001
69.7	491	Holstein Heifers	Hasler, 2001
78.8	590	Holstein Heifers	Hasler, 2001
60.7	84	Holstein Heifers	Hasler, 2001
68.6	2,512	Holstein Heifers	Hasler, 2001
67.3	2,716	Holstein Heifers	Hasler, 2001
70.1	1,960	Holstein Heifers	Hasler, 2001
67.2	1,960	Holstein Heifers	Hasler, 2001
68.5	7,457	Holstein Heifers	Hasler, 2001

(*cont.)*

Table 61 (*cont.*). Percentage of recipients receiving a fresh IVD embryo that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
67.3	1,566	Holstein Heifers	Hasler, 2001
70.5	6,612	Holstein Heifers	Hasler, 2001
65.9	267	Holstein Heifers	Hasler, 2001
68.6	835	Holstein Heifers	Hasler, 2001
66.0	320	*Bos taurus*- ET Center Managed	Hasler et al., 1995

Table 62. Parameters for the percentage of recipients receiving a fresh IVD embryo that were pregnant at d 21 of gestation.

Weighted Mean Adjusted 21 d Pregnancy Rate (%)	Adjusted Standard Deviation Across Sample Means
78.0	9.0

Figure 15. Estimated probability distribution of the true mean for the percentage of recipients receiving a fresh IVD embryo that were pregnant at d 21 of gestation.

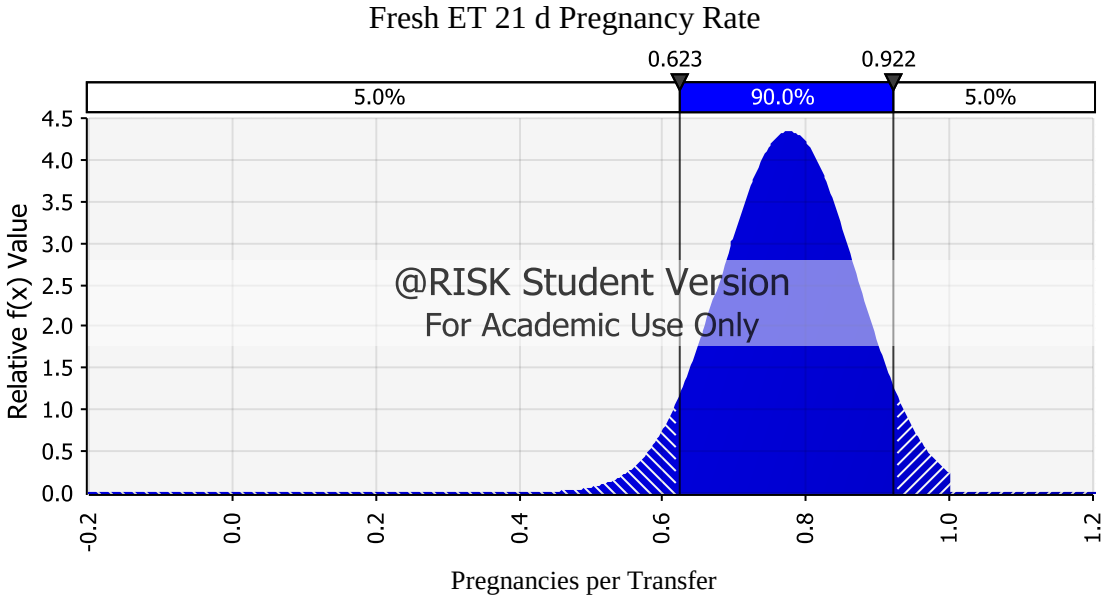


Table 63. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients receiving a fresh IVD embryo that were pregnant at d 21 of gestation.

5th Percentile	Mode	95th Percentile
0.62	0.78	0.92

21 d Pregnancy Rate Following Transfer of Fresh IVP Embryos

The same method as described before is implemented to adjust ~60 d pregnancy rate data from Table 64 to a ~21 d pregnancy rate value. An adjusted, weighted mean and standard deviation across the mean of data samples are also calculated by the method previously described. The resulting normal distribution with a mean of 0.64 and standard deviation of 0.10 is built into the model (Figure 16). Again, a binomial distribution is set into the formula calculating the number of pregnant recipients at 21 d gestation following the transfer of fresh IVP embryos.

Table 64. Percentage of recipients receiving a fresh IVP embryo that were pregnant at ~21-~30 d of gestation (14-23 d post-transfer).

~21-30 d Pregnancy Rate (%)	n	Recipient Type	Source
62.7	51	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Taverne et al., 2002
63.2	106	Bos taurus (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Taverne et al., 2002
70.0	44	Bos taurus (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Taverne et al., 2002
51.4	35	Hereford	Martinez et al., 2002

21 d pregnancy rate determined by return to estrus.
30 d pregnancy rate determined by ultrasound.

Table 65. Percentage of recipients receiving a fresh IVP embryo that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
53.8	1,220	*Bos taurus* (probably Holstein heifers)	Hasler et al., 1995
47.8	467	Holstein Heifers	Hasler, 2000
47.1	382	Holstein Heifers	Hasler, 2000
50.4	129	Holstein Heifers	Hasler, 2000
37.0	19	Angus Cross	Farin and Farin, 1995

Table 66. Parameters for the percentage of recipients receiving a fresh IVP embryo that were pregnant at d 21 of gestation.

Weighted Mean Adjusted 21 d Pregnancy Rate (%)	Adjusted Standard Deviation Across Sample Means (%)
64.0	10.0

Figure 16. Estimated probability distribution of the true mean for the percentage of recipients receiving a fresh IVP embryo that were pregnant at d 21 of gestation.

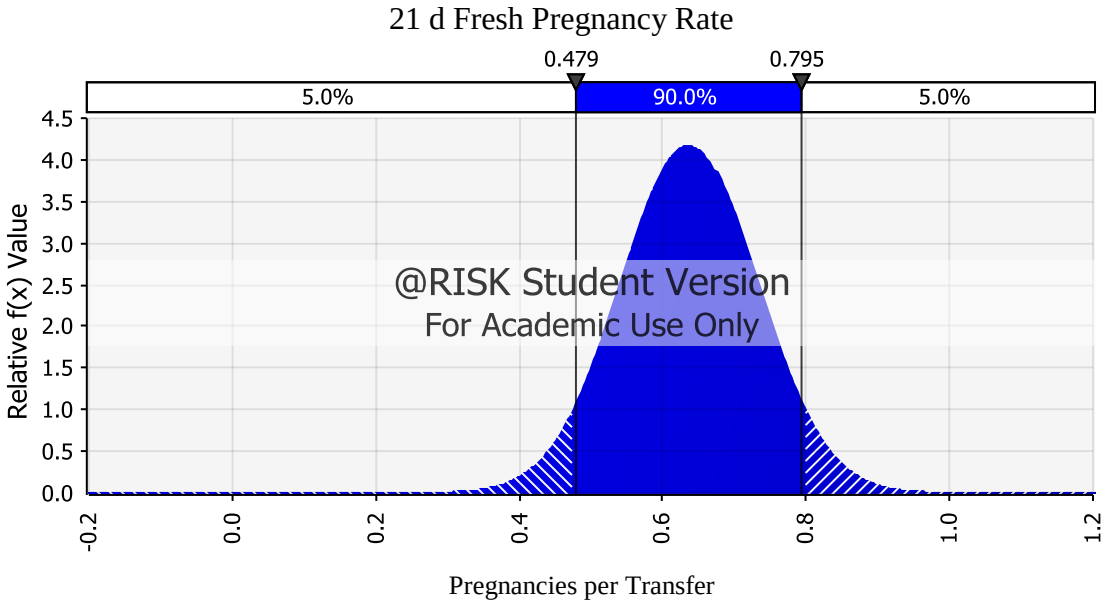


Table 67. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients receiving a fresh IVP embryo that were pregnant at d 21 of gestation.

5th Percentile	Mode	95th Percentile
0.48	0.64	0.79

21 d Pregnancy Rate Following Transfer of Frozen-Thawed IVD Embryos

Regarding the 21 d pregnancy rate and the subsequent number of pregnant recipients following the transfer of frozen-thawed IVD embryos, identical methodology as explained for fresh IVD embryos is applied to generate a normal distribution with a mean of 0.75 and a standard deviation of 0.16 (Figure 17)**.** The frozen-thawed embryonic loss values from Table 82 and Table 83 are used to make the appropriate adjustments. A binomial distribution is also applied to the model per previous description.

Table 68. Percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at ~21-~30 d of gestation (14-23 d post-transfer).

~21-30 d Pregnancy Rate (%)	n	Recipient Type	Source
62.7	83	Holstein Friesan Cows and Heifers	Chagas et al., 2002
62.8	196	Holstein Friesan Cows and Heifers	Chagas et al., 2002
69.0	326	Angus	Spell et al., 2001

21 d pregnancy rate determined by return to estrus.
30 d pregnancy rate determined by ultrasound.

Table 69. Percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
64.0	517	Holstein Heifers	Hasler, 2000
34.9	83	Holstein Friesan Cows and Heifers	Chagas et al., 2002
50.5	196	Holstein Friesan Cows and Heifers	Chagas et al., 2002
56.1	3,616	Holstein Heifers	Hasler, 2001
58.4	5,297	Holstein Heifers	Hasler, 2001
68.7	774	Holstein Heifers	Hasler, 2001

Table 70. Parameters for the percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at d 21 of gestation.

Weighted Mean Adjusted 21 d Pregnancy Rate (%)	Adjusted Standard Deviation Across Sample Means
75.0	16.0

Figure 17. Estimated probability distribution of the true mean for the percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at d 21 of gestation.

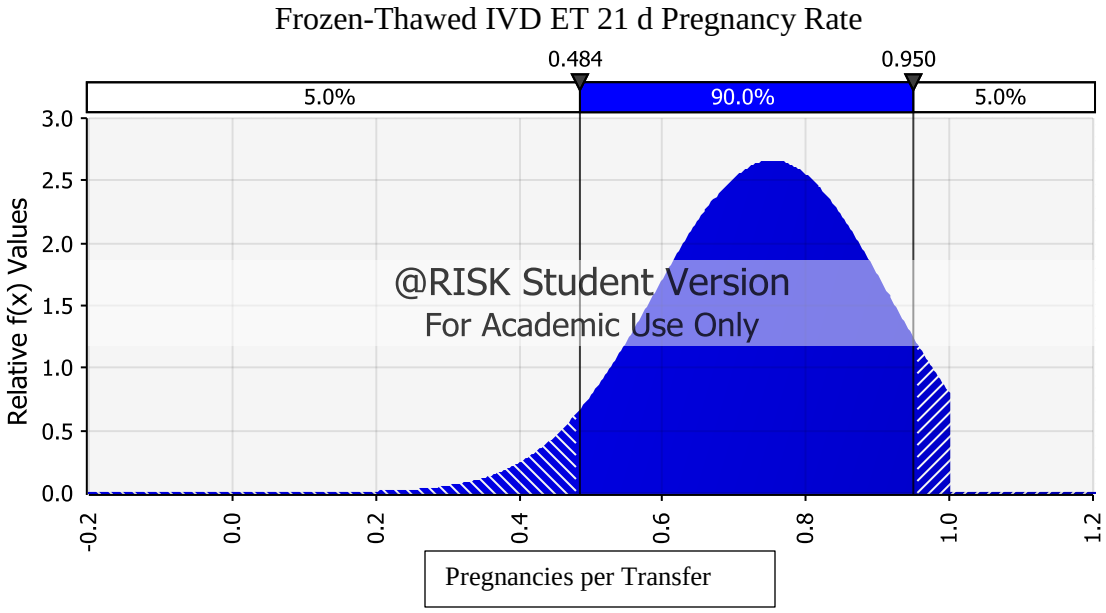


Table 71. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at d 21 of gestation.

5th Percentile	Mode	95th Percentile
0.48	0.75	0.95

21 d Pregnancy Rate Following Transfer of Frozen-Thawed IVP Embryos

Adjustments to ~60 d pregnancy rate data are made, following the standards already set, to generate a normal distribution with a mean of 0.50 and SD of 0.17 (Figure 18). The same IVF embryonic loss data is used for both fresh and frozen IVP transfers. A binomial distribution is used to account for different numbers of recipients receiving embryos.

Table 72. Percentage of recipients receiving a frozen-thawed IVP embryo that were pregnant at ~21-~30 d of gestation (14-23 d post-transfer).

~21-30 d Pregnancy Rate (%)	n	Recipient Type	Source
50.0	40	Hereford	Martinez, et al. 2002
45.0	40	Hereford	Martinez, et al. 2002

21 d pregnancy rate determined by return to estrus.
30 d pregnancy rate determined by ultrasound.

Table 73. Percentage of recipients receiving a frozen-thawed IVP embryo that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
42.0	67	*Bos taurus* (probably Holstein heifers)	Hasler et al., 1995
37.8	421	*Bos taurus*	Riha et al., 2002
40.9	3627	Chinese Yellow/Holstein	Xu et al., 2006
41.9	481	Chinese Yellow/Holstein	Xu et al., 2006
35.0	20	Holstein Friesian Heifers	Vajta et al., 1997
9.0	11	Holstein Cross Heifers	Donnay et al., 1998
0.0	11	Holstein Cross Heifers	Donnay et al., 1998
35.0	17	Hereford	Martinez et al., 1998

(*cont.*)

Table 73 (*cont.*). Percentage of recipients receiving a frozen-thawed IVP embryo that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
44.0	16	Hereford	Martinez et al., 1998
22.0	76	Crossbred *Bos taurus*	Pugh et al., 2000
50.0	40	Hereford	Martinez et al., 2002
40.0	40	Hereford	Martinez et al., 2002
30.0	10	*Bos taurus*	Nedambale et al., 2004
24.0	85	Dutch-Friesian Heifers	Wurth et al., 1994
14.0	35	Dutch-Friesian Heifers	Wurth et al., 1994

Table 74.Parameters for the percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at d 21 of gestation.

Weighted Mean Adjusted 21 d Pregnancy Rate (%)	Adjusted Standard Deviation Across Sample Means
50.0	17.0

Figure 18. Estimated probability distribution of the true mean for the percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at d 21 of gestation.

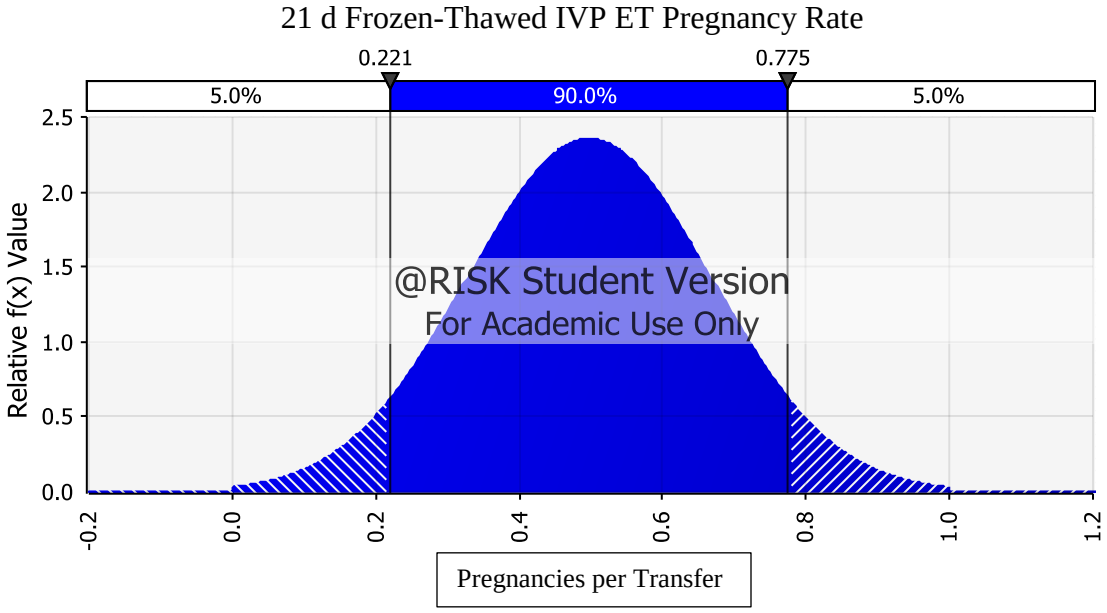


Table 75. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients receiving a frozen-thawed IVD embryo that were pregnant at d 21 of gestation.

5th Percentile	Mode	95th Percentile
0.22	0.50	0.77

21 d Pregnancy Rate Following Service by Natural Sire

Without any actual 21 d pregnancy rate data, the weighted mean 60 d pregnancy rate based on Table 76 is adjusted using the sample means of 21 d-60 d pregnancy loss (Table 88) to create a mean 21 d pregnancy rate of 0.77. The potential variation between 21 d sample means is accounted

for using the adjustment technique previously described to produce a SD across means of .074. A normal probability distribution (Figure 19) based on the parameters just described provided the means for stochastic sampling per LHS. A binomial distribution is also included to account for the possible variation in the number of females serviced by a natural sire.

Table 76. Percentage of naturally serviced females that were pregnant at ~60 d of gestation.

~60 d Pregnancy Rate (%)	n	Recipient Type	Source
70.7	1,705	Beef Cattle (Small number of *Bos indicus* cross. Significantly different *Bos indicus* influenced herd was removed from data)	Lamb et al., 2008
76.3	76	*Bos taurus* Beef	Whittier et al., 1991
86.1	79	*Bos taurus* Beef	Whittier et al., 1991

Table 77. Parameters for the percentage of naturally serviced females that were pregnant at ~21 d of gestation.

Weighted Mean Adjusted 21 d Pregnancy Rate (%)	Adjusted Standard Deviation Across Sample Means (%)
77.0	7.4

Figure 19. Estimated probability distribution of the true mean for the percentage of naturally serviced females that were pregnant at ~21 d of gestation.

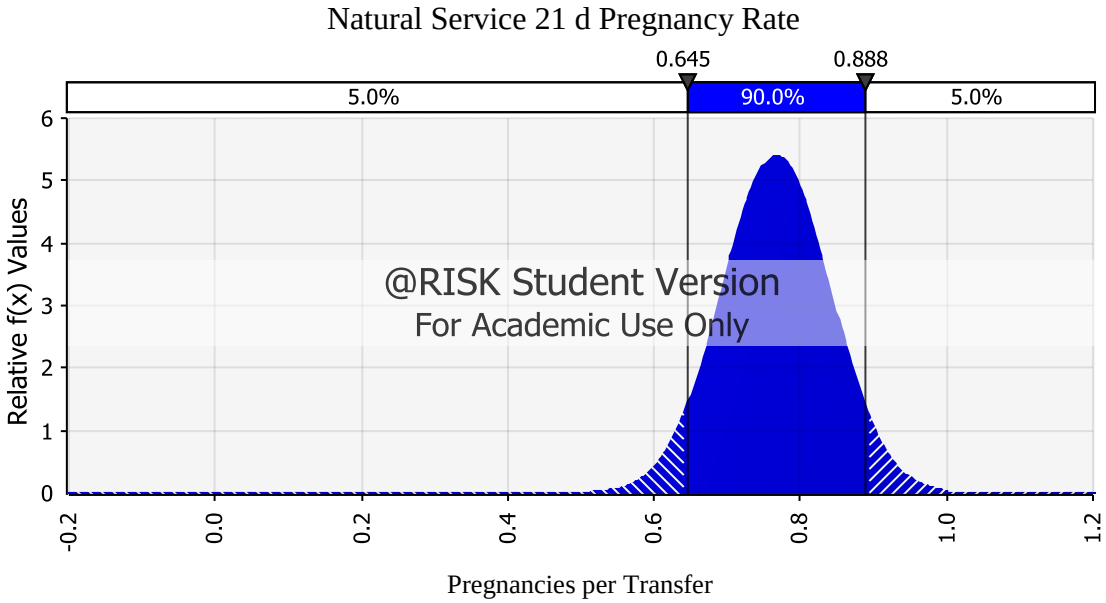


Table 78. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of naturally serviced females that were pregnant at ~60 d of gestation.

5th Percentile	Mode	95th Percentile
0.65	0.77	0.89

Pregnancy Loss Between d 21 and d 60 of Gestation Following Transfer of Fresh IVD Embryos

Pregnancy loss in the interval of d 21 and d 60 of gestation (number of recipients losing pregnancy between d 21 and d 60 divided by the number of recipients pregnant at d 21) is accounted for by calculating a weighted mean, 0.11, and a SD, 0.06, between sample means from Table 79. A normal distribution, truncated at 0 and 1, is based off the aforementioned parameters. A binomial distribution based on the n number of recipients pregnant at d 21 and probability p, 1 minus probability of pregnancy loss, is embedded into the formula used to calculate the number of pregnant recipients at d 60 of gestation following transfer of fresh IVD embryos.

Table 79. Percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of fresh IVD embryos.

Pregnancy Loss Between d ~21 and d ~60 of Gestation (%)	n	Recipient Type	Source
10.5	16	Angus Cross	Farin and Farin 1995
5.0	192	Chinese Yellow/Holstein	Xu et al., 2006
8.5	142	Hereford x Angus	Markette et al., 1985
14.6	526	Hereford x Angus	Markette et al., 1985
8.6	736	Hereford x Angus	Markette et al., 1985
20.9	86	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002

Table 80. Parameters for the percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of fresh IVD embryos. From Table 79.

Weighted Mean Pregnancy Loss d ~21 to d ~60 (%)	SD Across Sample Means (%)
11.0	6.0

Figure 20. Estimated probability distribution of the true mean for the percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of fresh IVD embryos.

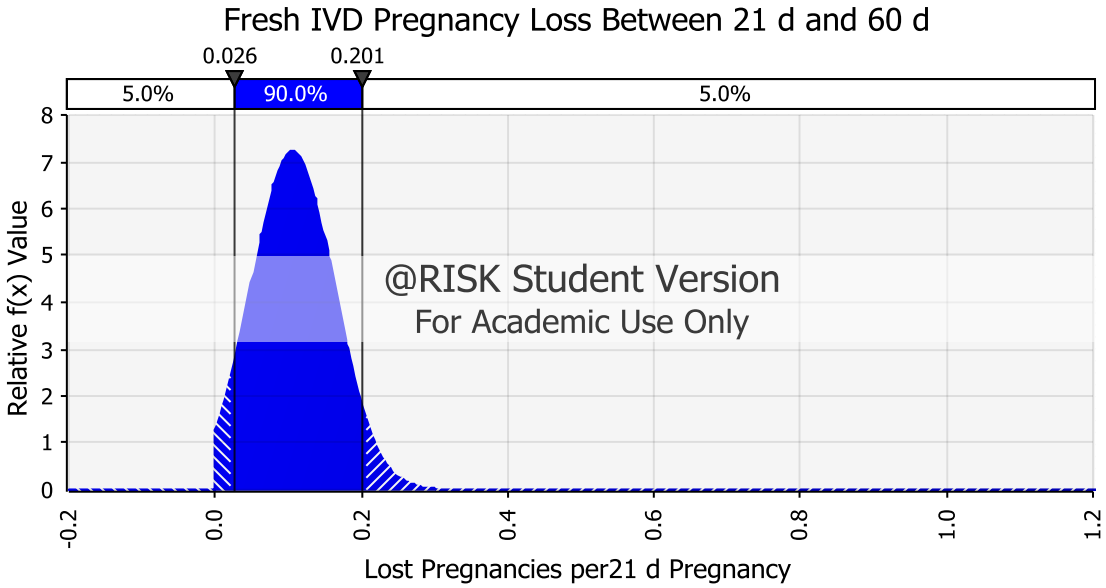


Table 81. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of fresh IVD embryos.

5th Percentile	Mode	95th Percentile
0.03	0.11	0.20

Pregnancy Loss Between d 21 and d 60 of Gestation Following Transfer of Frozen-Thawed IVD Embryos

Using Table 82, the same approach as previously described to calculate pregnancy loss between d 21 and d 60 is applied to arrive at a normal probability distribution with mean 0.23 and

SD 0.08 and truncation at 0 and 1. A binomial distribution is also built into the formula for the number of recipients pregnant at d 60.

Table 82. Percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of frozen-thawed IVD embryos.

Pregnancy Loss Between d ~21 and d ~60 of Gestation (%)	n	Recipient Type	Source
32.0	28	Heifers	Heyman, 1985
17.1	152	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
26.8	175	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002

Table 83. Parameters for the percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of frozen-thawed IVD embryos. From Table 82.

Weighted Mean Pregnancy Loss d ~21 to d ~60 (%)	SD Across Sample Means (%)
23.0	8.0

Figure 21. Estimated probability distribution of the true mean for the percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of frozen-thawed IVD embryos.

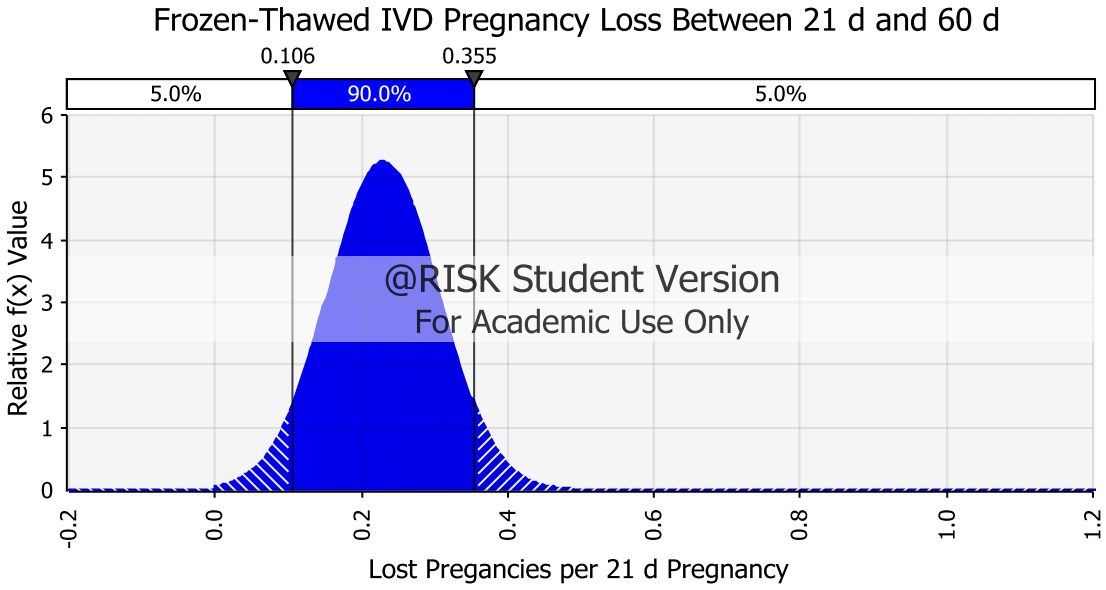


Table 84. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients pregnant at d 21 and not pregnant at d 60 of gestation following transfer of frozen-thawed IVD embryos.

5th Percentile	Mode	95th Percentile
0.11	0.23	0.35

Pregnancy Loss Between d 21 and d 60 of Gestation Following Transfer of IVP Embryos

Using Table 85, the same approach as previously described to calculate pregnancy loss between d 21 and d 60 is applied to arrive at a normal probability distribution with mean 0.20 and SD 0.08 and truncation at 0 and 1. A binomial distribution is also built into the formula for the number of recipients pregnant at d 60. Unlike d 21 to d 60 pregnancy loss for IVD embryos, fresh or frozen-thawed is not distinguished regarding d 21 to d 60 pregnancy loss for IVP embryos.

Table 85. Percentage of recipients pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following transfer of IVP embryos.

Pregnancy Loss Between d ~21 and d ~60 of Gestation (%)	n	Embryo Type	Recipient Type	Source
30.0	117	Fresh	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
18.5	54	Fresh	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
10.5	19	Fresh	Angus Cross	Farin and Farin, 1995
21.3	32	Fresh	Charolais and Crossbred	Heyman et al., 2002
0.0	18	Fresh	Polled Hereford	Martínez et al., 2002
0.0	20	Frozen	Polled Hereford	Martínez et al., 2002
11.0	18	Frozen	Polled Hereford	Martínez et al., 2002

Table 86. Parameters for the percentage of recipients pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following transfer of IVP embryos. From Table 85.

Weighted Mean Pregnancy Loss d ~21 to d ~60 (%)	SD Across Sample Means (%)
20.0	8.0

Figure 22. Estimated probability distribution of the true mean for the percentage of recipients pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following transfer of IVP embryos.

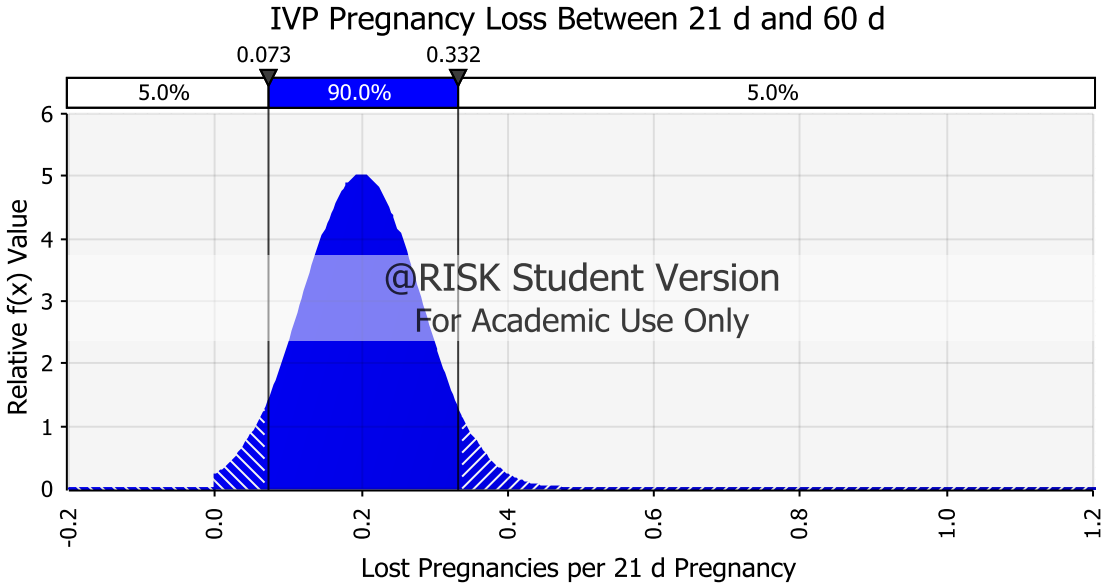


Table 87. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following transfer of IVP embryos.

5th Percentile	Mode	95th Percentile
0.07	0.20	0.33

Pregnancy Loss Between d 21 and d 60 of Gestation Following Service by Natural Sire

Using Table 88, the same approach as previously described to calculate pregnancy loss between d 21 and d 60 is applied to arrive at a normal probability distribution with mean 0.07 and

SD 0.03 and truncation at 0 and 1. A binomial distribution is also built into the formula for the number of recipients pregnant at d 60.

Table 88. Percentage of females pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following service by natural sire.

Pregnancy Loss Between d ~21 and d ~60 of Gestation (AI/Natural Service) (%)	n	Recipient Type	Source
10.20	147	Dairy Heifers	Rivera et al., 2004
6.05	131	Dairy Heifers	Silke et al., 2002
6.50	138	Lactating Beef Cows	Beal et al., 1992
10.80	223	Lactating Beef Cows	Stevenson et al., 2003
4.00	149	Beef Heifers	Lamb et al., 1997
4.10	271	Beef Heifers	Lamb et al., 1997
4.80	105	Beef Heifers	Lamb et al., 1997

Table 89. Parameters for the percentage of females pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following service by natural sire. From Table 88.

Weighted Mean Pregnancy Loss d ~21 to d ~60 (%)	SD Across Sample Means (%)
7.0	3.0

Figure 23. Estimated probability distribution of the true mean for the percentage of females pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following service by natural sire.

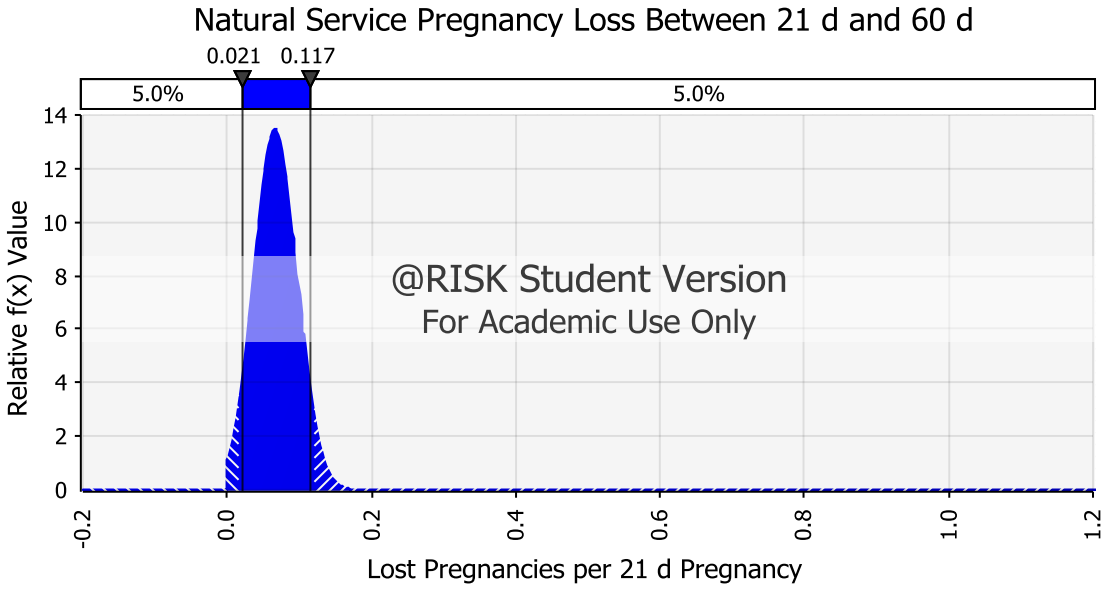


Table 90. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of females pregnant at d 21 that experience pregnancy loss between d 21 and d 60 of gestation following service by natural sire.

5th Percentile	Mode	95th Percentile
0.02	0.07	0.12

Fetal Loss After d 60 of Gestation Following Transfer of IVD Embryos

Nearly identical to the approach in estimating fetal loss between d 21 and d 60, fetal loss of IVD embryos between d 60 and term (number of recipients experiencing fetal loss after d 60 of gestation divided by number of recipients pregnant at d 60) is based on Table 91. A normal distribution with a mean of 0.05 and SD of 0.03 is used to represent the potential range of a true population mean. Truncation is placed at 0 and 1. A binomial distribution is then embedded in the formula to determine the number of recipients maintaining pregnancy to term. The same

distribution is used for both fresh and frozen-thawed IVD embryos, as literature suggests no significant difference (Chagas e Silva et al., 2002).

Table 91. Percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVD embryos.

Pregnancy Loss Between d ~60 of Gestation and Term (%)	n	Recipient Type	Source
11.0	19	Angus Cross	Farin and Farin, 1995
7.0	228	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
11.8	449	Hereford x Angus	Markette et al., 1985
5.7	674	Hereford x Angus	Markette et al., 1985
4.7	5,457	Holstein Heifers	Hasler et al., 1987
2.9	70	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002

(*cont.*)

Table 91 (*cont.*). Percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVD embryos.

Pregnancy Loss Between d ~60 of Gestation and Term (%)	n	Recipient Type	Source
5.6	126	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
5.9	68	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
7.0	128	Holstein Friesian (no significant difference between heifers and lactating cows)	Chagas et al., 2002
1.1	2,242	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw, 2000
5.3	1,776	Beef Breeds and Holstein Heifers	King et al., 1985

Table 92. Parameters for the percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVD embryos. From Table 91.

Weighted Mean Pregnancy Loss Between d ~60 and term (%)	SD Across Sample Means (%)
5.0	3.0

Figure 24. Estimated probability distribution of the true mean for the percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVD embryos.

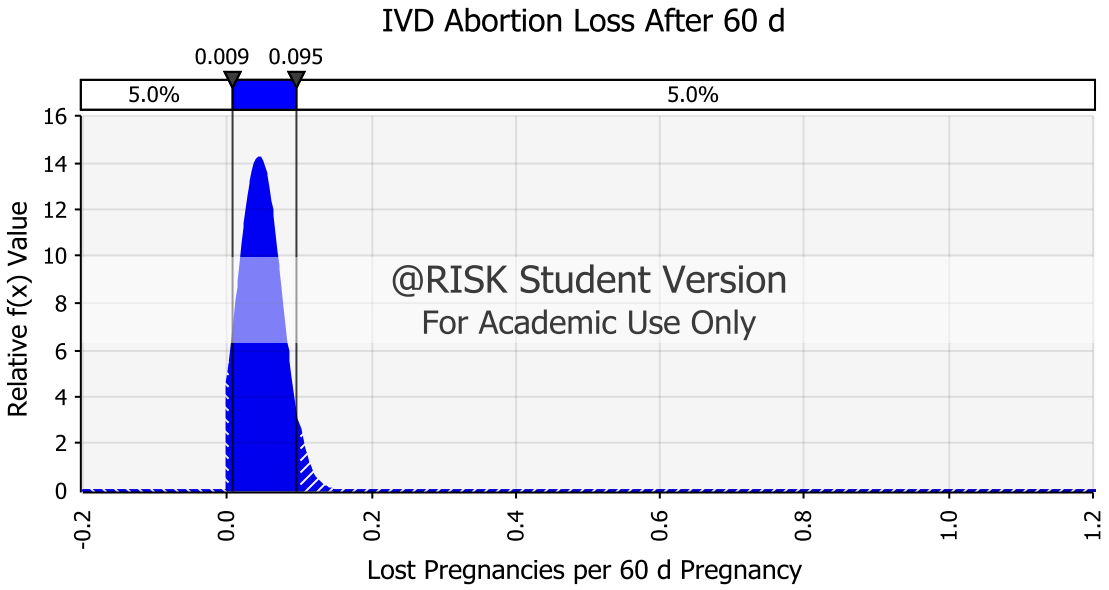


Table 93. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVD embryos.

5th Percentile	Mode	95th Percentile
0.006	0.05	0.13

Fetal Loss After d 60 of Gestation Following Transfer of IVP Embryos

The same methodology is used to determine fetal loss following the transfer of IVP embryos as that of IVD embryos. Applying the data from Table 94, the resulting normal

distribution had a mean of 0.05 and SD across sample means of 0.05, with truncation at 0 and 1. Again, a binomial distribution is incorporated into the calculation of the number of IVP ET recipients maintaining pregnancy to term. Fresh and frozen-thawed IVP embryos are not distinguished from one another.

Table 94. Percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVP embryos.

Pregnancy Loss Between d ~60 of Gestation and Term (%)	n	Recipient Type	Source
5.5	711	Chinese Yellow/Holstein	Xu et al., 2006
5.4	481	Chinese Yellow/Holstein	Xu et al., 2006
13.0	75	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
6.8	58	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
2.6	1,452	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
13.1	273	Holstein Heifers	Hasler, 2000

(*cont.*)

Table 94 (*cont*.). Percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVP embryos.

Pregnancy Loss Between d ~60 of Gestation and Term (%)	n	Recipient Type	Source
11.9	201	Holstein Heifers	Hasler, 2000
10.7	65	Holstein Heifers	Hasler, 2000
0.0	110	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian)	Wagtendonk-de Leeuw et al., 2000
1.3	152	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian)	Wagtendonk-de Leeuw et al., 2000

Table 95. Percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVP embryos. From Table 94.

Weighted Mean Pregnancy Loss Between d ~60 and term (%)	SD Across Sample Means (%)
5.0	5.0

Figure 25. Estimated probability distribution of the true mean for the percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVP embryos.

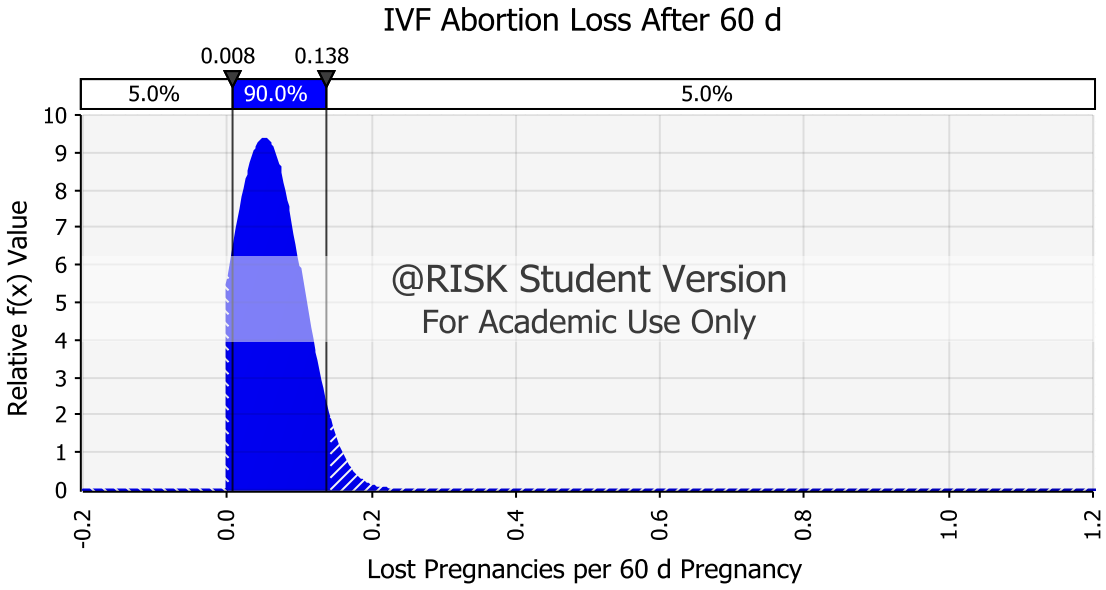


Table 96. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of recipients pregnant at d 60 of gestation that do not maintain pregnancy to term following transfer of IVP embryos.

5th Percentile	Mode	95th Percentile
0.008	0.05	0.14

Fetal Loss After d 60 of Gestation Following Service by Natural Sire

Applying the same procedure in estimating the other fetal loss measurements, using Table 97, a normal probability distribution with a mean of 0.02 and SD across sample means of 0.01 describes fetal loss after d 60 of gestation following service by a natural sire. The distribution is truncated at 0 and 1. A binomial distribution is included in the formula for calculating the number of naturally serviced females retaining pregnancy to term.

Table 97. Percentage of females pregnant at d 60 of gestation that do not maintain pregnancy to term following service by natural sire.

Pregnancy Loss Between d ~60 of Gestation and Term (AI/Natural Service) (%)	n	Recipient Type	Source
1.3	5,353	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
0.5	1,764	*Bos taurus* (Breed not disclosed, Probably Holstein-Friesian and beef cattle)	Wagtendonk-de Leeuw et al., 2000
2.8	10,595	Beef Cattle	Dziuk and Bellows, 1983

Table 98. Parameters for the percentage of females pregnant at d 60 of gestation that do not maintain pregnancy to term following service by natural sire.

Weighted Mean Pregnancy Loss Between d ~60 and term (%)	SD Across Sample Means (%)
2.0	1.0

Figure 26. Estimated probability distribution of the true mean for the percentage of females pregnant at d 60 of gestation that do not maintain pregnancy to term following service by natural sire.

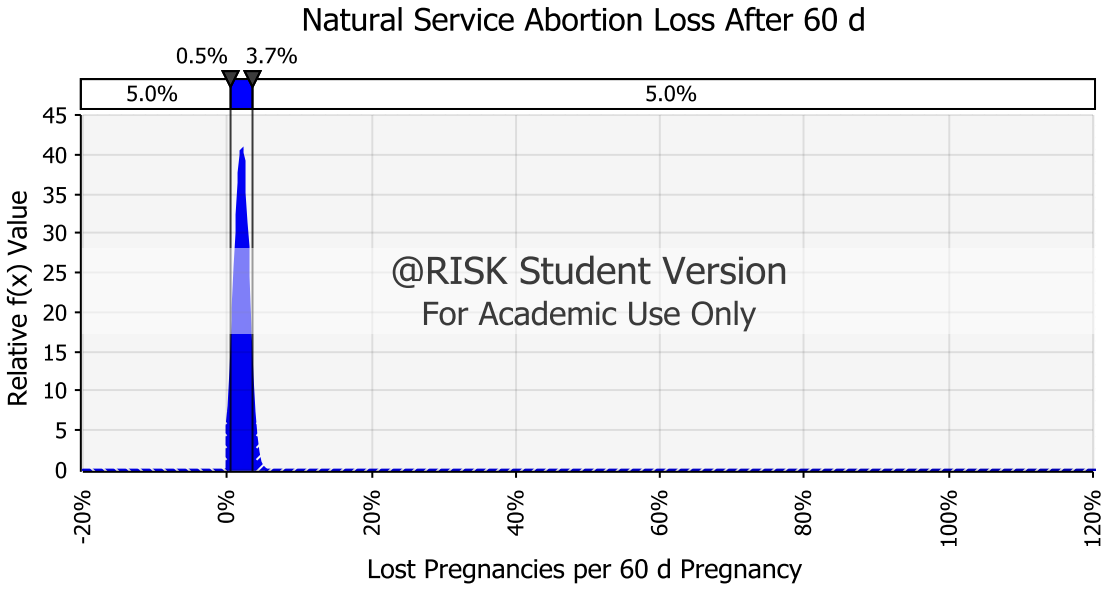


Table 99. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of females pregnant at d 60 of gestation that do not maintain pregnancy to term following service by natural sire.

5th Percentile	Mode	95th Percentile
0.01	0.02	0.04

Percentage of Desired Sex Resulting From Use of Sex- Sorted Semen

Using Table 100 for reference, a normal probability distribution (Figure 27) represents the mean accuracy and standard deviation of sexed semen accuracy across sample populations (Table 101). The distribution is truncated at 0.8 and 1. The 0.8 can be justified by the likelihood of some type of quality control measures used by the sex-sorted semen manufacturer, or the potential that any accuracy issues of that magnitude would not be tolerated by industry. The stochastically sampled mean value, drawn from the normal distribution described per LHS, is included in the

binomial distribution that is inserted into the equation computing the number of progeny of the desired sex.

Table 100. Percentage of calves of the selected sex resulting from the use of sex-sorted semen.

Sexed Semen Accuracy (%)	n	Source
94.0	966	Morotti et al., 2014
91.0	7,763	Pontes et al., 2010
87.0	2,286	Pontes et al., 2010
96.5	458	Xu et al., 2006

Table 101. Parameters for the percentage of calves of the selected sex resulting from the use of sex-sorted semen.

Weighted Mean of Sexed Semen Accuracy	SD Across Sample Means
91.0	4.0

Figure 27. Estimated probability distribution of the true mean for the percentage of calves of the selected sex resulting from the use of sex-sorted semen.

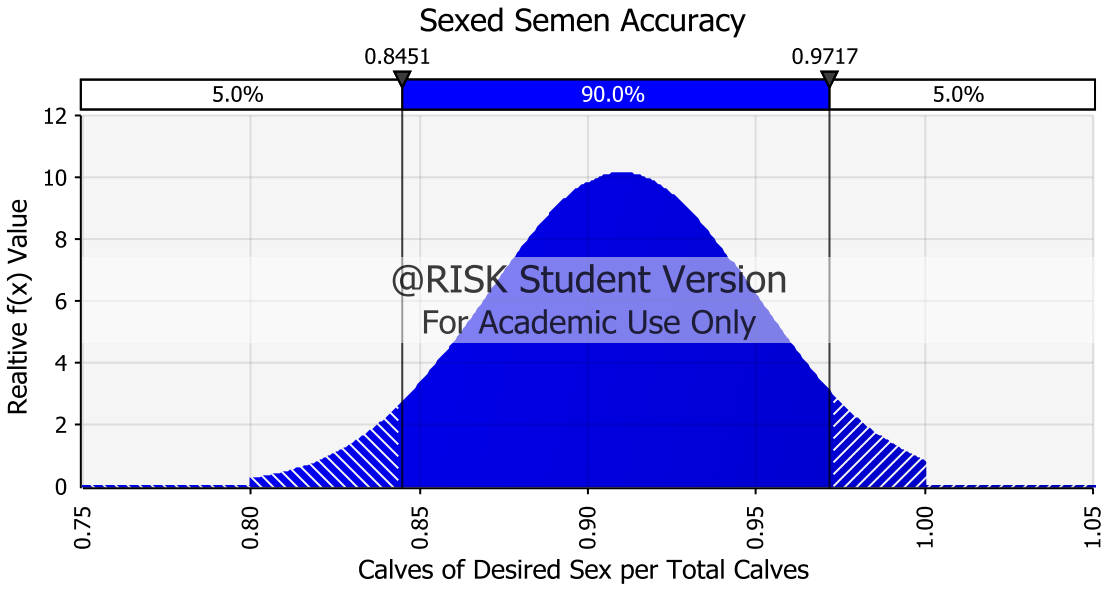


Table 102. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of calves of the selected sex resulting from the use of sex-sorted semen.

5th Percentile	Mode	95th Percentile
0.84	0.91	0.97

Number of Progeny of Either Sex Resulting From Use of Non-Sorted Semen or Natural Service Sire

Although some literature reports variation from the expected, naturally occurring 1:1 sex ratio when using ET technology (Hasler et al., 1995; King et al., 1985); it was determined that a 1:1 sex ratio is sufficient for use in the model because it is difficult to confidently attribute causality of sex ratio variation to ET and not the natural laws of probability. Thus, a probability (p) of 0.5 is used in the binomial distribution calculating the expected number of progeny of each sex when using non-sorted semen or natural service.

Percentage of Calves Weaned per Calving Cow for Pregnancies Derived from IVD Embryo or Natural Service

To determine an estimate for the mean calf mortality from the time of calving through weaning, the weighted mean of perinatal calf mortality from Table 103 is combined with the weighted mean of calf mortality after the perinatal period through weaning, from Table 104. The potential variation across sample means is then computed as the standard deviation of the range of values resulting from the combination of every sample mean of perinatal calf mortality with every sample mean post-perinatal calf mortality. The inverse of mean calf mortality, calf survival, and the SD across samples (Table 105) are used as parameters for a normal probability distribution describing calf survival to weaning. The distribution is truncated at 0 and 1. A LHS derived value

for the calf survival distribution is then used as the probability, p, of survival within a binomial distribution predicting the number calves that survive through weaning.

Table 103. Percentage of calf births resulting in mortality during the perinatal period following a natural service, AI, or MOET derived pregnancy.

Perinatal Calf Mortality (%)	n	Origin of Pregnancy	Recipient Type	Source
5.2	1,682	MOET	Primarily Beef, some Holstein Heifers	King et al., 1985
5.3	4,949	AI	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
4.6	2,180	MOET	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
4.7	1,651	AI	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
2.9	34	MOET	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
7.9	10,300	Natural Service	Beef (Cows and Heifers)	Dziuk and Bellows, 1983
3.6	56	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973

(*cont.*)

Table 103 (*cont.*). Percentage of calf births resulting in mortality during the perinatal period following a natural service, AI, or MOET derived pregnancy.

Perinatal Calf Mortality (%)	n	Origin of Pregnancy	Recipient Type	Source
0.5	184	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
2.3	216	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
0.5	205	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
1.5	206	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1974
0.5	189	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1975
9.0	636	MOET	Beef (Cows and Heifers) (Estimation)	Markette et al., 1985

Table 104. Percentage of calves alive at the end of the perinatal period that do not survive to weaning following a natural service, AI, or MOET derived pregnancy.

Calf Mortality After Perinatal Stage until Weaning (%)	n	Origin of Pregnancy	Recipient Type	Source
3.8	1,682	MOET	Primarily Beef, some Holstein Heifers	King et al., 1985
3.6	10,300	Natural Service	Beef	Dziuk, 1983
3.6	56	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
6.0	184	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
13.9	216	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
4.4	205	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
4.9	206	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973
3.2	189	Natural Service	Beef (Cows and Heifers)	Dearborn et al., 1973

Table 105. Parameters for the percentage of calf births that result in a live calf at weaning following a natural service, AI, or MOET derived pregnancy**.**

Weighted Mean of Calf Survival to Weaning (%)	SD Across Sample Means (%)
90.0	4.0

Figure 28. Estimated probability distribution of the true mean for the percentage of calf births that result in a live calf at weaning following a natural service, AI, or MOET derived pregnancy.

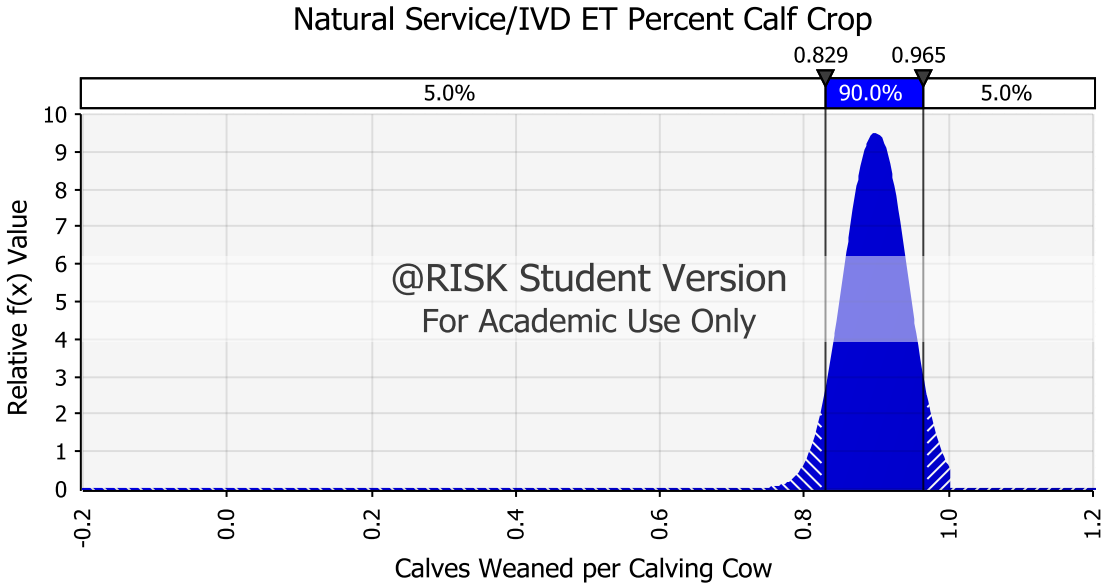


Table 106. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of calf births that result in a live calf at weaning following a natural service, AI, or MOET derived pregnancy.

5th Percentile	Mode	95th Percentile
0.83	0.90	0.97

Percentage of Calves Weaned per Calving Cow for Pregnancies Derived from IVP Embryos

As literature (Wagtendonk-de Leeuw et al., 2000) reported an increase in perinatal calf mortality with IVP derived pregnancies when compared to natural service, AI, or MOET derived pregnancies, a separate calf survival distribution is modeled for calves resulting from IVP derived pregnancies. Following the same methodology as described in the previous section, calf mortality of IVP calves is estimated using mean sample values from Table 107 and Table 104. An LHS derived value, sampled from the resulting distribution of calf survival, is used in the binomial distribution estimated calf survival through weaning.

Table 107. Percentage of calf births that result in a live calf at weaning following an IVP derived pregnancy.

Perinatal Calf Mortality (%)	n	Culture Type	Recipient Type	Source
7.5	1,374	co-culture	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
7.3	107	co-culture	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
5.2	149	SOF	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
3.1	32	co-culture	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
3.0	33	SOF	Likely Holstein-Friesian	Wagtendonk-de Leeuw et al., 2000
21.2	113	B2-BRL Serum	Holstein Heifers	Hasler, 2000
18.6	70	B2-BRL No Serum	Holstein Heifers	Hasler, 2000
8.9	45	TCM-199-BRL Serum	Holstein Heifers	Hasler, 2000
7.4	27	TCM-199-BRL	Dutch- Friesian Heifers	Wurth et al., 1994

Table 108. Parameters for the percentage of calf births that result in a live calf at weaning following an IVP derived pregnancy.

Weighted Mean of Calf Survival to Weaning (%)	SD Across Sample Means (%)
88.0	7.0

Figure 29. Estimated probability distribution of the true mean for the percentage of calf births that result in a live calf at weaning following an IVP derived pregnancy.

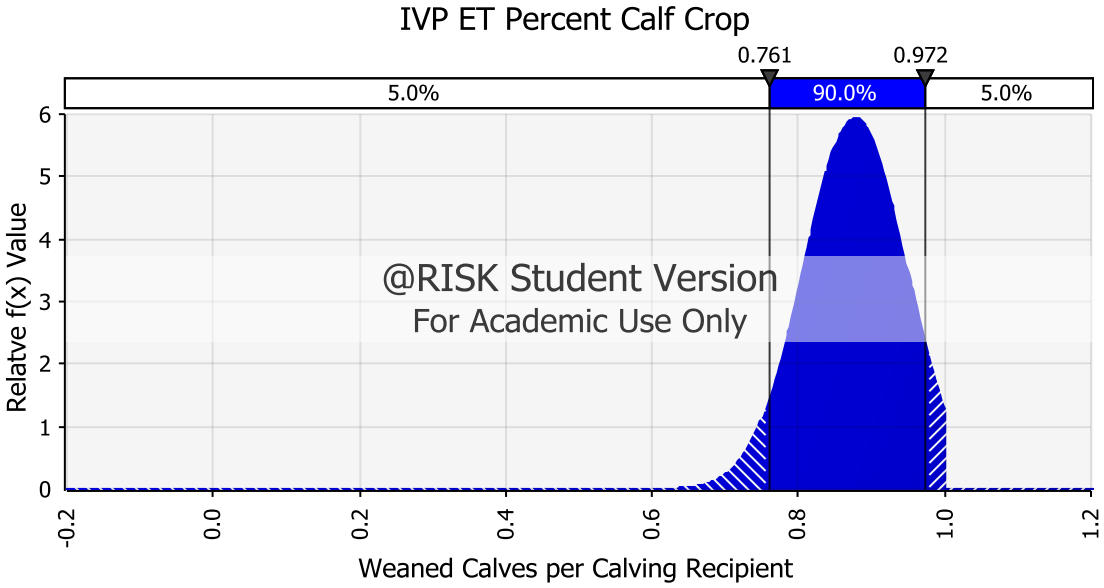


Table 109. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the percentage of calf births that result in a live calf at weaning following an IVP derived pregnancy.

5th Percentile	Mode	95th Percentile
0.76	0.88	0.97

Revenue Distribution of Developed Bulls and Heifers

To attempt a realistic representation of the inherent variation in revenue received through the marketing of developed progeny, likely for use as seedstock, a distribution of the mean value of such progeny is inputted into the model (Figure 30, Figure 31). While the distributions for the market value of developed bulls and heifers used within the scenarios in question are derived from industry knowledge and current market trends, there is also opportunity to develop a price distribution based on past marketing of ET derived progeny. The distributions within the current model representing the mean sale value of developed bulls and heifers are truncated at the 5th percentile of the original distributions, effectively creating a new bull revenue distribution with a

floor price of $2,730 and a heifer revenue distribution with a floor price of $2,013. These distributions can be modified to match the operation, value of genetics, and current market. For each iteration of the model, a mean value, sampled per LHS from the bull and heifer revenue distributions, is multiplied by the number of bulls and heifers, respectively, to yield total revenue from the sale of developed bulls and heifers.

Table 110. Parameters for the distribution of the true mean for the revenue received per head from the sale of developed, ET derived bulls.

Mean of ET Derived Bull Sale Averages ($)	SD ($)
6,710.42	4,000.00

Figure 30. Estimated probability distribution of the true mean for the revenue received per head from the sale of developed, ET derived bulls.

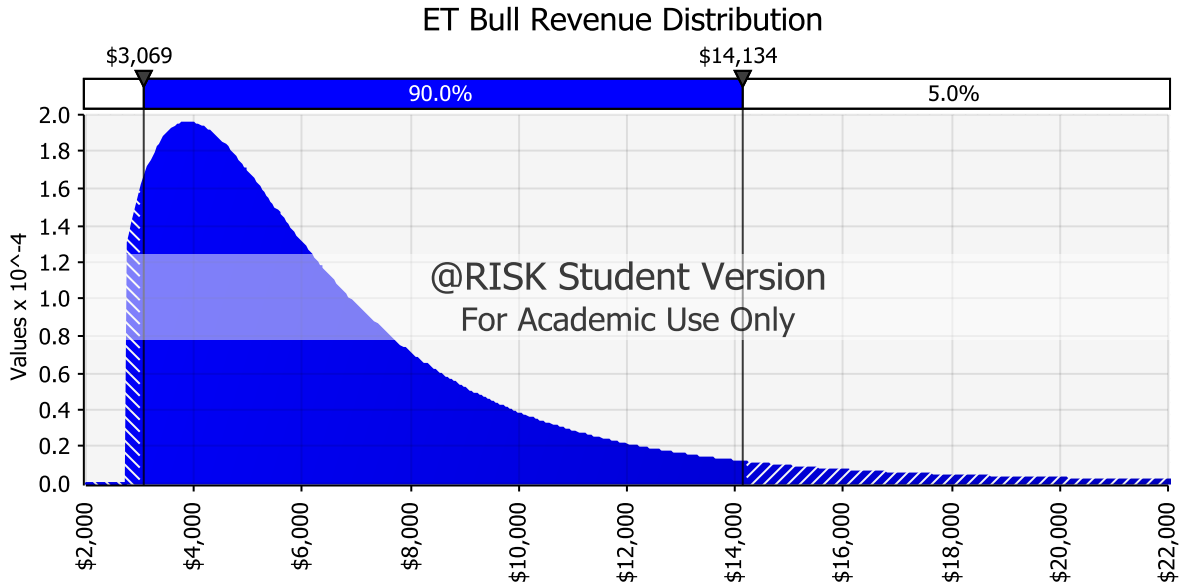


Table 111. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the revenue received per head from the sale of developed, ET derived bulls.

5th Percentile ($)	Mode ($)	95th Percentile ($)
3,063.36	3,880.70	14,095.35

Table 112. Parameters for the distribution of the true mean for the revenue received per head from the sale of developed, ET derived heifers.

Mean of ET Derived Bull Sale Averages ($)	SD ($)
5,155.63	4,000.00

Figure 31. Estimated probability distribution of the true mean for the revenue received per head from the sale of developed, ET derived heifers.

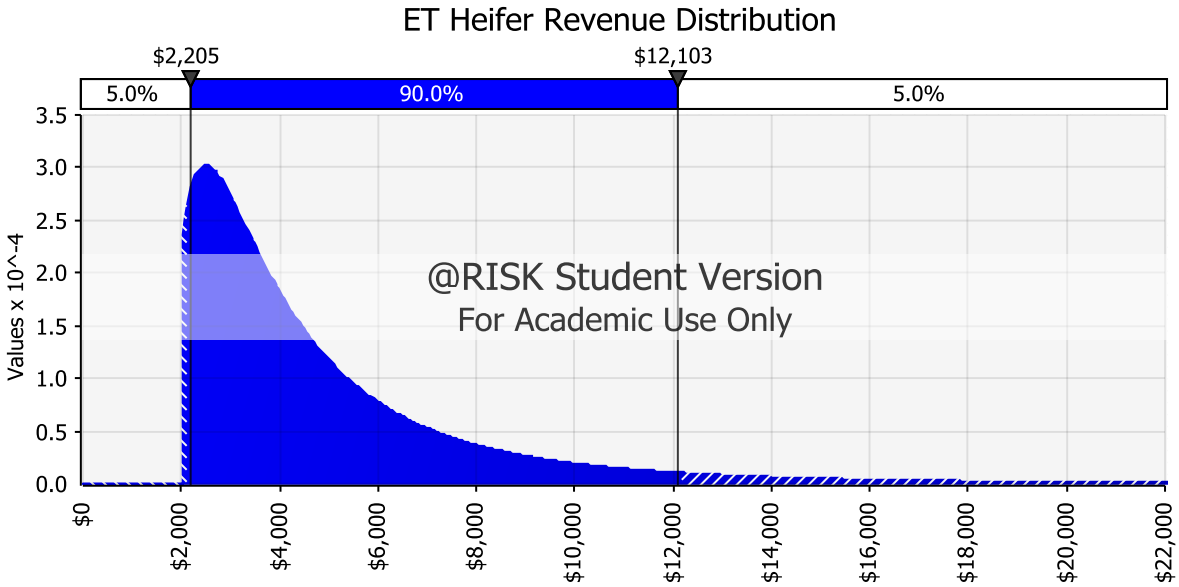


Table 113. 5th percentile, mode, and 95th percentile of the estimated probability distribution of the true mean for the revenue received per head from the sale of developed, ET derived heifers.

5th Percentile ($)	Mode ($)	95th Percentile ($)
2,203.42	2,499.41	12,077.54

Deterministic Variables

Accompanying the stochastic variables characterized by the distributions previously described are the user-defined deterministic variables listed in the following tables. The values used in the current simulation study are included in the tables.

ET Production

Table 114. User-defined, deterministic variables describing the ET program scenario under consideration.

	MOET	IVP
Number of Flushes per Donor	2	5
Number of Fresh Transfer Events	2	5
Number of Frozen Transfer Events	1	1
Days Between Flushes/OPU	45	14
Total Number of Donors	5	5
Total Recipients in Herd	100	100
Days between final Fresh Transfer Event and Thawed Transfer Event	17	14
Number of Days Exposed to Bull	45	45
Number of Open Cows Exposed per Bull	30	30

Table 115. User- defined, deterministic expenses associated with the ET program scenario under consideration.

	MOET	IVP
Average Donor Purchase Cost/Head ($)	15,000.00	15,000.00
Annual Donor Feed Cost/Head ($)	550.00	550.00
Annual Donor Health Program Cost/Head ($)	15.00	15.00
Average Bull Purchase Cost/Head ($)	6,000.00	6,000.00
Annual Bull Feed Cost/Head ($)	400.00	400.00
Annual Bull Health Program Cost/Head ($)	50.00	50.00
Average Open Recipient Purchase Cost/Head ($)	1200.00	1200.00
Annual Bred Recipient Feed Cost/Head ($)	500.00	500.00
Annual Calving Recipient Feed Cost/Head minus Calving Season Feed Cost ($)	450.00	450.00
Recipient Health Program Cost/Head ($)	15.00	15.00

Protocol

Table 116. User-defined, deterministic variables describing synchronization and stimulation protocols for the ET program scenario under consideration.

	MOET	IVP NS	IVP SS
GnRH Doses/Donor/Protocol	1	0	1
CIDR Doses/Donor/Protocol	1	0	0
Doses/Donor/Protocol	4	0	0
FSH Doses/Donor/Protocol	8	0	4
Unsorted Semen Doses/Donor/Protocol	3	300 oocytes/straw	300 oocytes/straw
Sexed Semen Doses/Donor/Protocol	6	100 oocytes/straw	100 oocytes/straw
GnRH Doses/Recipient/Protocol	1	1	1
CIDR Doses/Recipient/Protocol	1	1	1
PGF Doses/Recipient/Protocol	1	1	1
FSH Doses/Recipient/Protocol	0	0	0

Estimated Market Value

In some situations, the variables in Table 117, currently represented as deterministic, user-defined variables, could also be represented stochastically through distributions. Depending on the marketing and ownership scenario it is common for products such as ET pregnancies and weaned calf premiums to be fixed values determined through a contract. In other situations, the values of all variables in Table 117 could be exposed to market, genetic, and animal weight variation that could induce fluctuation in value. While the current model does not account for the potential variation in the values of the variables in Table 117, proper industry review could allow for their stochastic representation in an improved model.

Table 117. User-defined, deterministic variables estimating the fair market value of potential marketing avenues associated with the ET program under consideration.

	Average Value ($)
Unsexed Embryo	300.00
Sexed Bull Embryo	400.00
Sexed Heifer Embryo	400.00
ET Bred Recipient	3,200.00
ET Bred Recipient- Bull Calf Pregnancy	3,500.00
ET Bred Recipient- Heifer Calf Pregnancy	3,000.00
Bull Bred Recipient	2,000.00
ET Weaned Bull Calf Premium	700.00
ET Weaned Heifer Calf Premium	500.00
Bull Salvage Value	1,500.00
Donor Salvage Value	1,000.00
Recipient Salvage Value	1,000.00

Table 118. Estimated feeder calf price scale based on the sale of feeder steers resulting from the ET program under consideration.

Weaned Calf Base Pricing		
Price Index/lb:	**Weight (lbs)**	**Price/lb ($)**
Between (lbs)	375.00 – 425.01	1.57
	425.01 – 475.00	1.54
	475.01 – 525.00	1.50
	525.01 – 575.00	1.47
	575.01 – 625.00	1.43
	625.01 – 675.00	1.39
	675.01 – 725.00	1.35
	725.01 – 775.00	1.31
	775.01 – 825.00	1.27
	825.01 – 875.00	1.24
	875.01 – 925.00	1.20
	925.01 – 975.00	1.16
	975.01 – 1025.00	1.12

From Farmers and Ranchers Weekly Market Report (November 2016).

Protocol Expenses

Table 119. User-defined, deterministic variables representing the estimated expense of protocol factors associated with the ET program under consideration.

	MOET	IVP
GnRH Cost/Dose ($)	2.50	2.50
CIDR Cost/Dose ($)	11.00	11.00
PGF Cost/Dose ($)	3.40	3.40
FSH Cost/Dost ($)	18.00	18.00
MOET Flush Procedure Cost ($)	350.00	NA
IVF OPU Cost ($)	NA	750.00
Unsorted Semen Cost/Straw ($)	20.00	20.00
Sex-Sorted Cost/Straw ($)	50.00	50.00
Embryo Freezing Cost/Embryo ($)	45.00	45.00
Cost/Transfer ($)	50.00	50.00
Pregnancy Determination Cost/Head ($)	6.00	6.00
Pregnancy Sex Determination Cost/Head ($)	10.00	10.00

Bred Recipient Program

Table 120. User-defined, deterministic variables describing the length of ownership specific to each classification of recipient at the conclusion of an ET program marketing bred recipients.

Recipient Type	Average Length of Ownership From Purchase of Open Recipients (months)
Open Recipients	4
ET Bred Recipients	4
Natural Service Bred Recipients	4

Weaned Calf Program

Table 121. User-defined, deterministic variables specific to factors associated with an ET program marketing weaned calves.

Average Length of Ownership (in months) From Purchase of Open Recipients (Open Recipients)	4
Ration Cost/lb DM during Calving Season ($)	0.10
3rd Trimester Daily DMI (lbs)	25
Post-Partum Daily DMI (lbs)	32
Length of Calving Season Before Grazing Season (Days)	40

Anticipated Calf Performance

The variables in Table 122, currently represented as user-defined variables, could also be represented stochastically through distributions. While the current model does not account for the potential variation in the values of the variables in Table 122, proper review of the distribution of performance trait values could allow for their stochastic representation in an improved model.

Table 122. User-defined, deterministic variables estimating calf performance for the ET program under consideration.

Bull Birthweight (lbs)	80.0
Heifer Birthweight (lbs)	76.0
Bull Average Daily Gain (ADG) Preweaning (lbs)	2.4
Bull ADG Postweaning (lbs)	2.5
Heifer ADG Preweaning (lbs)	2.3
Heifer ADG Postweaning (lbs)	2.4
Oldest Calves' Age @ Weaning (days)	205.0

Preconditioning/Development Factors

Several variables, such as treatment cost per head, in Table 123 could also be represented by stochastic, rather than deterministic, variables, if such a feature is deemed pertinent to the usefulness of the model. In the current model, the variables in Table 123 are user defined and deterministic.

Table 123. User-defined, deterministic variables describing factors associated with the preconditioning and development of calves resulting from the ET program under consideration.

Preconditioning Days	45
Daily Backgrounding Cost/Head ($)	2.20
Vaccine Cost/Head ($)	2.00
Treatment Cost/Head ($)	1.80
Misc. Bull Development Cost ($)	100.00
Misc. Heifer Development Cost ($)	80.00
Daily Bull Development Cost ($)	2.45
Daily Heifer Development Cost ($)	1.40
Postweaning Development Days- Bulls	180
Postweanig Development Days- Heifers	180
Expected Cull Rate (%)	20.0

Labor

Table 124. User-defined, deterministic variables estimating labor costs and requirements associated with the ET program under consideration.

Cost/Non-Vet Man-Hour ($)	10.00
Non-Vet Man-Hours/Flush	6
Non-Vet Man-Hours/IVP NS OPU	0.25
Non-Vet Man-Hours/IVP SS OPU	1
Non-Vet Man-Hours/IVD Recipient	1
Non-Vet Man-Hours/IVP NS Recipient	1
Non-Vet Man-Hours/IVP SS Recipient	1

Investment Variables

Table 125. User-defined, deterministic variables describing the investment parameters associated with the ET program under consideration.

Discount Rate (%)	5
Number of Weaned Calf Crops	6
Number of Developed Bull/Heifer Calf Crops	6

Statistical Analysis

Statistical analysis was performed using StatTools 7.5 ©. Using the individual results generated from each iteration of the simulation, a standardized, stepwise regression analysis was executed for each scenario with each stochastic variable serving as an independent variable and ROI as the dependent variable. Adjusted R-squared values were determined for each regression model.

Sensitivity analysis allows for the determination of which independent variable(s) has(have) the greatest influence on the outcome of the dependent variable. Regression can serve as a means of sensitivity analysis. Basic stepwise regression creates a regression model by adding one independent variable at a time. The first independent variable used in the model is the variable with the greatest correlation coefficient value (Iman et al., 1985). Additional variables are added to the model in order of the greatest to least partial correlation coefficient value (Iman et al., 1985). Only variables with a significant impact on the R-squared value of the final model will be included in the final regression model (Iman et al., 1985).

With a value ranging from 0 to 1, the R-squared value, also called the coefficient of determination, discloses the percent of variation in the dependent variable that is explained by the variable(s) included in the regression model (Iman et al., 1985). In stepwise regression, the

individual contribution of each variable to the final R-squared value provides a comparison of the influence of each independent variable.

The R-squared value of a model can also be adjusted based on the number of variables in the model. When an additional variable is included in the model the R-squared value will always increase; however, the adjusted R-squared value will only increase if the mean square error of the model decreases simultaneously (Mendenhall and Sincich, 2012).

By standardizing the regressions coefficients of the regression model equation based on one standard deviation of the variable, the regression coefficients of the independent variables also allow for a relative comparison of the impact of each variable (Iman et al., 1985). If regression coefficients are not standardized, the unit of measure can distort the relative magnitude of the coefficient value (Iman et al., 1985).

Chapter 4 - Results and Discussion

A simulation of 100,000 iterations was run using the stochastic model with scenario parameters as described in the previous sections. The use of 100,000 iterations balances a negligible amount of variation between simulation experiments of identical input, while still allowing for a reasonably short simulation run-time. While numerous scenarios utilizing sex-sorted or unsorted semen with variations in ownership of donors and recipients and alternative marketing avenues can be compared simultaneously, a select few scenarios were chosen for analysis.

When analyzing the results of the model, it must be remembered that the overhead costs of facilities, equipment, enterprise financing, taxes, labor not directly associated with ET procedures, and any other potential non-direct expenses are not included in the model. Fuel, as well as facility and equipment depreciation and maintenance expense are also unaccounted for. Thus, the economic model represents variable costs directly attributed to the ET program. If the user chose to embed overhead costs within other cost parameters within the model, it is possible to consider such expenses, but it is assumed in the current model that any differences between the scenarios are negligible.

The intent of this model is not to provide a means for industry wide assessment of the application of a specific reproductive technology or the profitability of a given marketing strategy, in general. The following results are merely circumstantial predictors of the economic value and risk associated with a given scenario based on the user-defined, deterministic variables and default stochastic elements as described in the preceding sections. The subsequent figures (Figure 32 – Figure 58) and tables (Table 126 – Table 135) describe the characteristics of the distribution of NPV, ANPV, and ROI for the scenarios in question. Although a multitude of ownership and marketing strategies are combined with all 8 embryo production methods described in the

preceding chapter, to create a variety of scenarios that are simulated concurrently, only the following scenario results are reported in this thesis.

Scenario A1:

- Embryo Production Method: MOET using unsorted semen.
- Ownership: Own donors and own recipients.
- Marketing: Sell developed bulls and females per the pricing distribution described in the previous chapter. Sell all cull progeny and naturally sired calves by weight, as feeder cattle, per the feeder calf pricing index. Market excess embryos using the user-defined price disclosed in the preceding chapter.

Scenario A2:

- Embryo Production Method: IVP NS, 14 d OPU interval using unsorted semen.
- Ownership: Own donors and own recipients.
- Marketing: Sell developed bulls and females per the pricing distribution described in the previous chapter. Sell all cull progeny and naturally sired calves by weight, as feeder cattle, per the feeder calf pricing index. Market excess embryos using the user-defined price disclosed in the preceding chapter.

Scenario A3:

- Embryo Production Method: IVP SS using unsorted semen.
- Ownership: Own donors and own recipients.
- Marketing: Sell developed bulls and females per the pricing distribution described in the previous chapter. Sell all cull progeny and naturally sired calves by weight, as feeder cattle, per the feeder calf pricing index. Market excess embryos using the user-defined price disclosed in the preceding chapter.

Scenario B1:

- Embryo Production Method: MOET using semen sex-sorted for males.
- Ownership: Own donors and own recipients.
- Marketing: Sell developed bulls and females per the pricing distribution described in the previous chapter. Sell all cull progeny and naturally sired calves by weight, as feeder cattle, per the feeder calf pricing index. Market excess embryos using the user-defined price disclosed in the preceding chapter.

Scenario B2:

- Embryo Production Method: IVP NS, 14 d OPU interval using semen sex-sorted for males.
- Ownership: Own donors and own recipients.
- Marketing: Sell developed bulls and females per the pricing distribution described in the previous chapter. Sell all cull progeny and naturally sired calves by weight, as feeder cattle, per the feeder calf pricing index. Market excess embryos using the user-defined price disclosed in the preceding chapter.

Scenario B3:

- Embryo Production Method: IVP SS using semen sex-sorted for males.
- Ownership: Own donors and own recipients.
- Marketing: Sell developed bulls and females per the pricing distribution described in the previous chapter. Sell all cull progeny and naturally sired calves by weight, as feeder cattle, per the feeder calf pricing index. Market excess embryos using the user-defined price disclosed in the preceding chapter.

Scenario C1:

- Embryo Production Method: MOET using unsorted semen.

- Ownership: Own recipients. Contracted to sell ET pregnant recipients back to owner of the donor/embryos.
- Marketing: Sell recipients at least 60 d pregnant to embryo transfer. Sex of pregnancy is determined via pregnancy ultrasound. Sell natural service and open recipients. All market prices subject to user-defined price disclosed in the preceding chapter.

Scenario C2:

- Embryo Production Method: IVP NS, 14 d OPU interval using unsorted semen.
- Ownership: Own recipients. Contracted to sell ET pregnant recipients back to owner of the donor/embryos.
- Marketing: Sell recipients at least 60 d pregnant to embryo transfer. Sex of pregnancy is determined via pregnancy ultrasound. Sell natural service and open recipients. All market prices subject to user-defined price disclosed in the preceding chapter.

Scenario C3:

- Embryo Production Method: IVP SS using unsorted semen.
- Ownership: Own recipients. Contracted to sell ET pregnant recipients back to owner of the donor/embryos.
- Marketing: Sell recipients at least 60 d pregnant to embryo transfer. Sex of pregnancy is determined via pregnancy ultrasound. Sell natural service and open recipients. All market prices subject to user-defined price disclosed in the preceding chapter.

Scenario A: Unsorted Semen- Owned Donors- Owned Recipients- Market Developed Bulls and Heifers

Results

Consider this scenario, with results shown below, as one in which an operation owns 100 potential recipients and 5 donors. Its primary business model is to market developed ET generated bulls and ET generated females of breeding quality. Cull ET progeny and natural service sired calves will be marketed as feeder cattle. Following the conclusion of all ET rounds, recipients are exposed to a natural service sire. Open females are sold at the conclusion of the breeding season, with the corresponding value of an open female.

Scenario A1: MOET

Figure 32. Probability distribution of the NPV resulting from the scenario of MOET- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

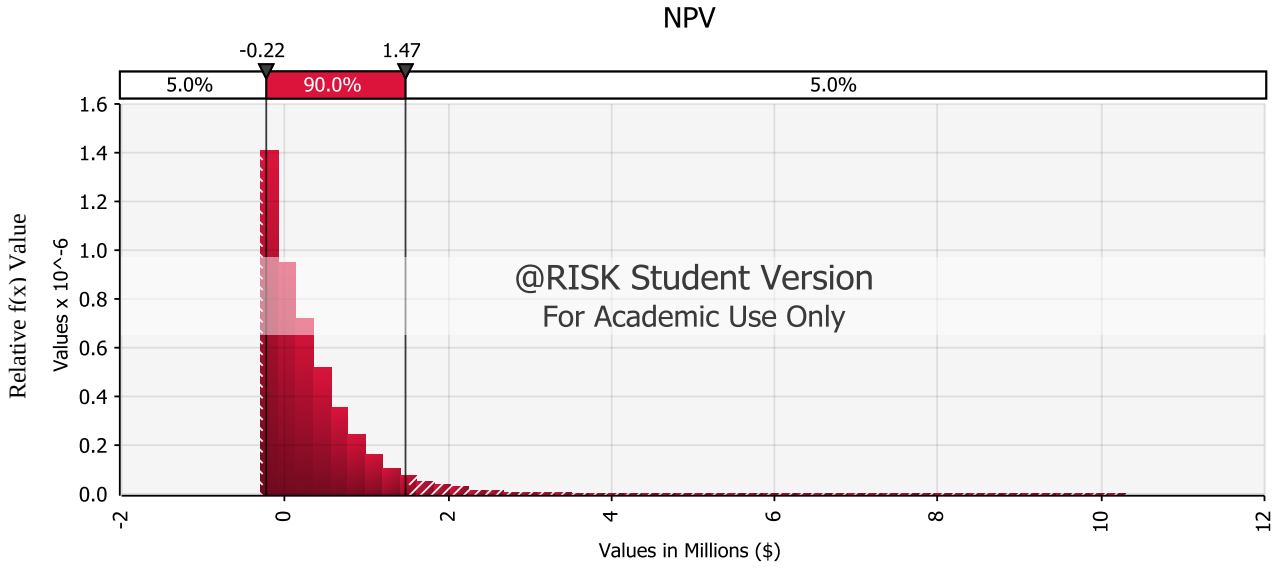


Figure 33. Probability distribution of the ANPV resulting from the scenario of MOET- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

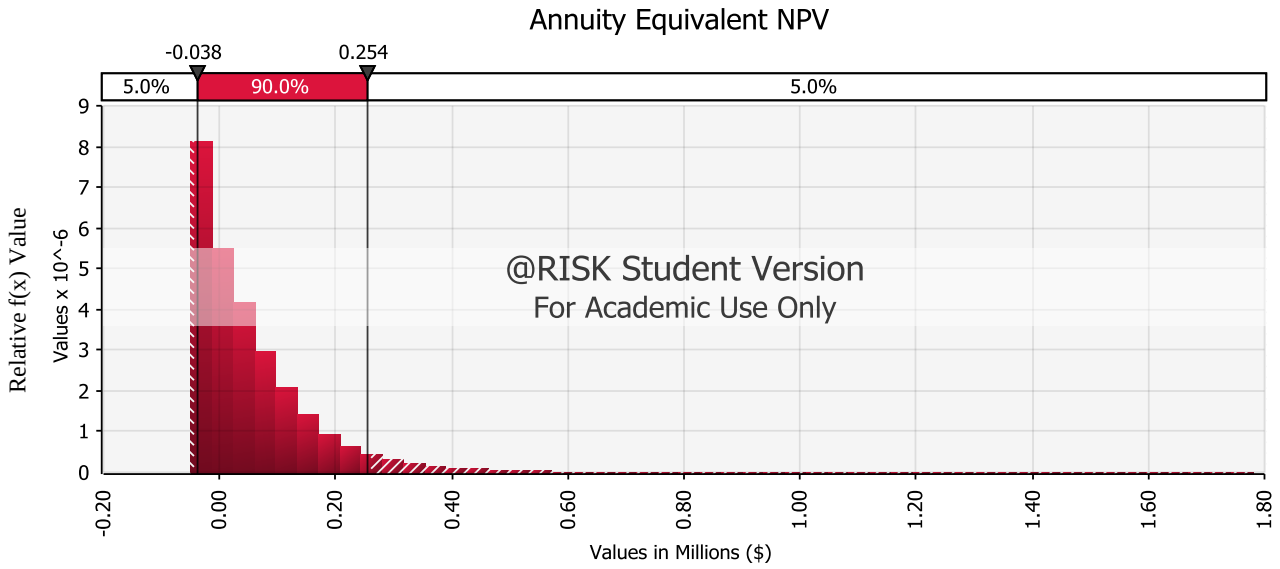


Figure 34. Probability distribution of the ROI resulting from the scenario of MOET- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

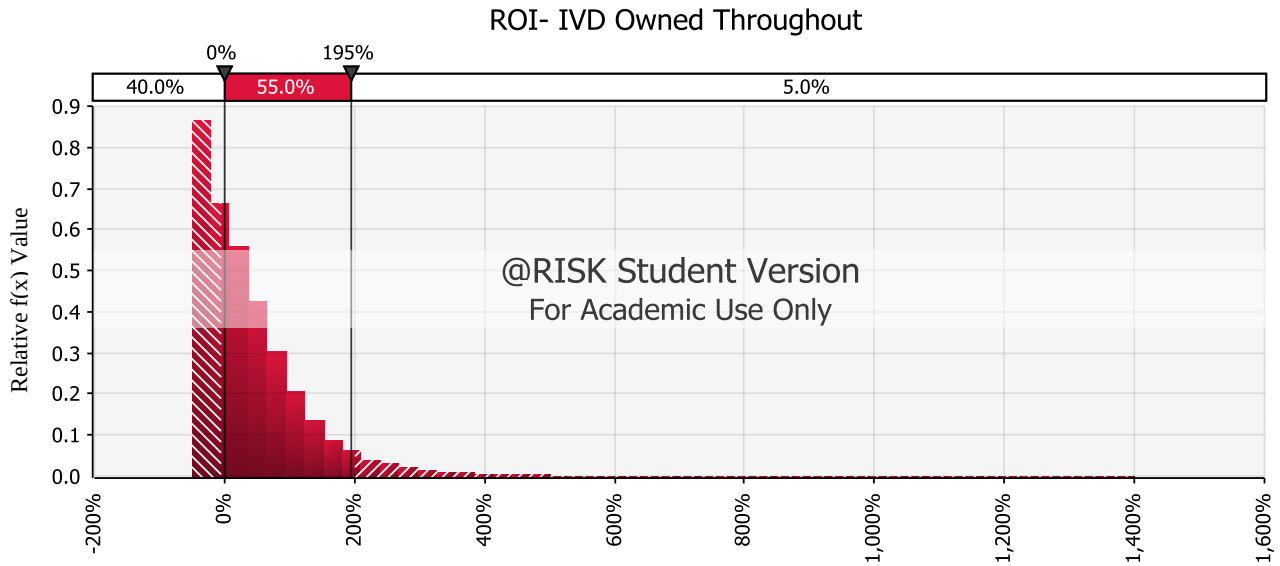
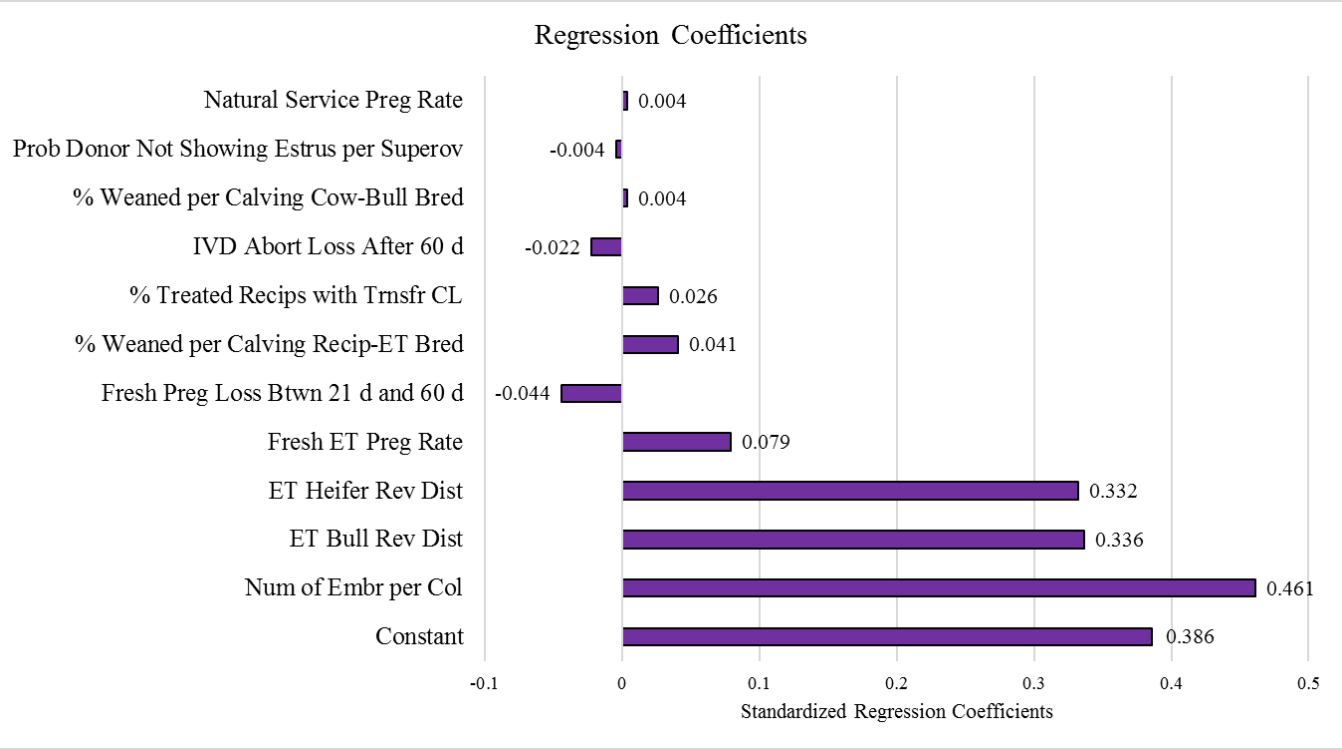
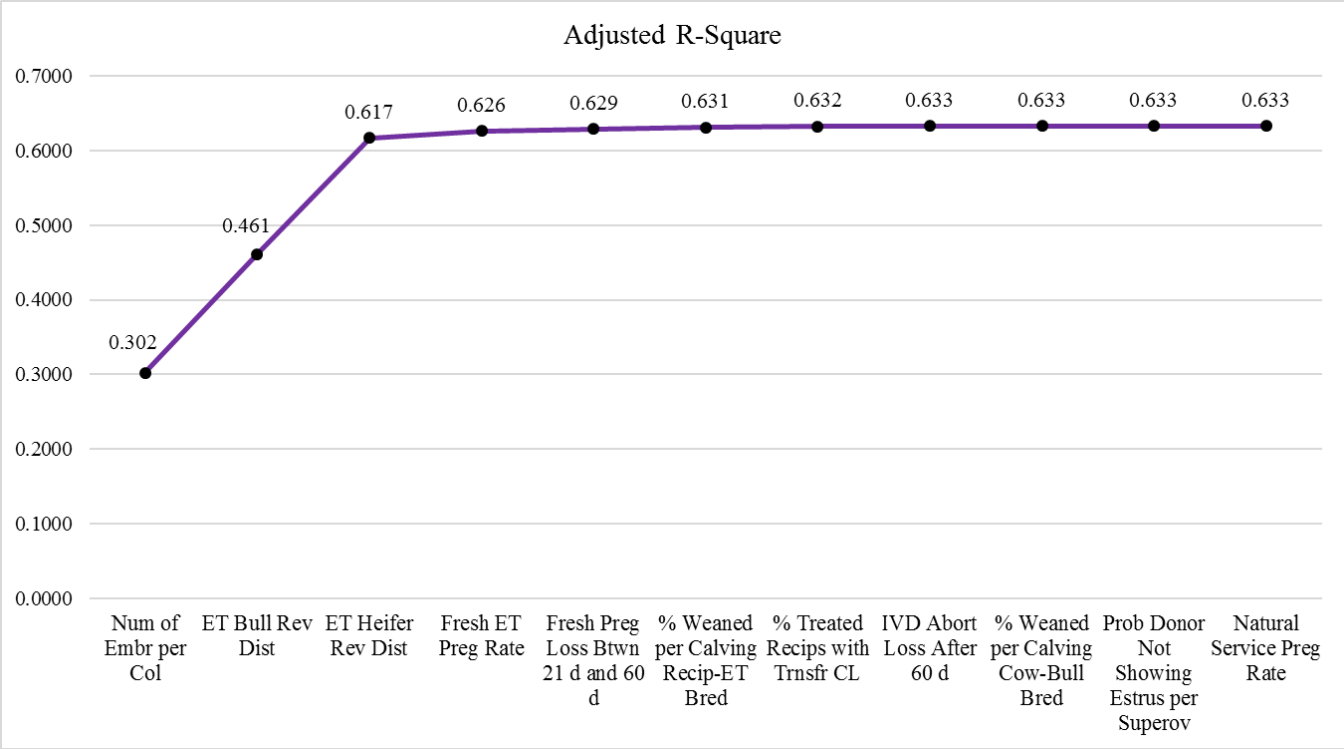


Figure 35. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of MOET- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.



Num of Embry per Col (number of transferable embryos per collection). Preg Rate (pregnancy rate at 21 days post-ovulation).

Figure 36. Cumulative distribution of the R-squared value associated with the stochastic variables influencing the scenario of MOET- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.



Num of Embry per Col (number of transferable embryos per collection). Preg Rate (pregnancy rate at 21 days post-ovulation).

Scenario A2: IVP NS

Figure 37. Probability distribution of the NPV resulting from the scenario of IVP NS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

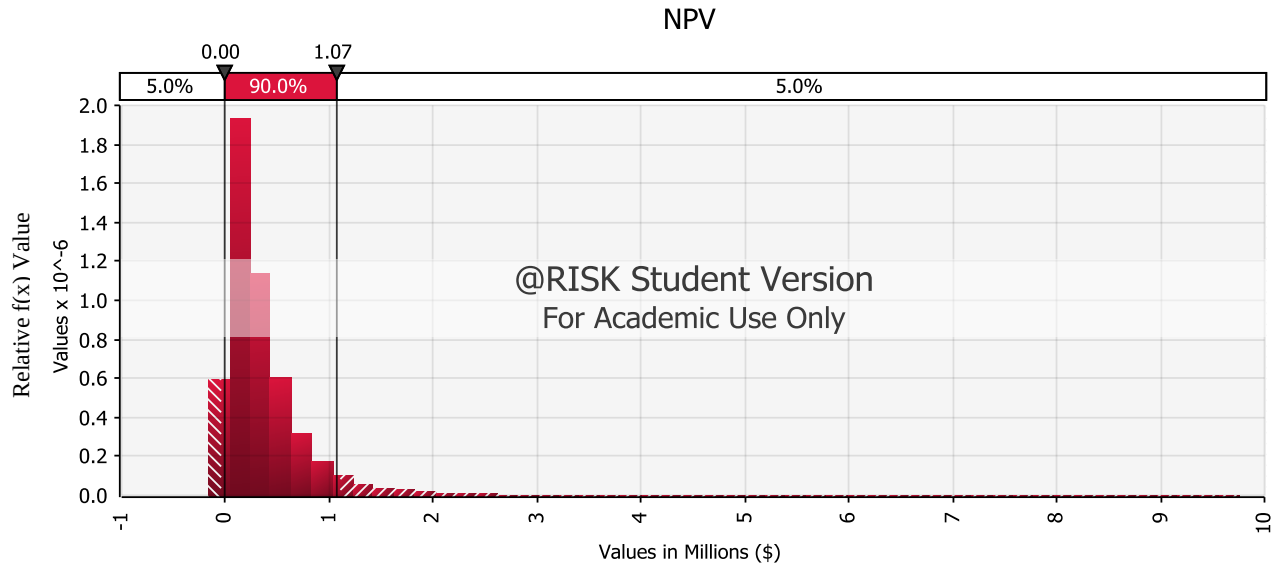


Figure 38. Probability distribution of the ANPV resulting from the scenario of IVP NS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

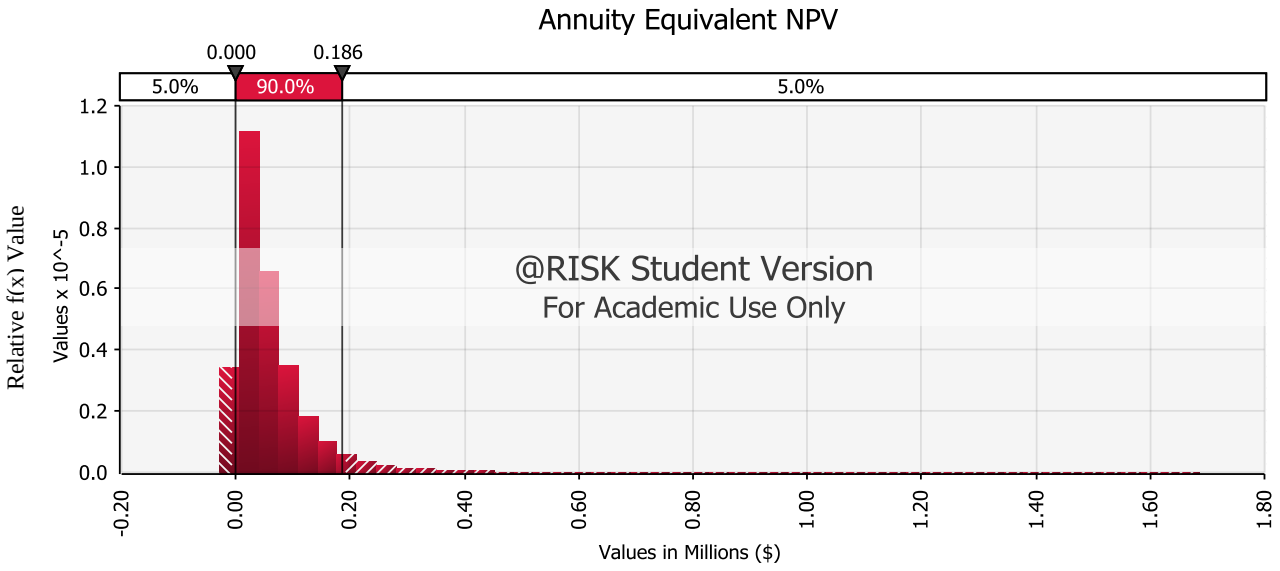


Figure 39. Probability distribution of the ROI resulting from the scenario of IVP NS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

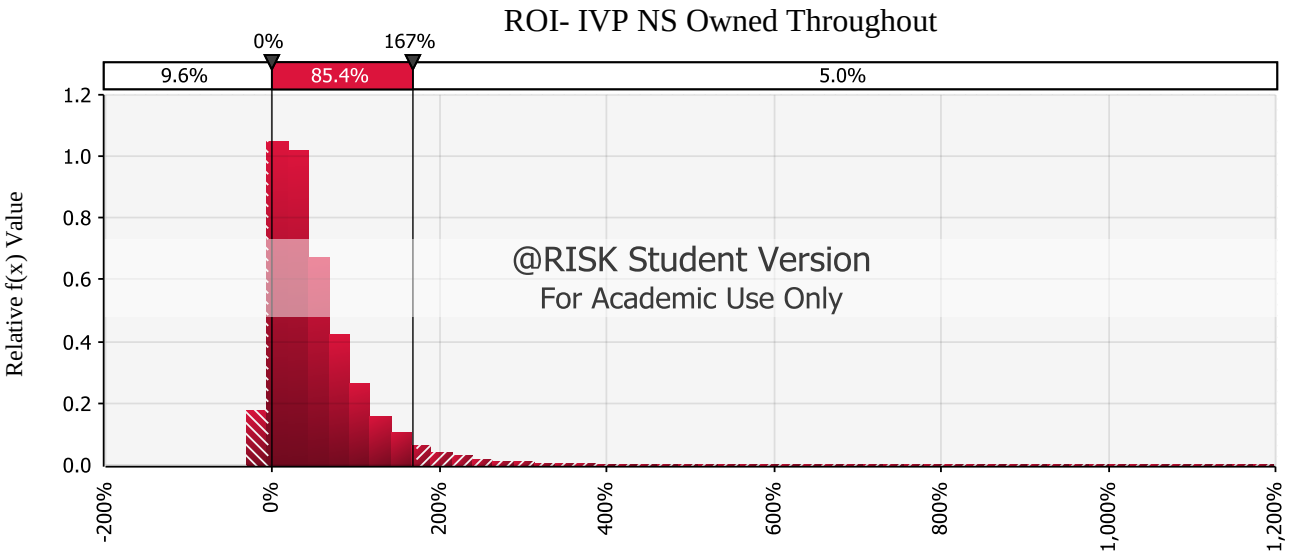
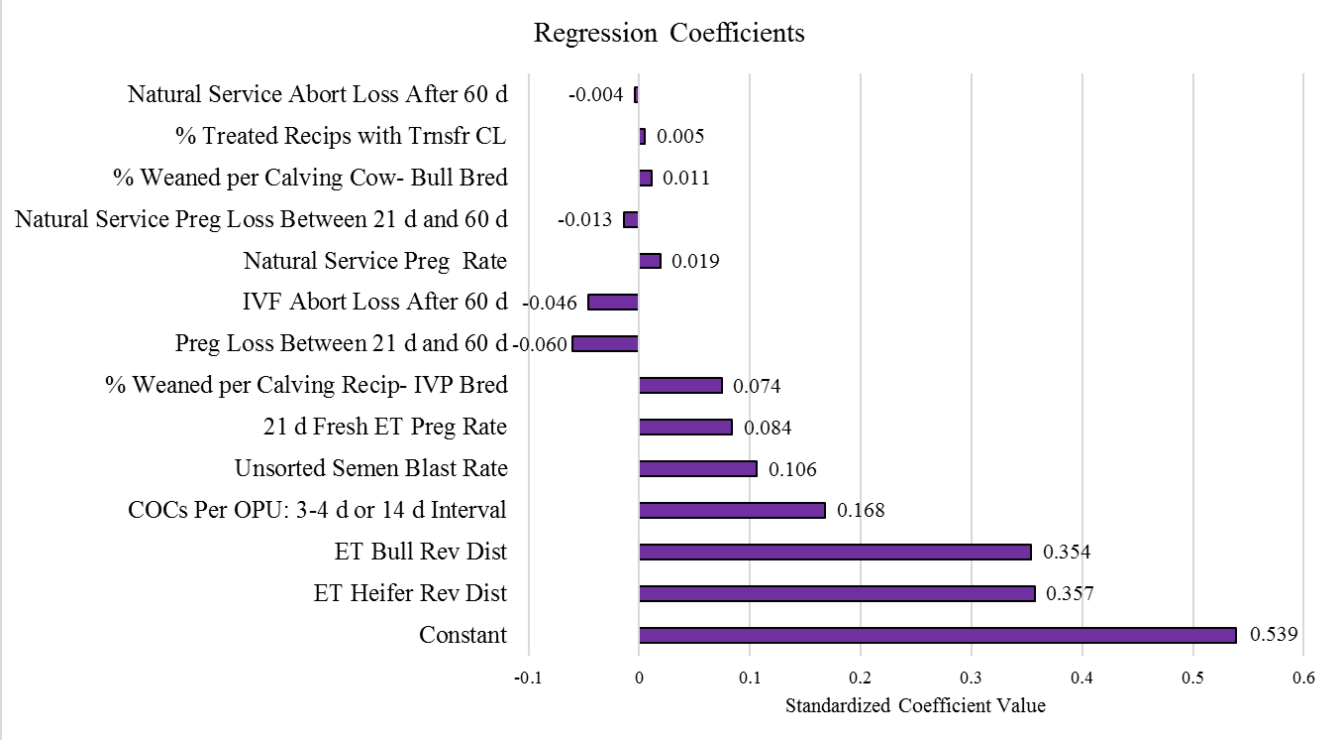
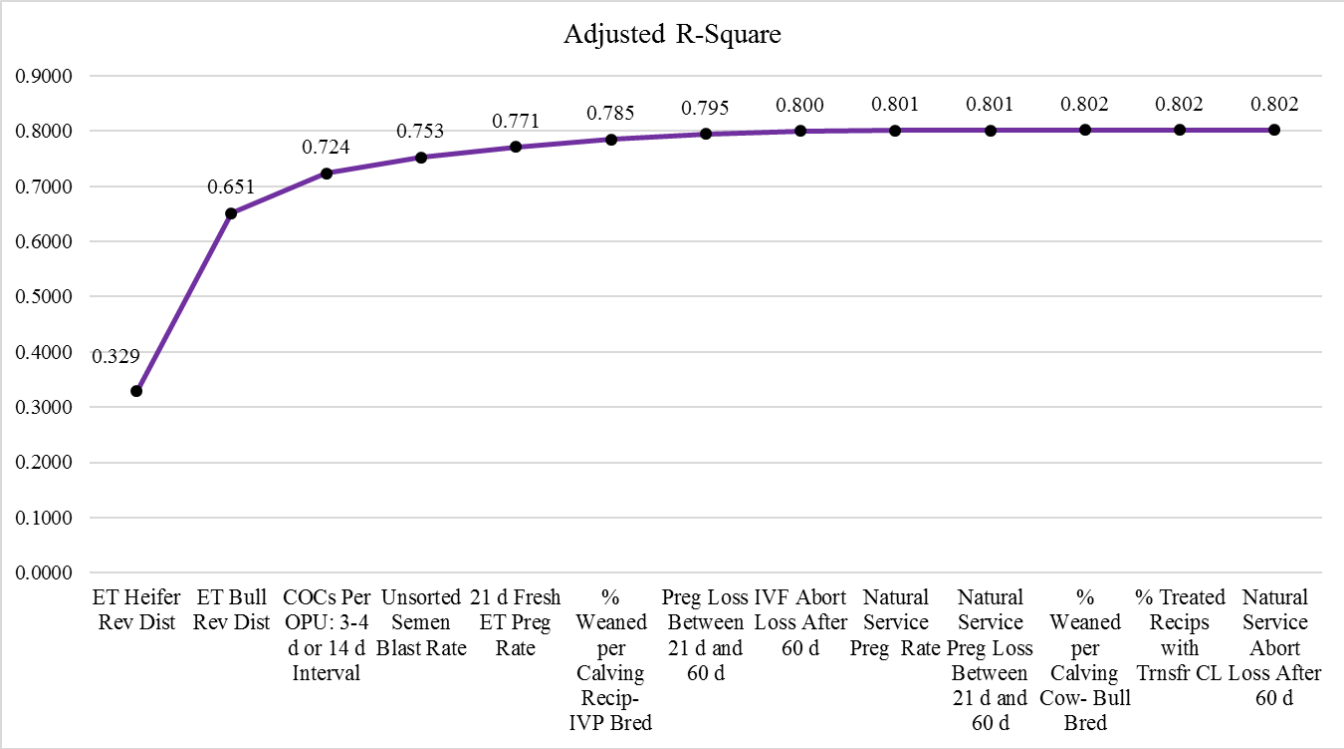


Figure 40. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of IVP NS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.



Preg Rate (pregnancy rate 21 d post-ovulation). Blast Rate (blastocyst rate). COCs Per OPU (number of cultured oocytes per OPU).

Figure 41. Cumulative distribution of the R-squared value associated with the stochastic variables influencing the scenario of IVP NS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.



Preg Rate (pregnancy rate 21 d post-ovulation). Blast Rate (blastocyst rate). COCs Per OPU (number of cultured oocytes per OPU).

Scenario A3: IVP SS

Figure 42. Probability distribution of the NPV resulting from the scenario of IVP SS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

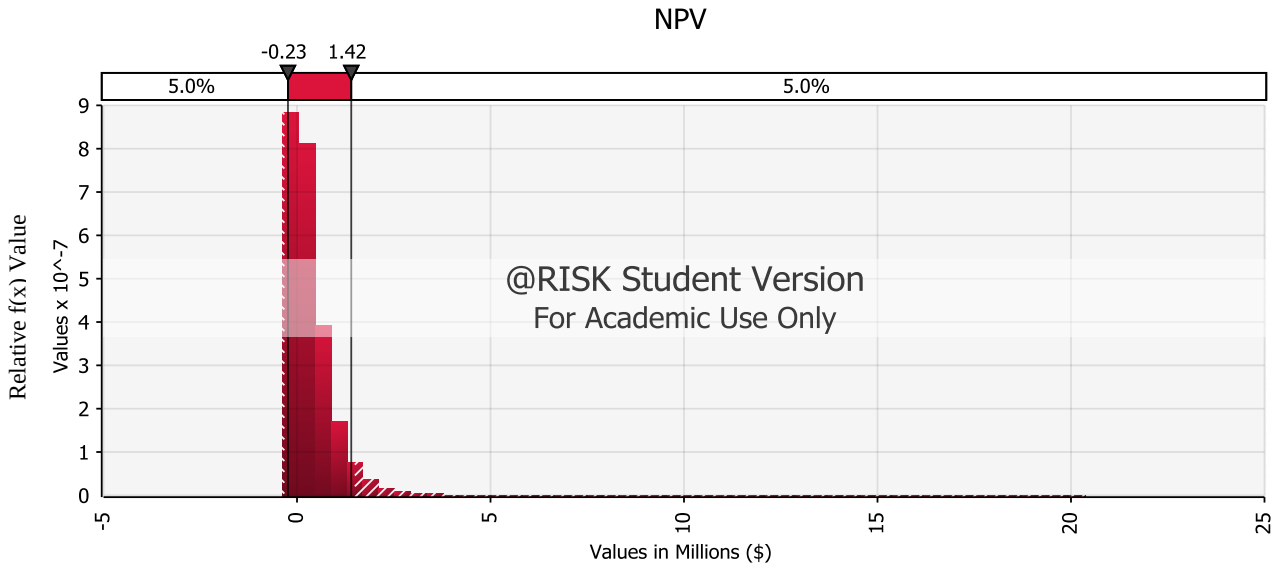


Figure 43. Probability distribution of the ANPV resulting from the scenario of IVP SS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

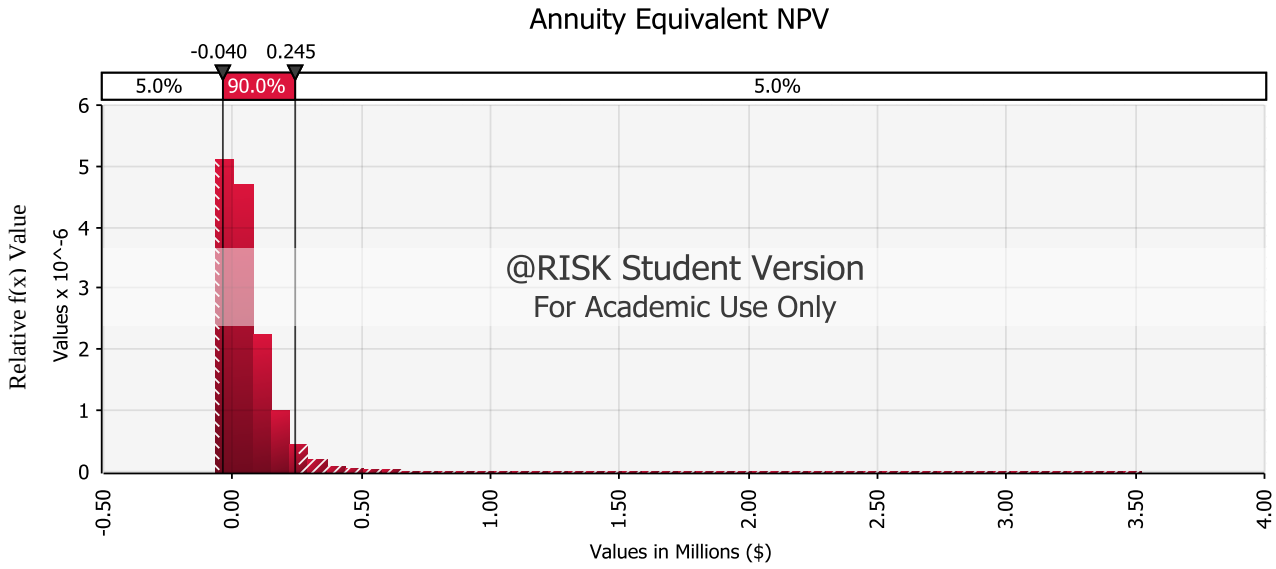


Figure 44. Probability distribution of the ROI resulting from the scenario of IVP SS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

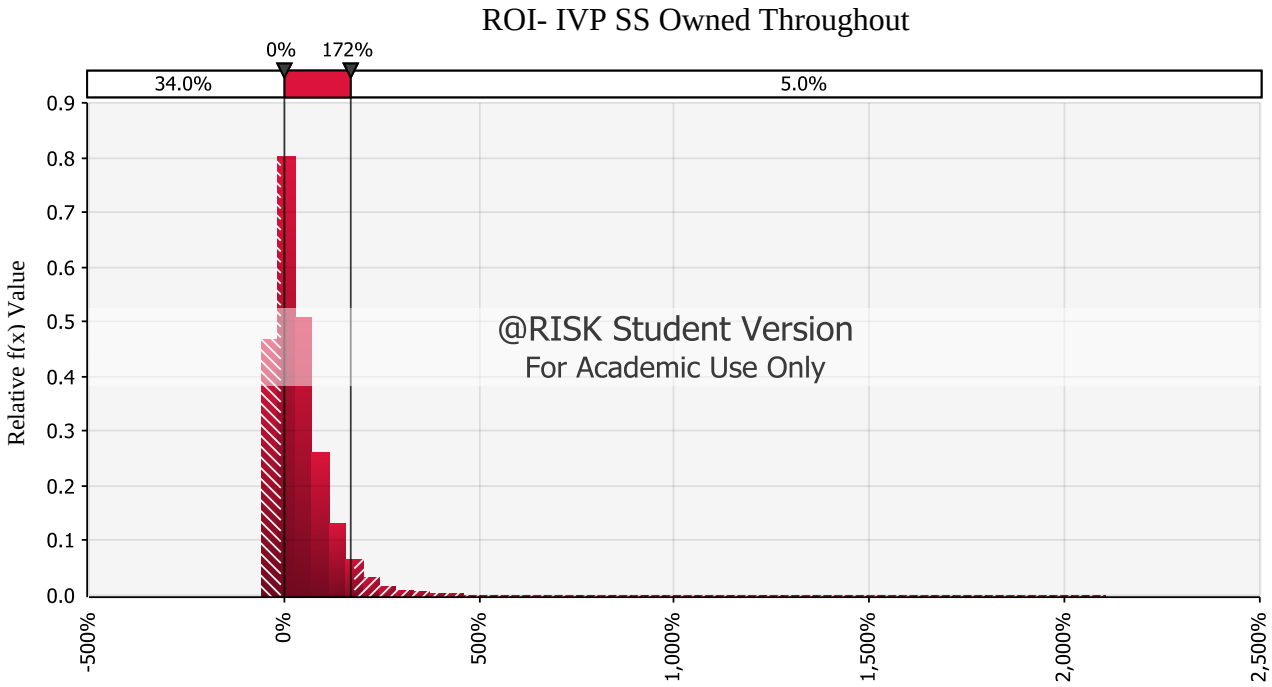
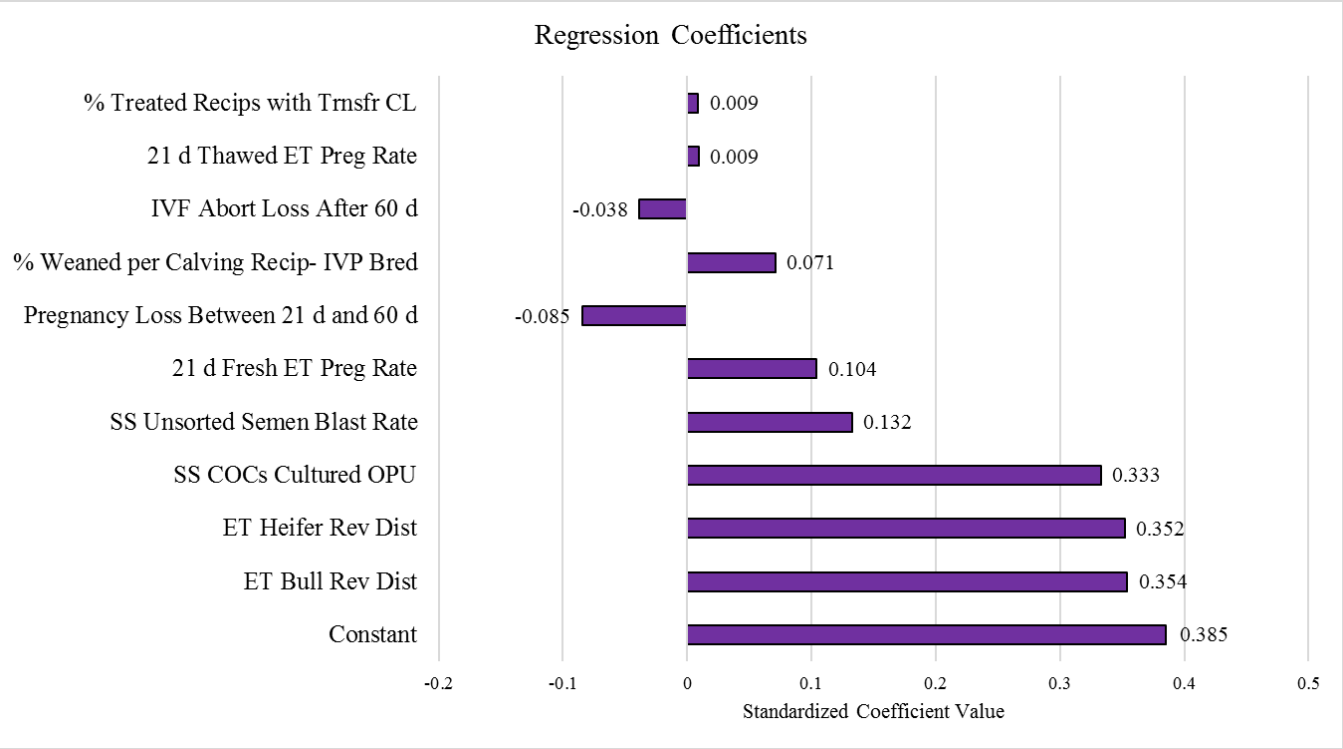
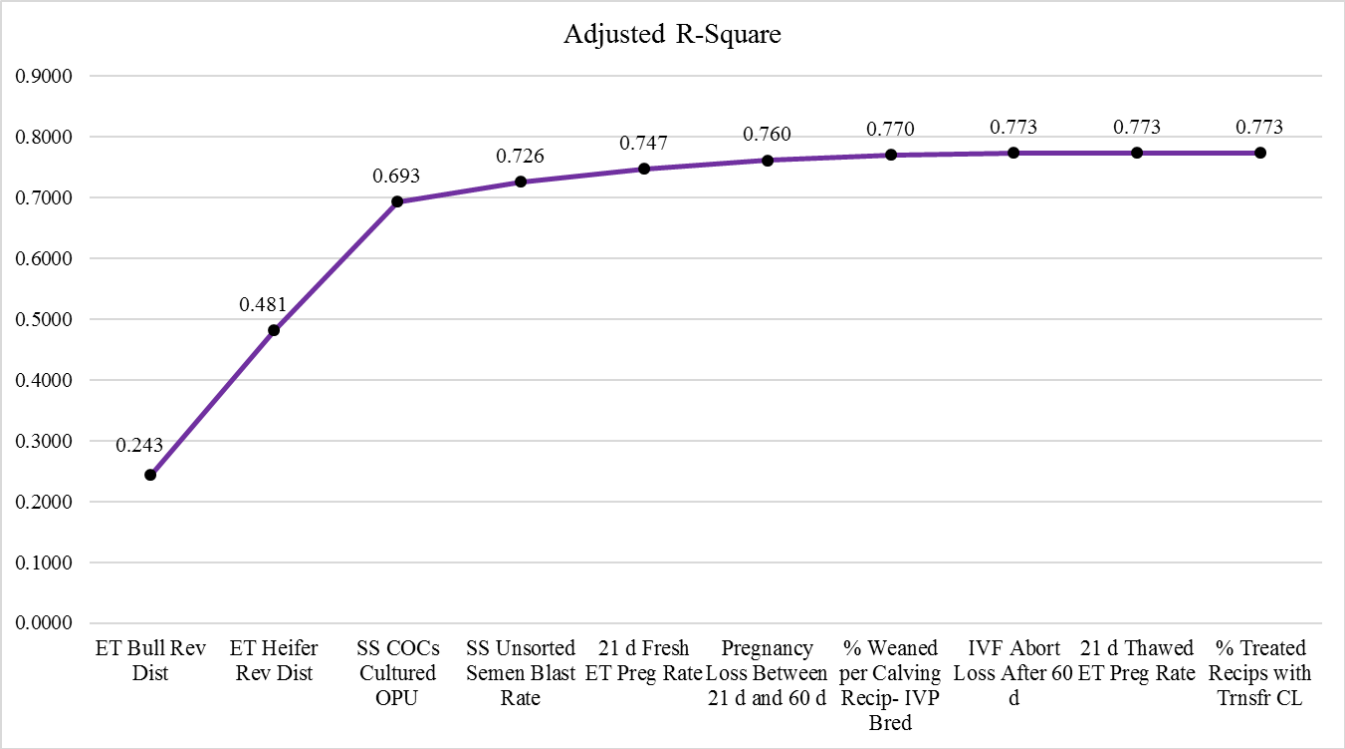


Figure 45. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of IVP SS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.



Preg Rate (pregnancy rate 21 d post-ovulation). Blast Rate (blastocyst rate). SS COCs Cultured OPU (number of cultured oocytes per OPU).

Figure 46. Cumulative distribution of the R-squared value associated with the stochastic variables influencing the scenario of IVP SS- unsorted semen- owned donors- owned recipients- market developed bulls and heifers.



Preg Rate (pregnancy rate 21 d post-ovulation). Blast Rate (blastocyst rate). SS COCs Cultured OPU (number of cultured oocytes per OPU).

Table 126. Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI resulting from the scenario of unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

NPV ($)	MOET	IVP NS	IVP SS
Mode	(209,099.09)	65,560.10	(77,242.45)
5%	(218,138.32)	(2,808.74)	(228,653.25)
25%	(114,843.39)	101,012.82	(56,566.14)
Median	138,715.60	235,102.40	178,597.22
75%	537,393.16	463,179.77	548,364.70
95%	1,469,577.26	1,073,926.10	1,419,572.53
Mean ± 90% C.I.	316,625.23± 3,195.40	349,168.90 ±2,085.64	336,604.50± 3,052.84
SD	614,319.01	400,967.19	586,912.18
ANPV ($)	**MOET**	**IVP NS**	**IVP SS**
Mode	(36,136.47)	11,330.08	(13,349.03)
5%	(37,698.63)	(485.41)	(39,515.81)
25%	(19,847.21)	17,457.02	(9,775.75)
Median	23,972.81	40,630.35	30,865.14
75%	92,872.19	80,046.64	94,768.29
95%	253,972.08	185,595.71	245,330.27
Mean ± 90% C.I.	54,719.12± 552.23	60,343.31 ± 360.44	58,171.93± 527.59
SD	106,166.50	69,295.08	101,430.06

(*cont.*)

Table 126 (*cont.*). Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI resulting from the scenario of unsorted semen- owned donors- owned recipients- market developed bulls and heifers.

ROI (%)	MOET	IVP NS	IVP SS
Mode	-37.4	13.5	-16.3
5%	-39.0	-5.5	-34.3
25%	-22.0	13.9	-10.2
Median	16.9	37.1	20.5
75%	71.3	74.1	66.0
95%	194.5	166.9	169.8
Mean ± 90% C.I.	38.6± 0.437	53.7 ± 0.326	38.4± 0.374
SD	84.0	62.6	71.8
Probability of Negative Return	40.0	9.6	34.0

Discussion

It should be noted that while assessing the means of economic and production measures is a reasonable method of comparing production strategies, the distributions of biological uncertainties embedded within the model cause many of the output distributions to vary greatly in shape, often straying far from a normal distribution. Thus, it is possible for distribution means and most likely outcomes to diverge from one another substantially. Therefore, equal, if not greater, attention should be paid to the percentiles and probabilities associated with each output distribution.

The mean ROI for MOET, 38.6%, and IVP SS, 38.4%, were not significantly different at 90% confidence (Table 126). Mean ROI for IVP NS, 53.7%, was significantly greater than the mean ROI for both MOET and IVP SS at 90% confidence (Table 126).

The mean ANPV for MOET: $54,719.12, was significantly lower (90% confidence) than the mean ANPV of both IVP NS: $60,343.31, and IVP SS: $58,171.93 (Table 126). The mean

ANPV of IVP NS and IVP SS were also significantly different at 90% confidence. These differences can be rationalized by investigating the mean annual expenses of $99,269.95, $82,134.23, and $119,675.40 (Table 133) for MOET, IVP NS, and IVP SS, respectively, under the given scenario. Mean annual revenues for each embryo production strategy were $201,230.60, $185,656.30, and $226,616.40, respectively (Table 132).

Donor and recipient protocol costs played a primary role behind the increased annual expenses of both MOET and IVP SS, when compared to IVP NS. Whereas, the donors in the IVP NS program did not require an exogenous hormone protocol, both MOET and IVP SS donors underwent an exogenous hormone protocol before the 2 embryo collections and 5 OPU sessions as associated with the respective scenario. The subsequent embryo collections and IVP procedures resulted in the mean production of 70 MOET produced embryos accompanied by 70 transfers; a mean of 70 IVP NS produced embryos complemented by 70 transfers; and a mean of 105 IVP SS produced embryos accompanied by 105 transfers. The mean number of recipients synchronized for the IVP SS scenario was 125 recipients, as compared to a mean of 84 recipients and 85 recipients for MOET and IVP NS, respectively. Keep in mind that there are synchronization/stimulation protocol costs and flush/OPU procedure costs associated with every flush/OPU and every transfer. The increased number of transfers within the IVP SS program inherently gives recipients more opportunities to become pregnant. Thus, the mean number of open recipients for MOET, IVP NS, and IVP SS were 13.1, 12.3, and 11.7 (Table 130), respectively. A reduction in opens also increases annual expense.

Driving the difference in mean annual revenues of $201,230.60, $185,656.30, and $226,616.40 (Table 132) for MOET, IVP NS, and IVP SS, respectively, were the variations in number of ET pregnancies and subsequent differences in the number of marketable ET calves. The

mean number of annual ET pregnancies at the conclusion of the ET season for MOET, IVP NS, and IVP SS were 34.4, 31.7, and 41.7 (Table 130), respectively, all significantly different. The increase in mean revenue for the MOET program versus the IVP NS program is derived from the distribution around the mean. Both distributions have a minimum possible value of zero and neither distribution is normal. While the standard deviation for the number of MOET ET bred recipients is 26.6, the standard deviation for the number of IVP NS ET pregnancies is only 15.0, simultaneously being skewed to the left (Table 130). Thus, there are 59 ET pregnancies and 40 ET pregnancies at the 75th percentile for MOET and IVP NS distributions, respectively (Table 130). Alternatively, there are 10 ET pregnancies and 21 ET pregnancies at the 25th percentile for the MOET distribution and IVP NS distribution, respectively (Table 130). As previously described, simply looking at means, without considering the shape of the distribution is detrimental to proper interpretation of this model. Despite the reduced pregnancy rates associated with IVP embryos, the sheer number of IVP SS embryos transferred allowed for an increase in ET pregnancies.

Along with noting the standard deviation of output means, an effective method of risk appraisal is an analysis of the probability distribution associated with each economic and production output. When considering ROI, the most likely outcomes for MOET, IVP NS, and IVP SS are -37.4%, 13.5%, and -16.3%, respectively (Table 126). The medians for each respective ROI distribution are 16.9%, 37.1%, and 20.5% (Table 126). Perhaps the greatest measurement of financial risk is the probability of negative return. Regarding this measurement, MOET, IVP NS, and IVP SS had probabilities of 40.0%, 9.6%, and 34.0% (Table 126), respectively. Although each individual firm may consider risk differently, using the most likely outcome and probability of negative return, one can argue that for the given scenario both the MOET and IVP SS programs are in contention for the economically riskiest methods of ET. Alternatively, if one defines risk as

an uncertainty of outcome, MOET also has the greatest standard deviation of ROI, at 84.0% (Table 126). Not surprisingly, considering many risk-reward trade-offs, MOET also has the greatest ROI at the 95th percentile (Table 126).

It seems rational that IVP NS has the lowest probability of negative return, because IVP NS is less influenced by the success or failure of expensive human intervention (no exogenous hormone protocols for synchronization or stimulation of donors) than either MOET or IVP SS. Depending on a firm's risk aversion, IVP NS could be an attractive method under the given scenario, as it also boasts the greatest most likely return and the smallest standard deviation around the mean. Simultaneously, the 95th percentile ROI of IVP NS, 166.9%, rivals that of IVP SS, 169.8% (Table 126).

The statistical results are shown in Figure 35 and 36, Figure 40 and 41, and Figure 45 and 46. For Scenario A1, the three largest regression values are the number of transferable embryos per collection, the revenue distribution for heifers, and the revenue distribution for bulls. For Scenario A2 and Scenario A3, the three largest regression coefficient values are the revenue distribution for heifers, the revenue distribution for bulls, and the number of oocytes incubated per OPU. According to the R-squared values, the regression model for each of the scenarios does not completely explain the outcome of the scenario. This is because of the incorporation of binomial distributions, which are not included in the regression analysis, as a method of implementing the stochastic variables that represent a mean probability, such as pregnancy rate. The results of the binomial distributions account for the proportion of the variation that the model utilizing only stochastic variables cannot explain.

Regardless of one's method for measuring economic success and the risk associated with that success, the numerical and logical analysis afforded through the stochastic simulation of

alternative scenarios through this model should allow for in-depth assessment. The caveat is that any model, no matter how robust, will never be completely accurate, as all are a simplified version of a complicated reality.

Scenario B: Semen Sex-Sorted for Males- Owned Donors- Owned Recipients- Market Developed Bulls and Heifers

Results

Consider this scenario, with results shown below, as one in which an operation owns 100 potential recipients and 5 donors. Its primary business model is to market developed ET generated bulls of breeding quality. Thus, the semen used to generate embryos is sex-sorted for males. Any ET heifers that are produced through inaccurate semen sorting will be marketed as replacement females. Cull ET progeny and natural service sired calves will be marketed as feeder cattle. Following the conclusion of all ET rounds, recipients are exposed to a natural service sire. Open females are sold at the conclusion of the breeding season, with the corresponding value of an open female.

Scenario B1: MOET

Figure 47. Probability distribution of the NPV resulting from the scenario of MOET- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

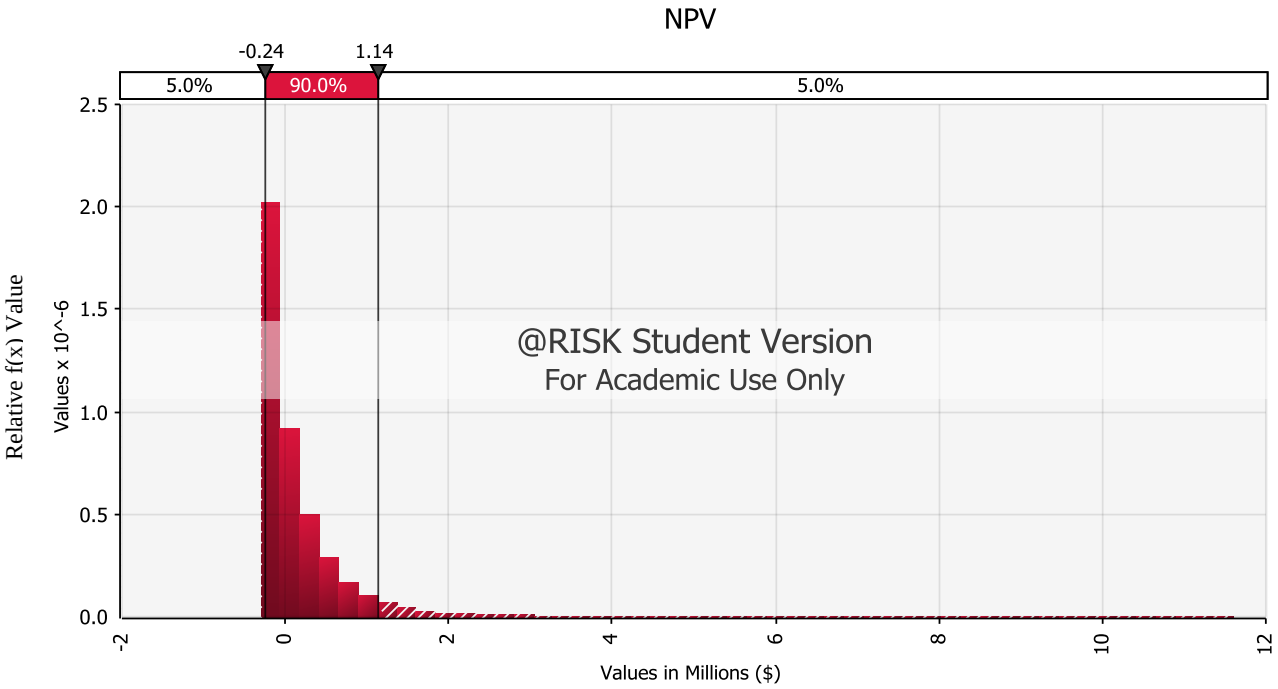


Figure 48. Probability distribution of the ANPV resulting from the scenario of MOET- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

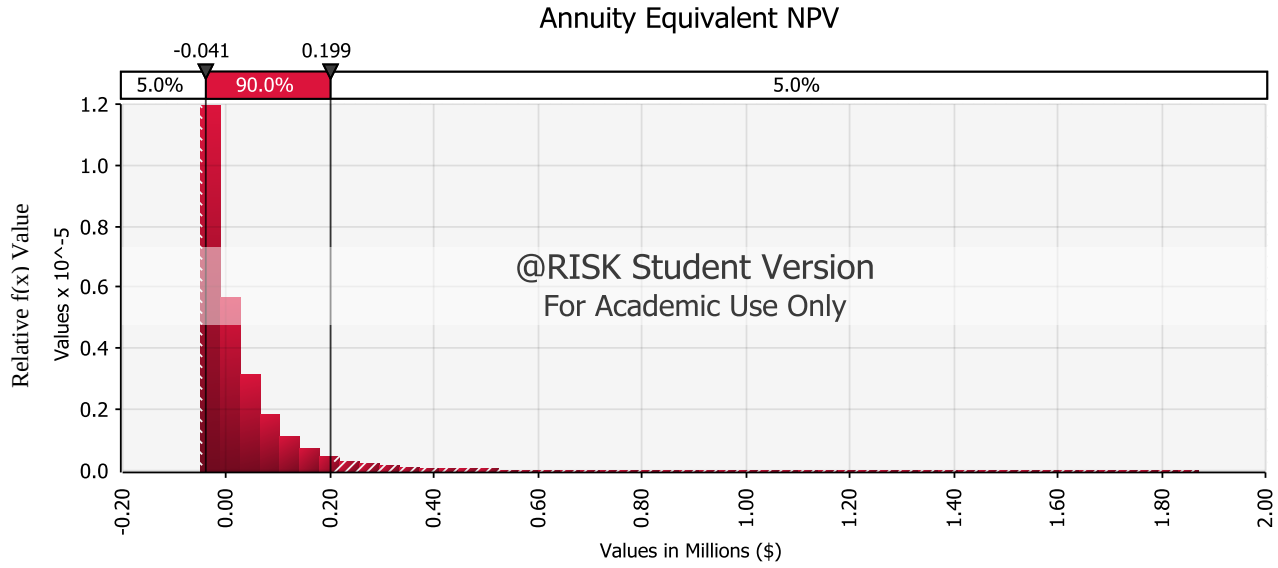


Figure 49. Probability distribution of the ROI resulting from the scenario of MOET- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

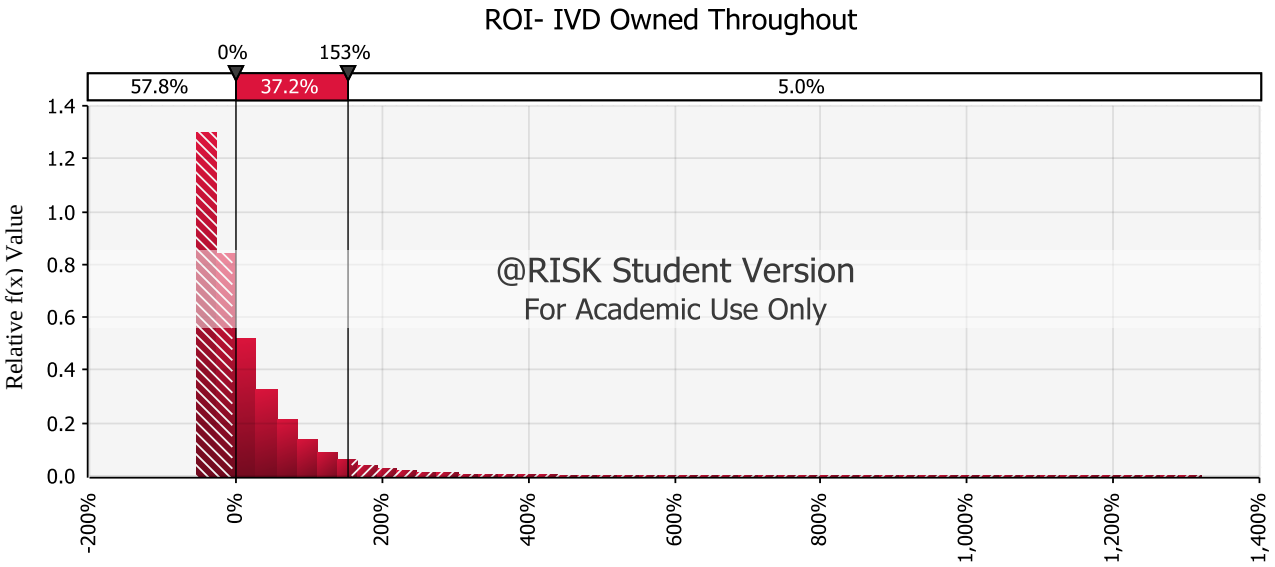
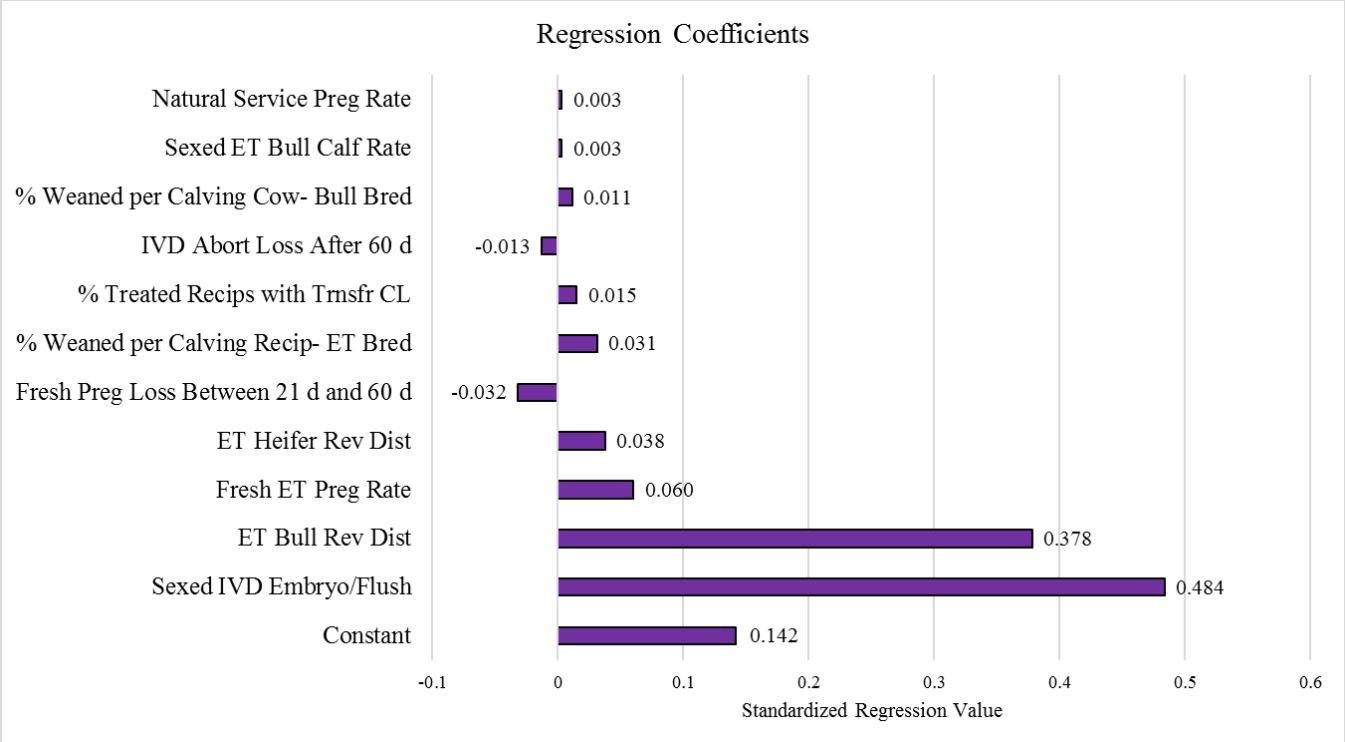
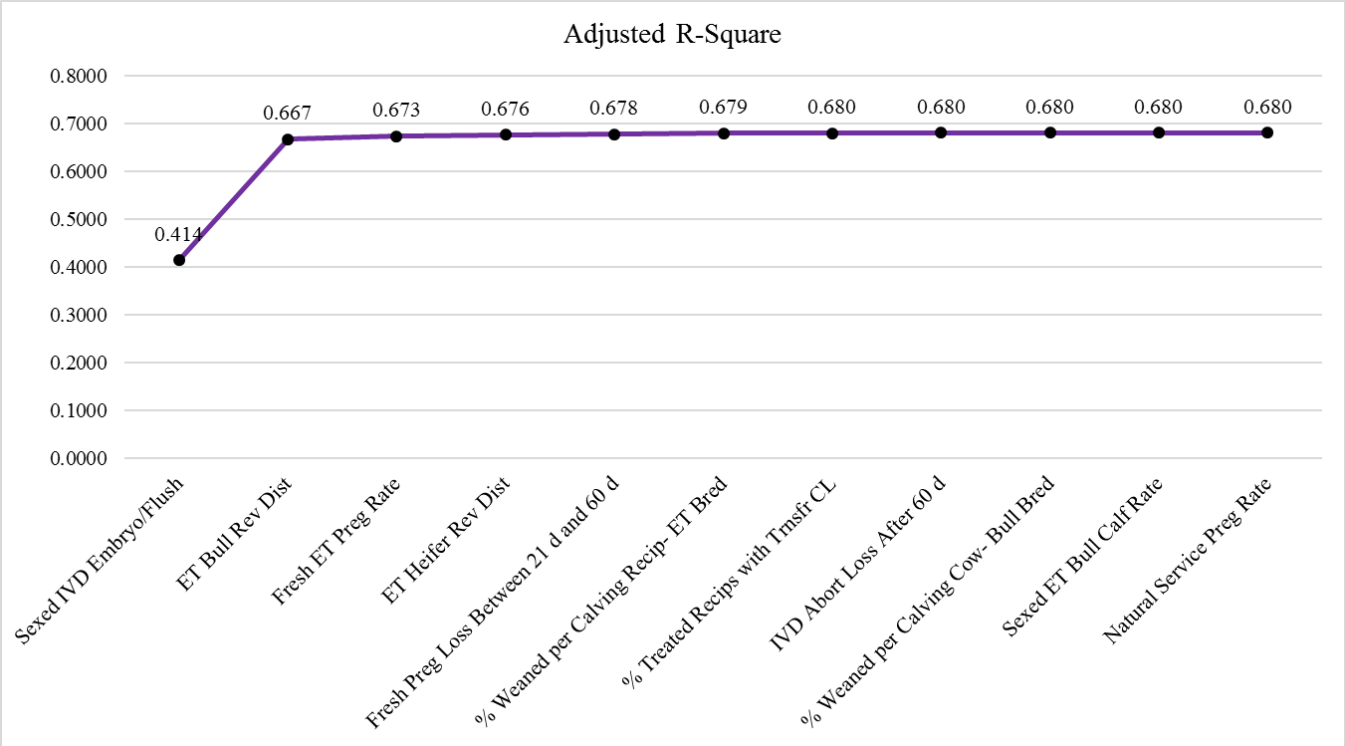


Figure 50. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of MOET- sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.



Preg Rate (pregnancy rate 21 d post-ovulation). Embryo/Flush (transferable embryos per collection).

Figure 51. Cumulative distribution of the R-squared value associated with the stochastic variables influencing the scenario of MOET- sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.



Preg Rate (pregnancy rate 21 d post-ovulation). Embryo/Flush (transferable embryos per collection).

Scenario B2: IVP NS

Figure 52. Probability distribution of the NPV resulting from the scenario of IVP NS- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

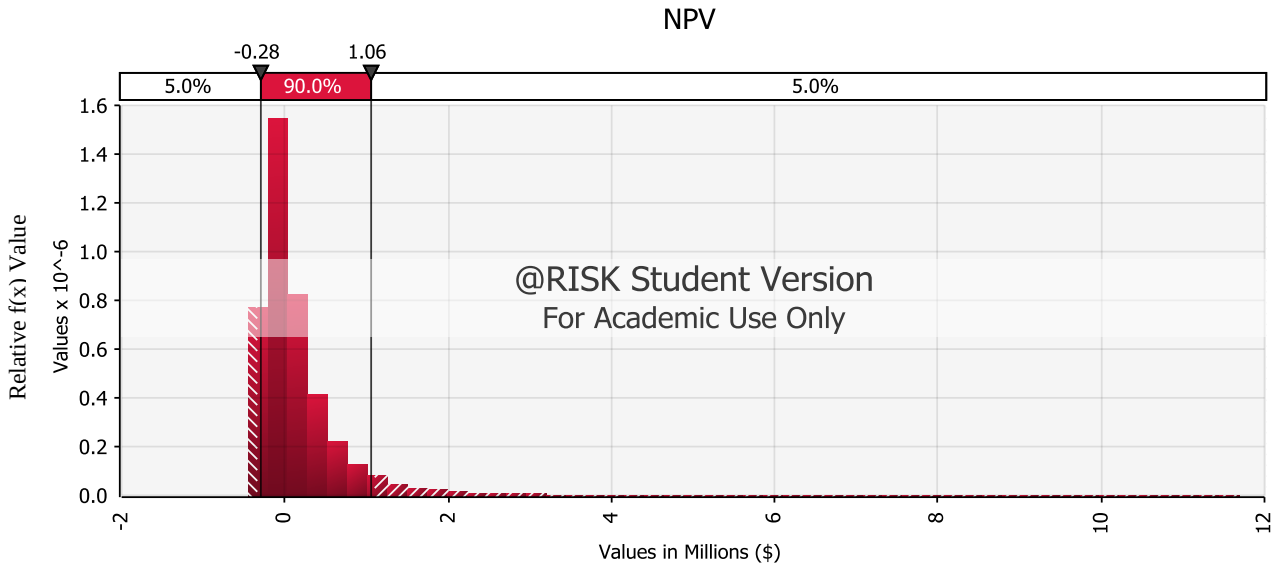


Figure 53. Probability distribution of the ANPV resulting from the scenario of IVP NS- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

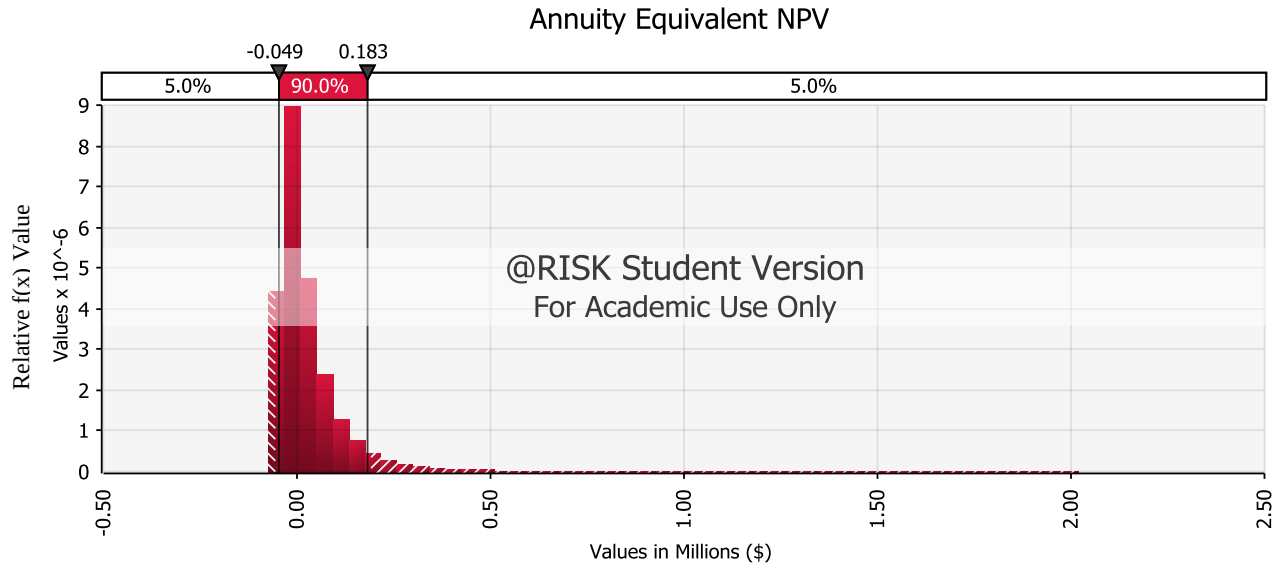


Figure 54. Probability distribution of the ROI resulting from the scenario of IVP NS- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

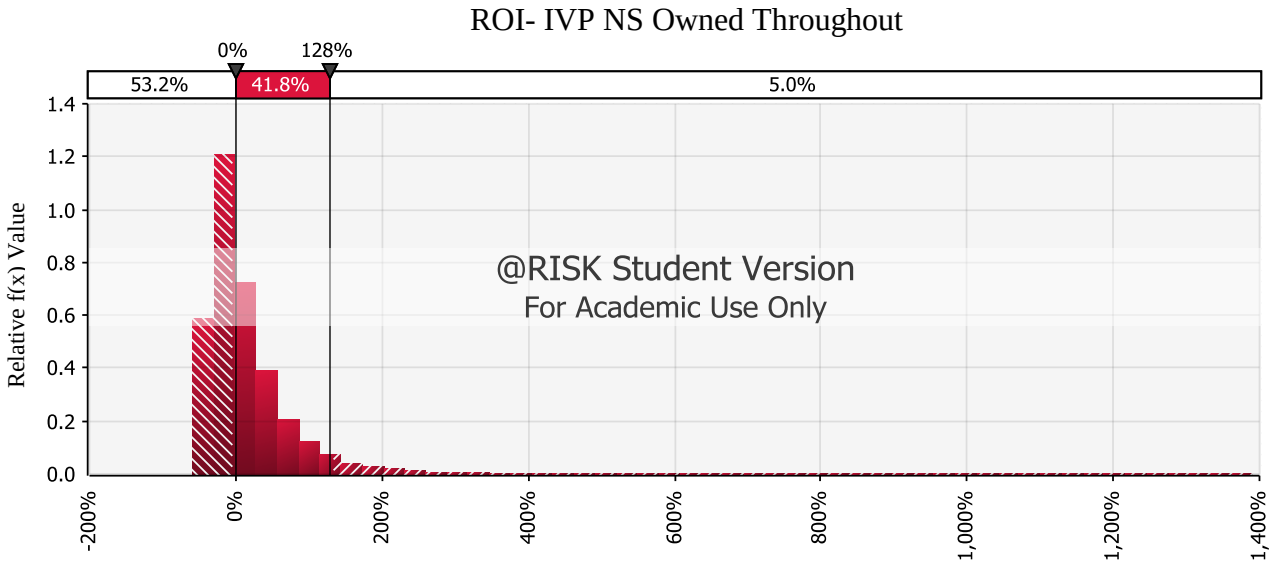
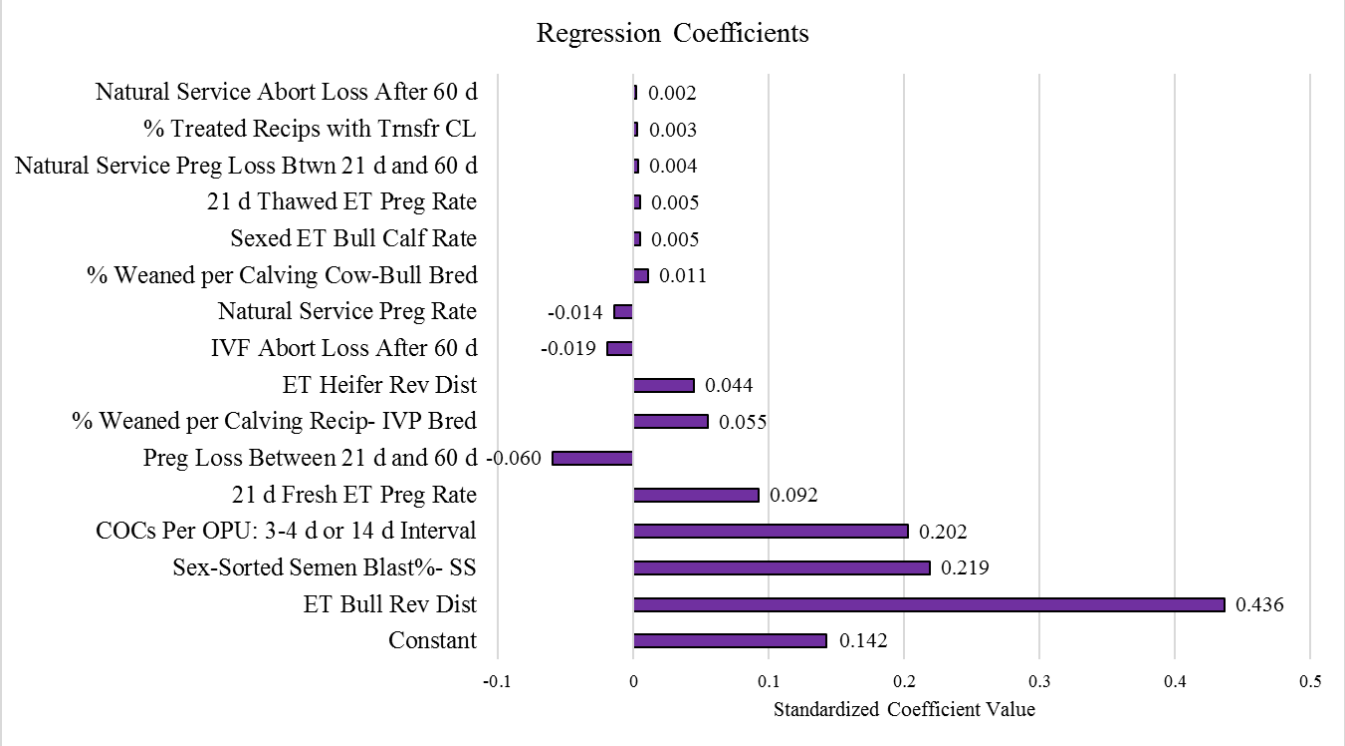
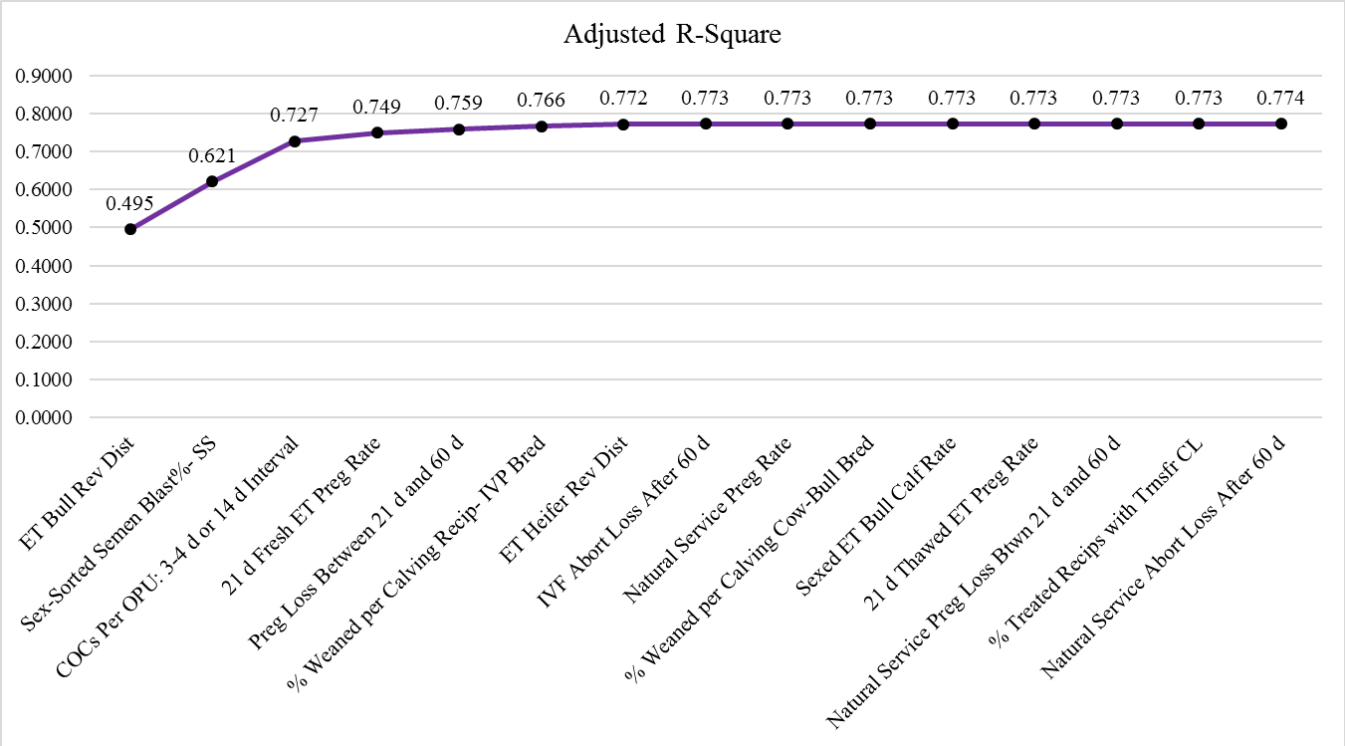


Figure 55. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of IVP NS- sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.



Trnsfr CL (CL qualified for recipient to receive an embryo). Preg Rate (pregnancy rate 21 d post-ovulation). COCs per OPU (number oocytes cultured per OPU). Blast% (blastocyst rate).

Figure 56. Cumulative distribution of the adjusted R-squared value associated with the stochastic variables influencing the scenario of IVP NS- sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.



Trnsfr CL (CL qualified for recipient to receive an embryo). Preg Rate (pregnancy rate 21 d post-ovulation). COCs per OPU (number oocytes cultured per OPU). Blast% (blastocyst rate).

Scenario B3: IVP SS

Figure 57. Probability distribution of the NPV resulting from the scenario of IVP SS- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

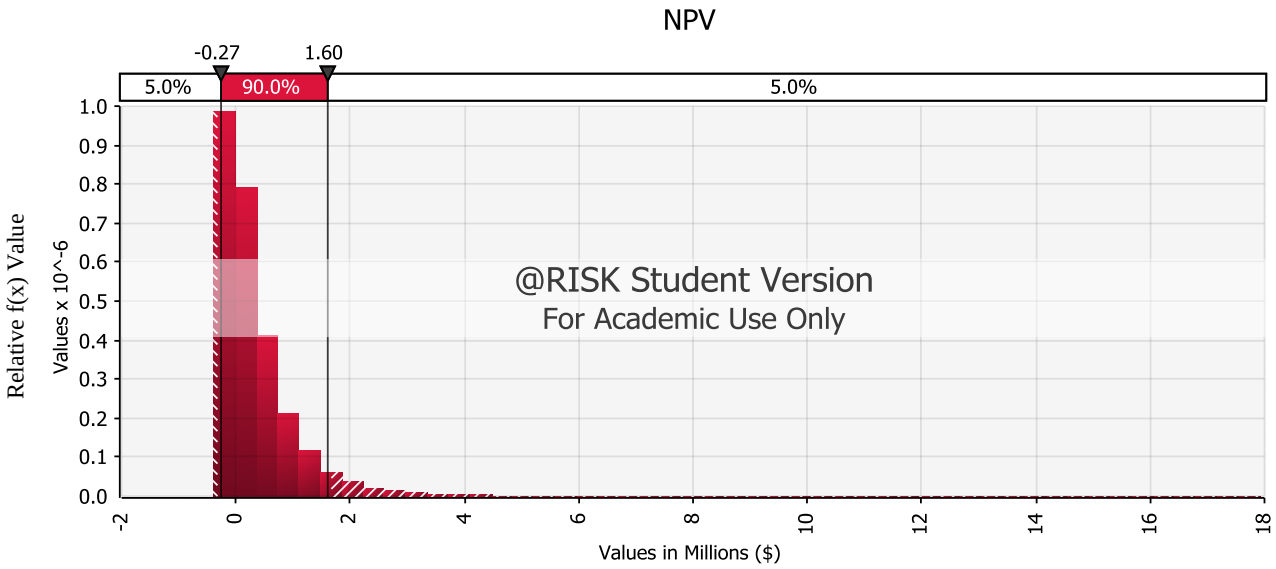


Figure 58. Probability distribution of the ANPV resulting from the scenario of IVP SS- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

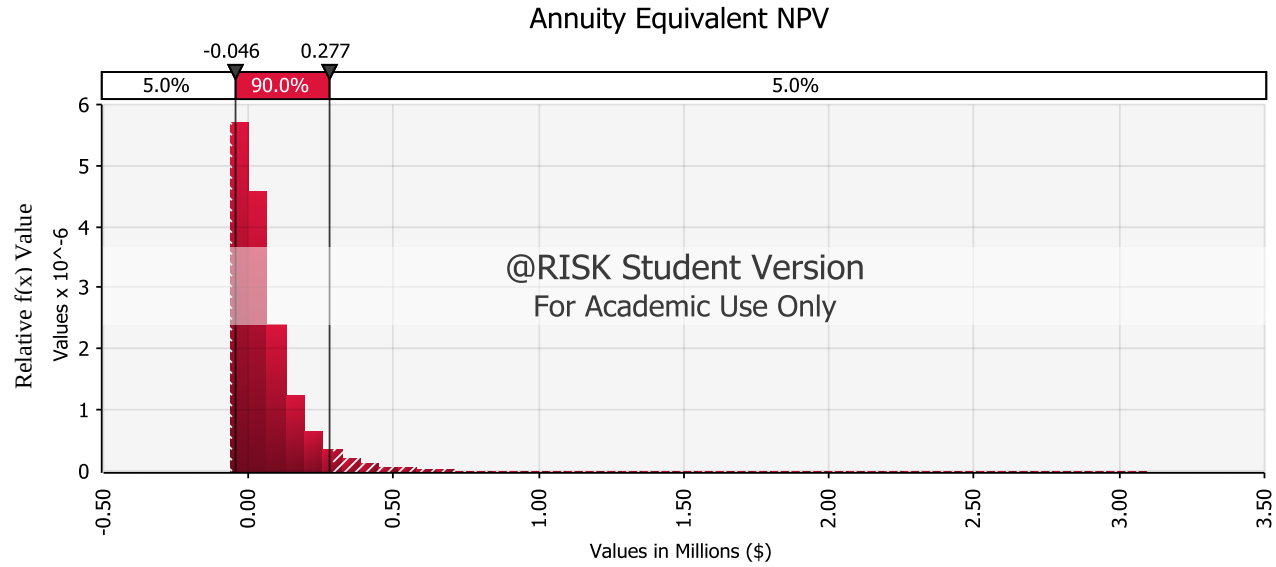


Figure 59. Probability distribution of the ROI resulting from the scenario of IVP SS- semen sex-sorted for males- owned donors- owned recipients- market developed bulls and heifers.

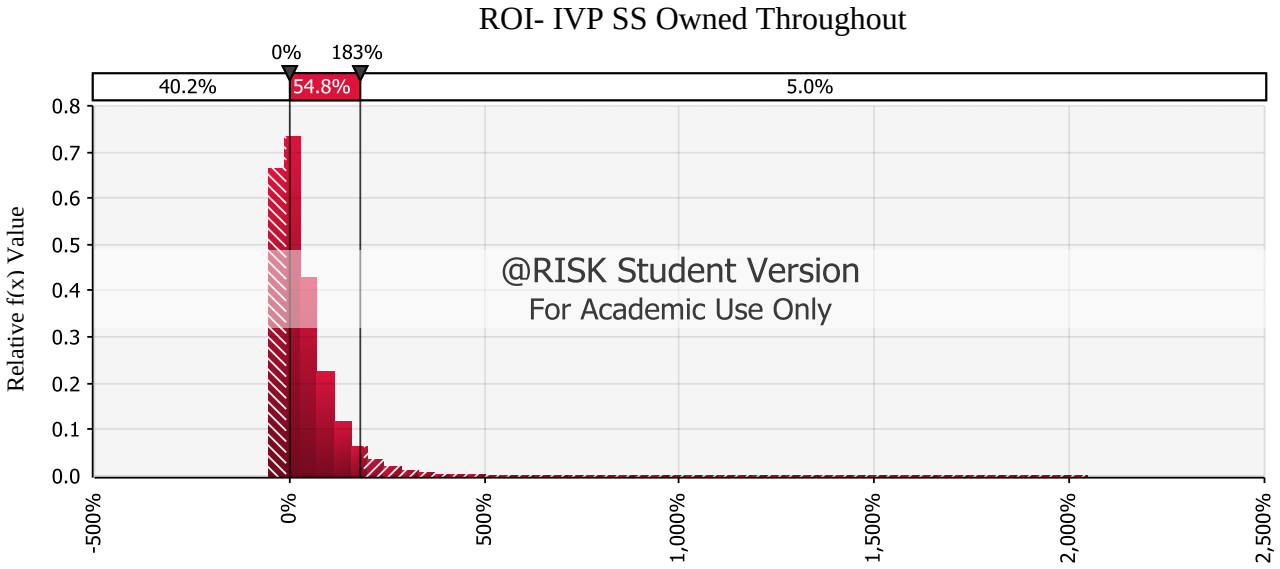
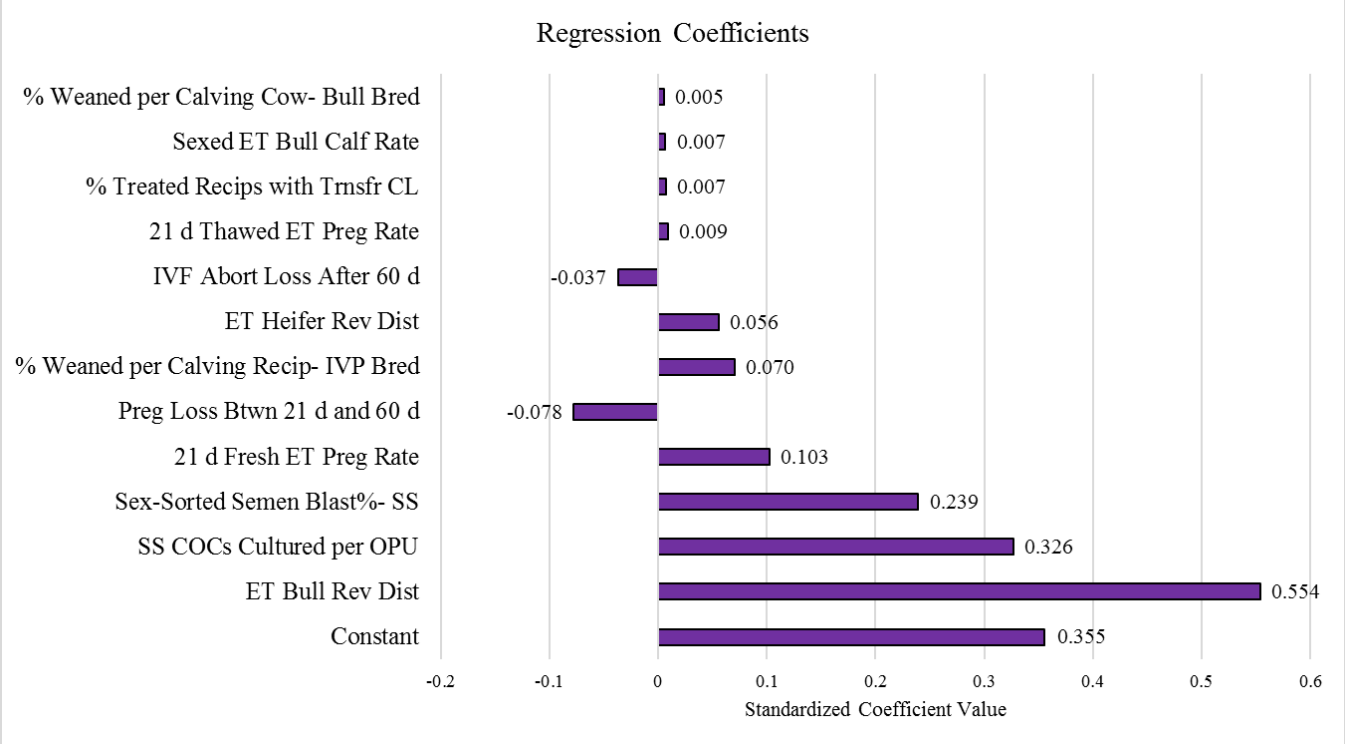
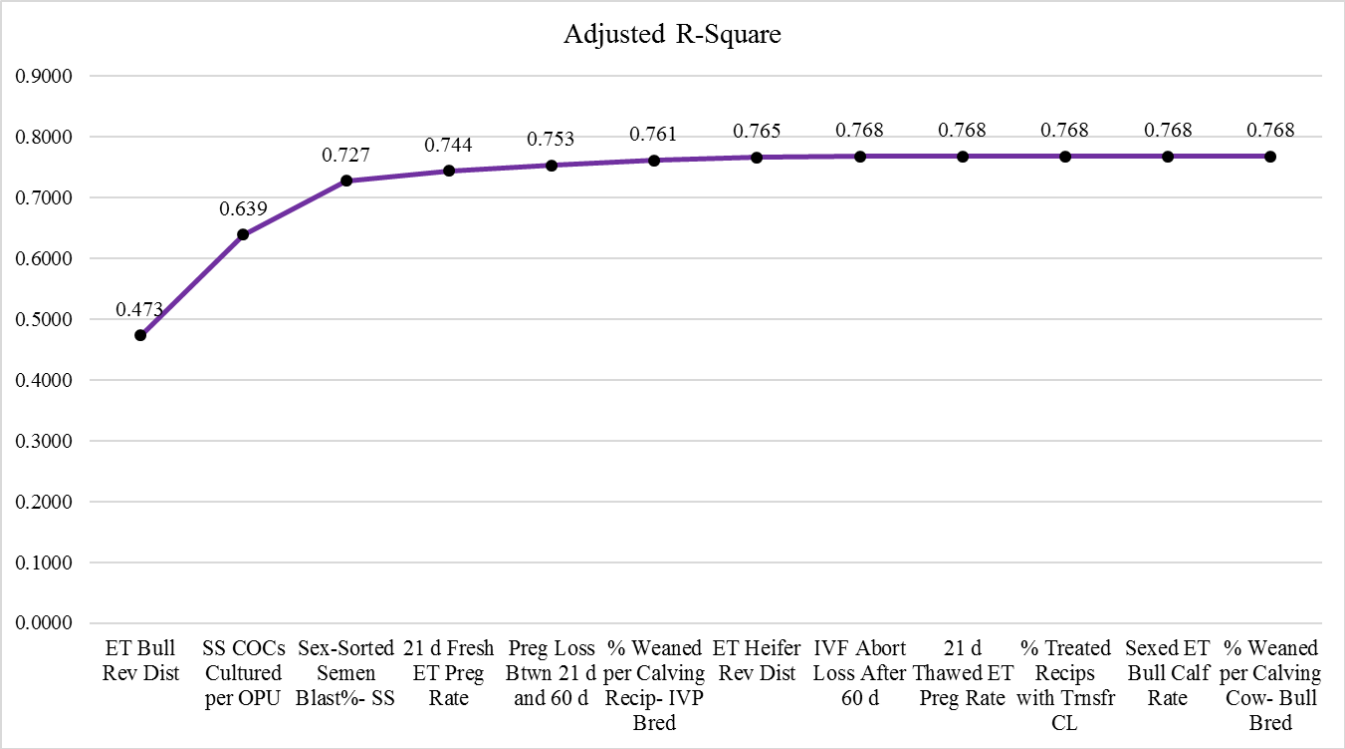


Figure 60. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of IVP SS- sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.



Trnsfr CL (CL qualified for recipient to receive an embryo). Preg Rate (pregnancy rate 21 d post-ovulation). Blast% (blastocyst rate).

Figure 61. Cumulative distribution of the adjusted R-squared value associated with the stochastic variables influencing the scenario of IVP SS- sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.



Trnsfr CL (CL qualified for recipient to receive an embryo). Preg Rate (pregnancy rate 21 d post-ovulation). Blast% (blastocyst rate).

Table 127. Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI resulting from the scenario of sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.

NPV ($)	MOET	IVP NS	IVP SS
Mode	(218,942.28)	(178,012.65)	(178,036.21)
5%	(235,131.25)	(283,366.77)	(267,604.13)
25%	(199,907.09)	(164,578.01)	(112,699.27)
Median	(45,370.47)	(5,653.10)	124,132.79
75%	262,440.91	268,401.13	534,619.76
95%	1,153,726.33	1,061,174.03	1,599,991.13
Mean ± 90% C.I.	144,819.10 ±2,889.04	142,195.34 ±2,633.37	330,273.26 ±3,621.28
SD	555,421.70	506,268.86	696,161.03
ANPV ($)	**MOET**	**IVP NS**	**IVP SS**
Mode	(37,837.56)	(30,764.11)	(30,768.19)
5%	(40,635.34)	(48,971.39)	(46,247.30)
25%	(34,547.91)	(28,442.34)	(19,476.67)
Median	(7,840.92)	(976.97)	21,452.61
75%	45,354.99	46,385.03	92,392.89
95%	199,386.78	183,391.90	276,510.18
Mean ± 90% C.I.	25,027.61 ± 499.28	24,574.17 ± 455.10	57,077.76 ± 625.83
SD	95,987.88	87,493.29	120,310.42

(*cont.*)

Table 127 (*cont.*). Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI resulting from the scenario of sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.

ROI (%)	MOET	IVP NS	IVP SS
Mode	-39.0	-26.9	-24.2
5%	-41.3	-40.7	-39.1
25%	-35.3	-24.3	-17.3
Median	-10.6	-3.24	13.2
75%	34.3	31.5	61.8
95%	153.4	127.9	182.8
Mean ± 90% C.I.	14.5 ± 0.394	14.3 ± 0.324	35.4 ± 0.422
SD	75.8	62.2	81.1
Probability of Negative Return	57.8	53.2	40.2

Discussion

Applying the IVP SS method of ET to the given scenario of sex selection generated a mean ROI of 35.4% (Table 127), significantly increasing the mean ROI with greater than 90% confidence when compared to MOET, 14.5%, and IVP NS, 14.3% (Table 127). At 90% confidence, the mean ROI for MOET and IVP NS was not significantly different. The standard deviation of the mean ROI for MOET, IVP NS, and IVP SS was 75.8%, 62.2%, and 81.1% (Table 127), respectively.

The initial investment expense for MOET, IVP NS, and IVP SS for the given scenarios were $213,000.00, $213,000.00, and $207,000.00, respectively. The difference in initial investment expense can be explained by the number of bulls required for each program. While 5 donors and 100 recipients were purchased for all three ET program scenarios, on average the IVP SS scenario requires one less natural service sire, valued at $6,000.00, because the average number

of recipients that do not settle to ET is below the threshold that requires two rather than one natural service sire.

Within the model there is an economic incentive to produce as many bulls as possible. Thus, in a situation where there is a high likelihood of each ET pregnancy being male (Table 100) (Morotti et al., 2014, Pontes et al., 2010, Xu et al., 2006), the differences in ROI can primarily be attributed to the number of ET pregnancies generated by each production method and the shape of the distribution associated with the number of ET pregnancies. The mean number of ET pregnancies for MOET, IVP NS, and IVP SS was 21.2 pregnancies, 28.3 pregnancies, and 37.6 pregnancies, respectively (Table 135). The shape of the distribution for the number of ET pregnancies contributes to the convergence of MOET and IVP to a similar mean ROI. Although only 5 ET pregnancies are expected at the 25th percentile for MOET, the mean number of pregnancies at the 95th percentile is 68 (Table 135). Conversely, 16 ET pregnancies are expected at the 25th percentile for IVP NS, while only 60 ET pregnancies are expected at the 95th percentile; illustrating how the distribution of pregnancies for IVP NS is skewed to the left (Table 135).

The number of embryos produced and transferred by each respective ET production method directly influences the number of pregnancies produced. For the given scenario, MOET generated a mean of 30 embryos coupled with 30 transfers; IVP NS produced 60 embryos and 60 transfers; while IVP SS generated 95 embryos accompanied by 95 transfers. The limited efficiency of MOET using sexed semen is explained further in Table 34 and its accompanying references (Schenk et al., 2006; Peippo et al., 2009; Hayakawa et al., 2009). The increase in embryo production with IVP SS as compared to IVP NS is driven by the application of exogenous hormone protocols to donors. Not only are the number of viable oocytes increased with the use of exogenous

hormone protocols (Table 10; Table 44) (C. Fernandes et al., 2014), but blastocyst rate is improved as well (Table 50) (De Roover et al., 2008).

The probability of negative return for MOET, IVP NS, and IVP SS is 57.8%, 53.2%, and 40.2%, respectively (Table 127). Along with having the lowest probability of negative return, the IVP SS method also generates the greatest return at the median, and 95th percentile (Table 127). Thus, depending on a firm's economic and production preferences, one could argue that for the given scenario, IVP SS is the lowest risk production option. When comparing MOET and IVP NS, the distribution of ROI follows closely with that of the number ET pregnancies. IVP NS has the lowest expected ROI at the 95th percentile, 127.9%. MOET has the lowest mode and median ROI, -39.0% and 10.6%, respectively. Furthermore, MOET has a greater standard deviation of mean ROI at 75.8%, compared to the lowest standard deviation of mean ROI at 62.2%, which describes the IVP NS method (Table 127). Actually, IVP SS has the greatest standard deviation of ROI at 81.1%. Again, depending on one's risk measurement preferences, one could reasonably conclude that any of the three scenarios create the greatest economic risk for the described ET program of sex-selection for male progeny.

Referencing the statistical results found in Figures 50 and 51, the two largest regression coefficients for Scenario B1 are the number of transferable embryos per flush and ET bull revenue distribution. For Scenario B2 and B3, Figures 55 and 56 and Figures 60 and 61 show that the three largest regression coefficient values are ET bull revenue distribution, number of incubated oocytes per OPU, and the blastocyst rate. Again, because of the incorporation of binomial distributions to implement stochastic variable representing a mean probability, the adjusted R-square for each scenario does not fully explain the variation of simulation results.

Scenario A and Scenario B

Table 128. Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the ROI resulting from the scenarios of unsorted semen or sex-sorted semen- owned donors- owned recipients- market developed bulls and heifers.

ROI (%)	MOET	IVP NS	IVP SS
Mode	-37.4	13.5	-16.3
5%	-39.0	-5.5	-34.3
25%	-22.0	13.9	-10.2
Median	16.9	37.1	20.5
75%	71.3	74.1	66.0
95%	194.5	166.9	169.8
Mean ± 90% C.I.	38.6± 0.437	53.7 ± 0.326	38.4± 0.374
SD	84.0	62.6	71.8
Probability of Negative Return	40.0	9.6	34.0
ROI (%)	**MOET**	**IVP NS**	**IVP SS**
Mode	-39.0	-26.9	-24.2
5%	-41.3	-40.7	-39.1
25%	-35.3	-24.3	-17.3
Median	-10.6	-3.24	13.2
75%	34.3	31.5	61.8
95%	153.4	127.9	182.8
Mean ± 90% C.I.	14.5 ± 0.394	14.3 ± 0.324	35.4 ± 0.422
SD	75.8	62.2	81.1
Probability of Negative Return	57.8	53.2	40.2

The results of all scenarios can be directly compared to each other using ANPV or ROI. The only difference between Scenarios A and B is the use of unsorted semen in Scenario A and sex-sorted semen in Scenario B (Table 128). The probability of negative return is greater for all

three sub-scenarios in Scenario B than in Scenario A. This can be attributed to the reduced number of transferable embryos collected for MOET and the reduced blastocyst rate for IVP typically found when using sex-sorted semen. Alternatively, because the use of sex-sorted semen is an attempt to capitalize on an economic incentive by maximizing the number of animals of a specific sex, each success is more lucrative.

The most drastic change in the distribution of ROI between Scenario A and Scenario B is found when using MOET or IVP NS (Table 126; Table 127). Although the mean ROI is still greater for Scenario A than Scenario B when considering IVP SS, the reduction is much less than when considering the other two methods of ET production. The 95th percentile for Scenario B3 is greater than Scenario A3. The increased success of IVP SS over MOET and IVP NS when using sex-sorted semen can be attributed to the number of transferable embryos produced. Within the model, the mean number of transferable embryos produced using MOET and sex-sorted semen is roughly half of what is produced when using MOET and unsorted semen. The difference in the mean blastocyst rate between IVP NS and IVP SS only changes by one percentage point when comparing Scenario A and Scenario B. The mean value of each ET calf is greater when using sex-sorted semen, thus the risk-reward ratio becomes more favorable for a scenario that typically generates more ET progeny, such as IVP SS.

Scenario C: Unsorted Semen- Custom Recipients- Market Ultrasound Sexed Pregnancies

Results

Consider this scenario, with results shown below, as one in which an operation owns 100 potential recipients and its business model is to set-up recipients and transfer embryos to those recipients for another operation that, in this scenario, owns 5 donors. After a 60 d, ET pregnancy

has been established the owner of the recipients then sells the recipient to the owner of the donor and the embryos. The sex of the pregnancy is determined, via ultrasound, before the sale of pregnant recipients. All recipients not pregnant to ET are exposed to a bull and sold at the conclusion of the breeding season, with the corresponding value of a commercial pregnancy or open female.

Scenario C1: MOET

Figure 62. Probability distribution of the NPV resulting from the scenario of MOET- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

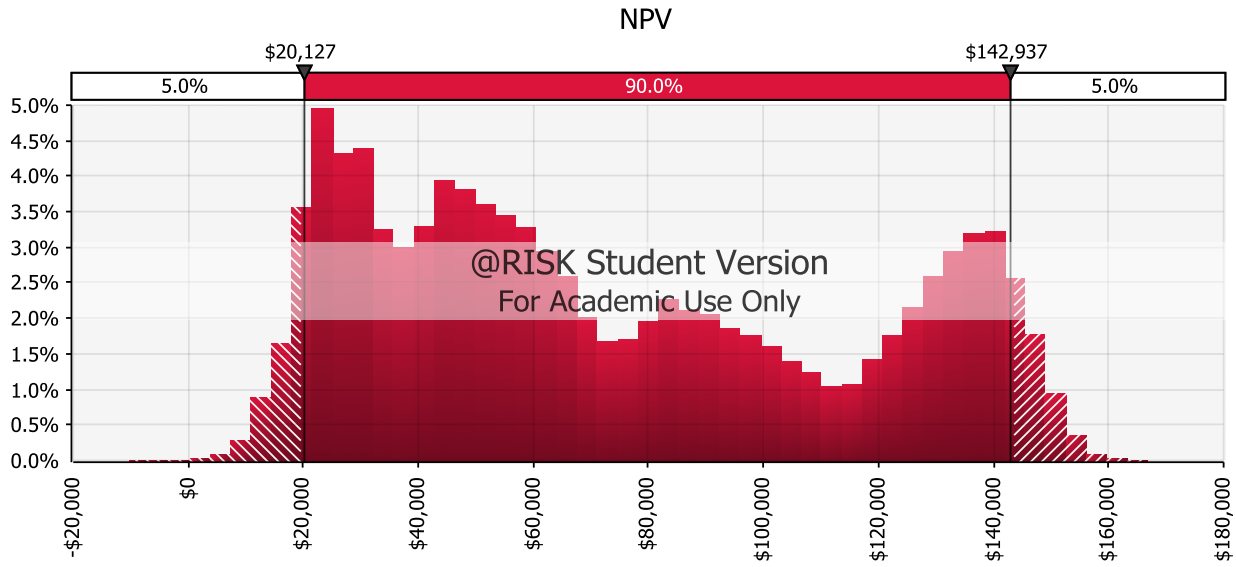


Figure 63. Probability distribution of the ANPV resulting from the scenario of MOET- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

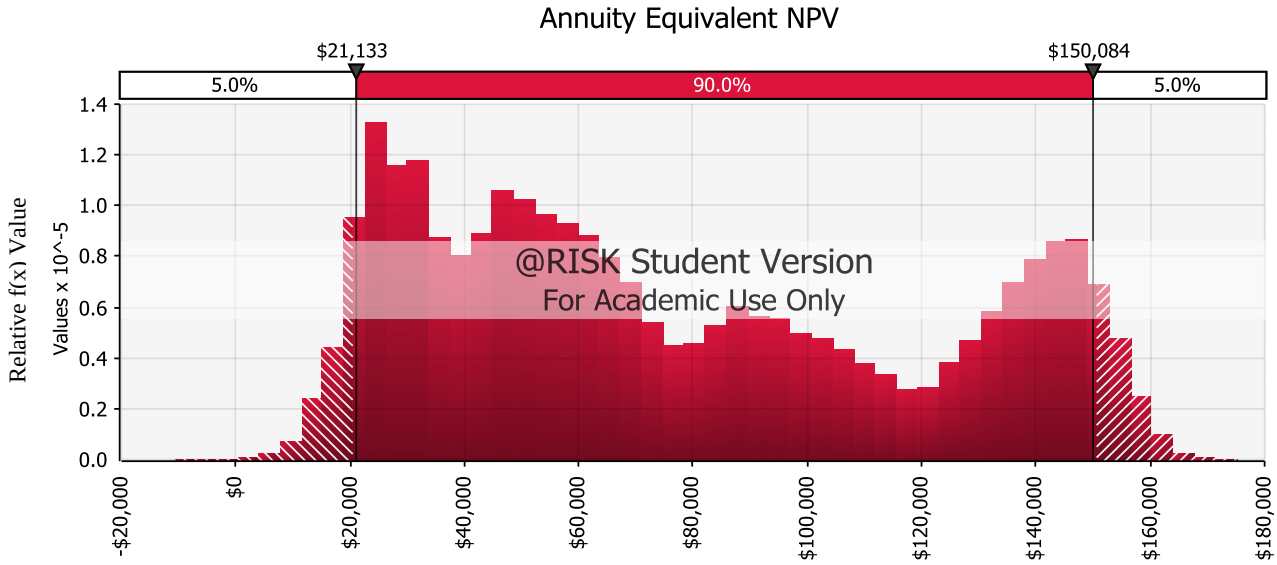


Figure 64. Probability distribution of the ROI resulting from the scenario of MOET- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

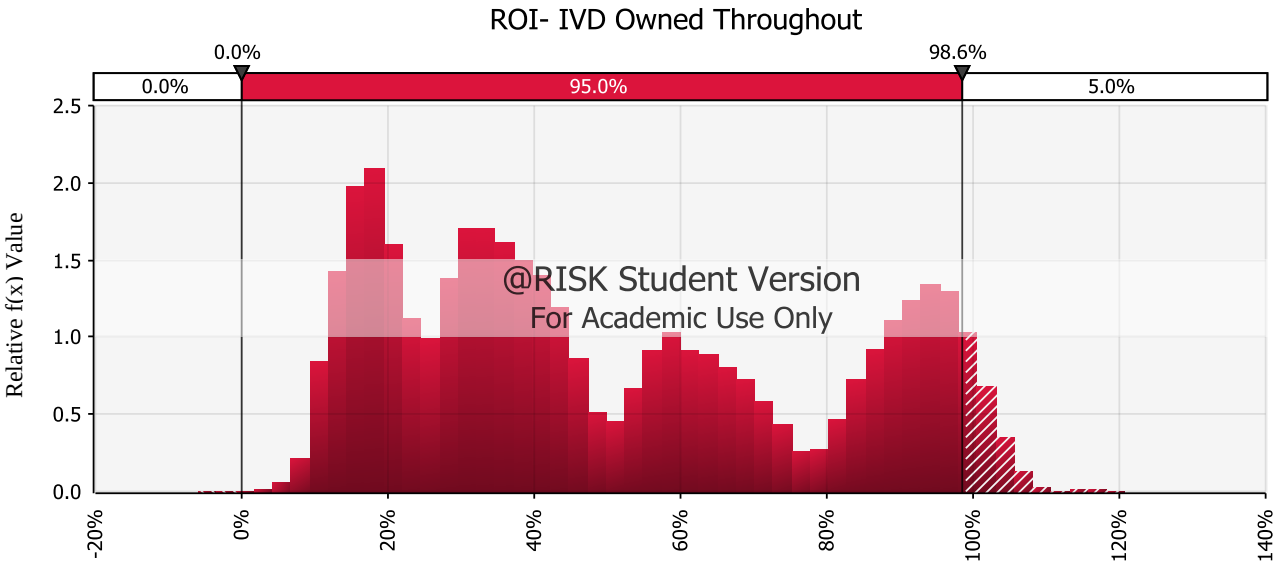
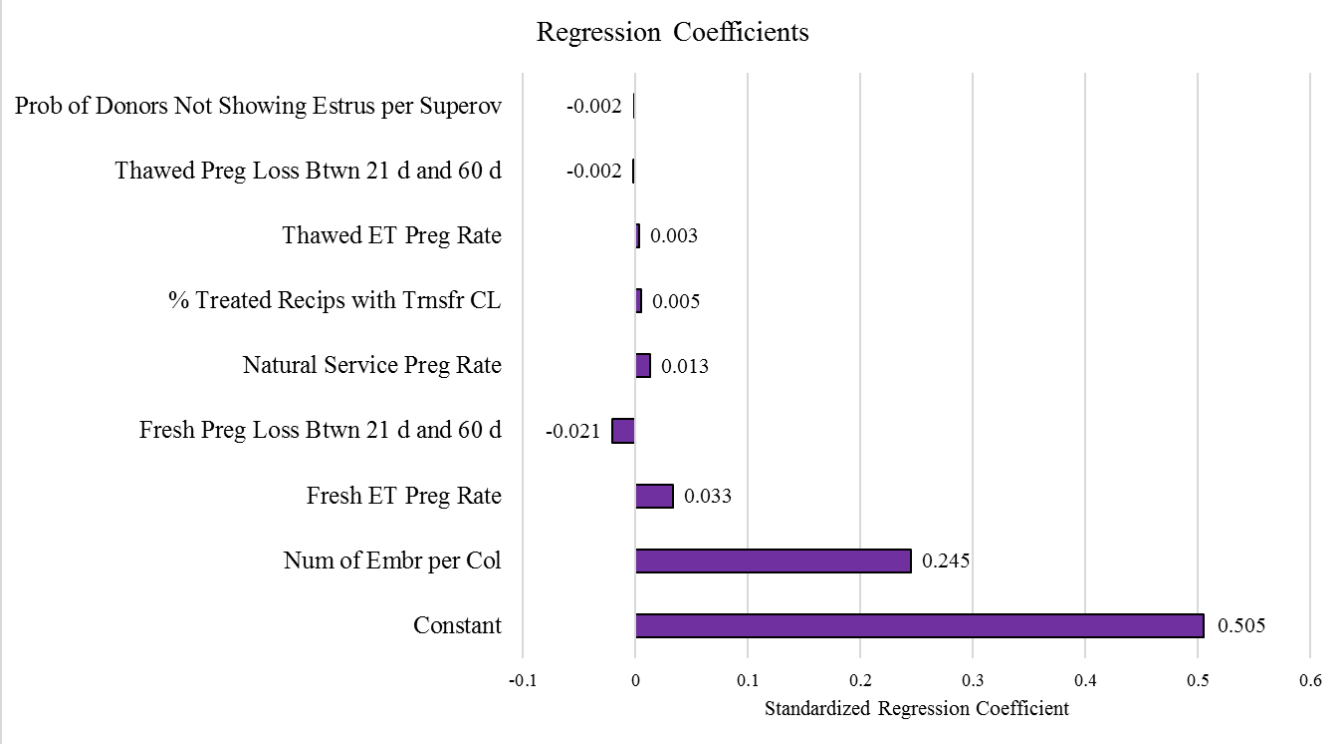
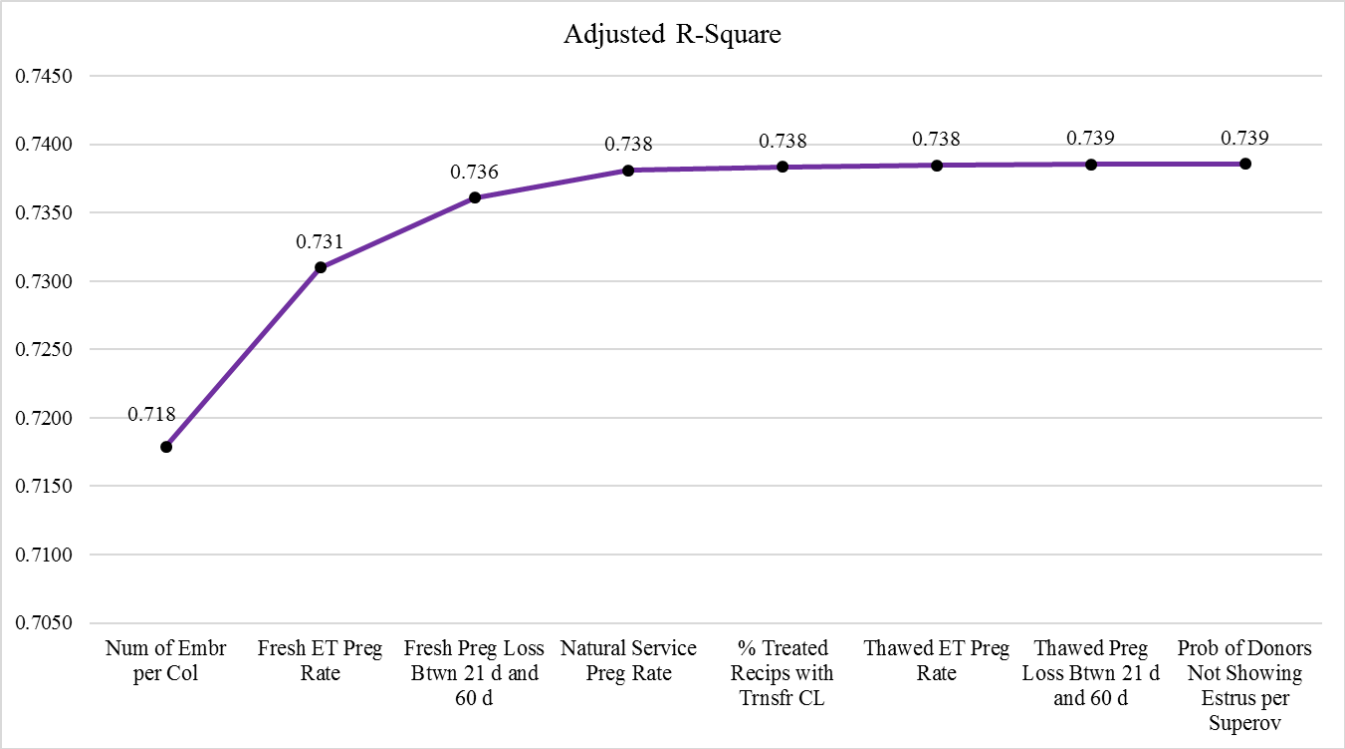


Figure 65. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of MOET- unsorted semen- custom recipients- market ultrasound sexed pregnancies.



Preg Rate (pregnancy rate 21 post-ovulation). Trnsfr CL (CL qualified for recipient to receive an embryo). Num of Embr per Col (number of transferable embryos per collection).

Figure 66. Cumulative distribution of the adjusted R-squared value associated with the stochastic variables influencing the scenario of MOET- unsorted semen- custom recipients- market ultrasound sexed pregnancies.



Preg Rate (pregnancy rate 21 post-ovulation). Trnsfr CL (CL qualified for recipient to receive an embryo). Num of Embr per Col (number of transferable embryos per collection).

Scenario C2: IVP NS

Figure 67. Probability distribution of the NPV resulting from the scenario of IVP NS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

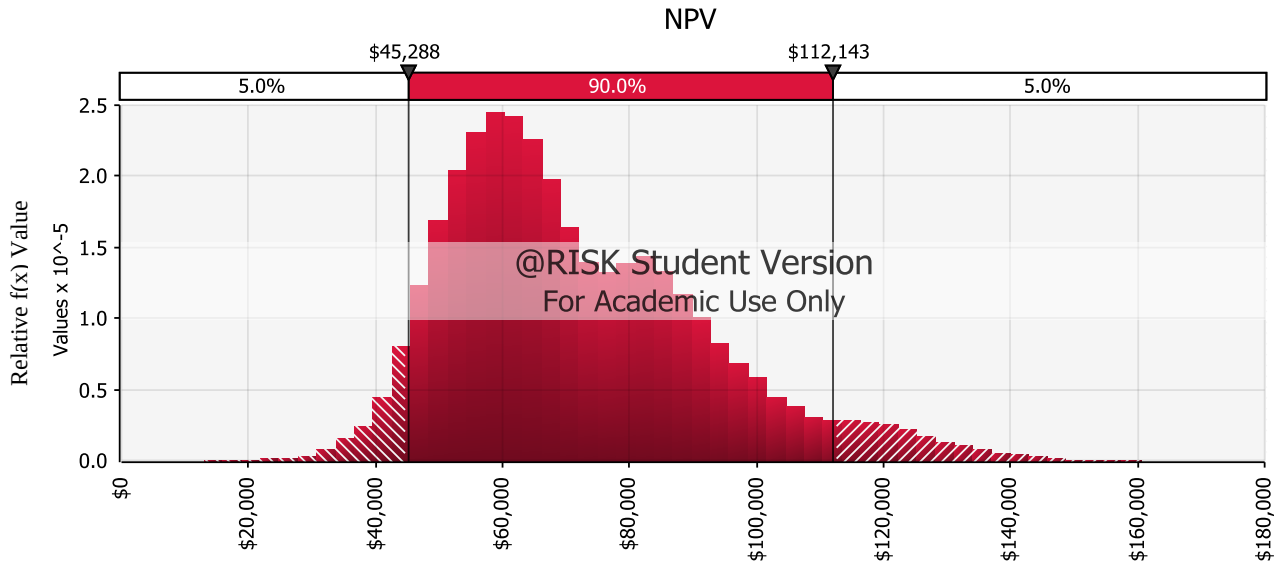


Figure 68. Probability distribution of the ANPV resulting from the scenario of IVP NS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

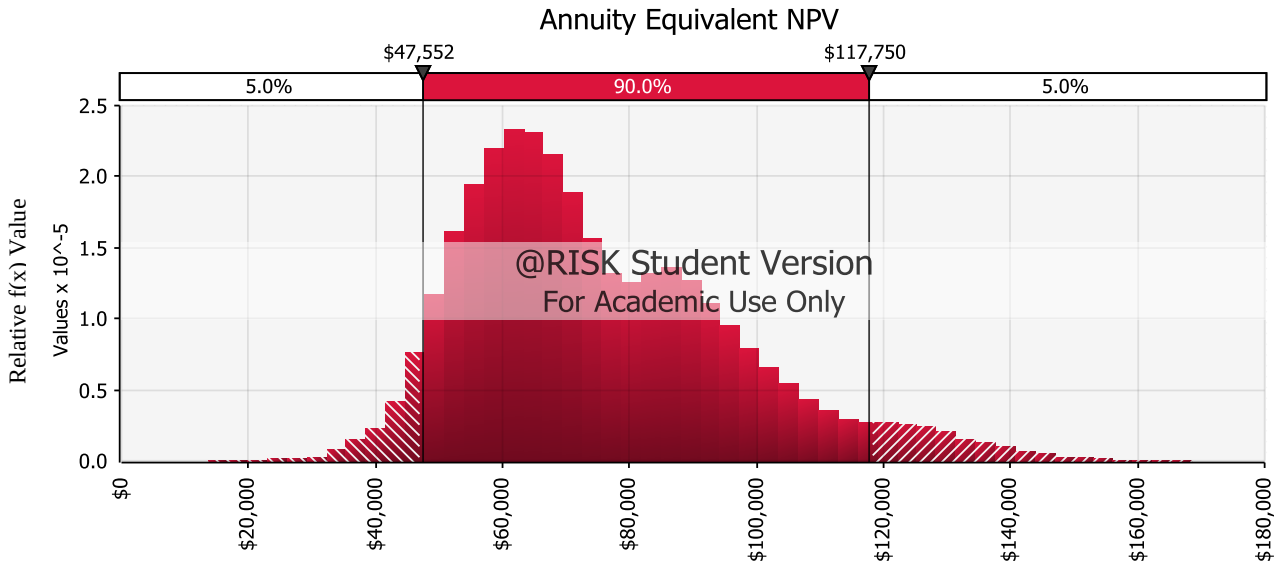


Figure 69. Probability distribution of the ROI resulting from the scenario of IVP NS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

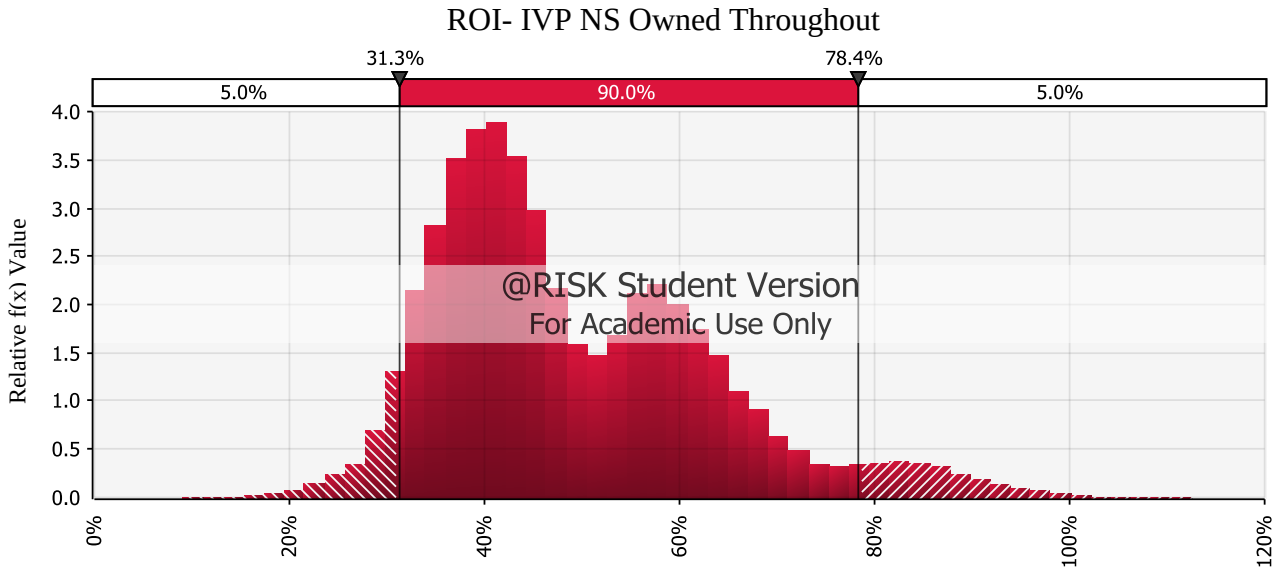
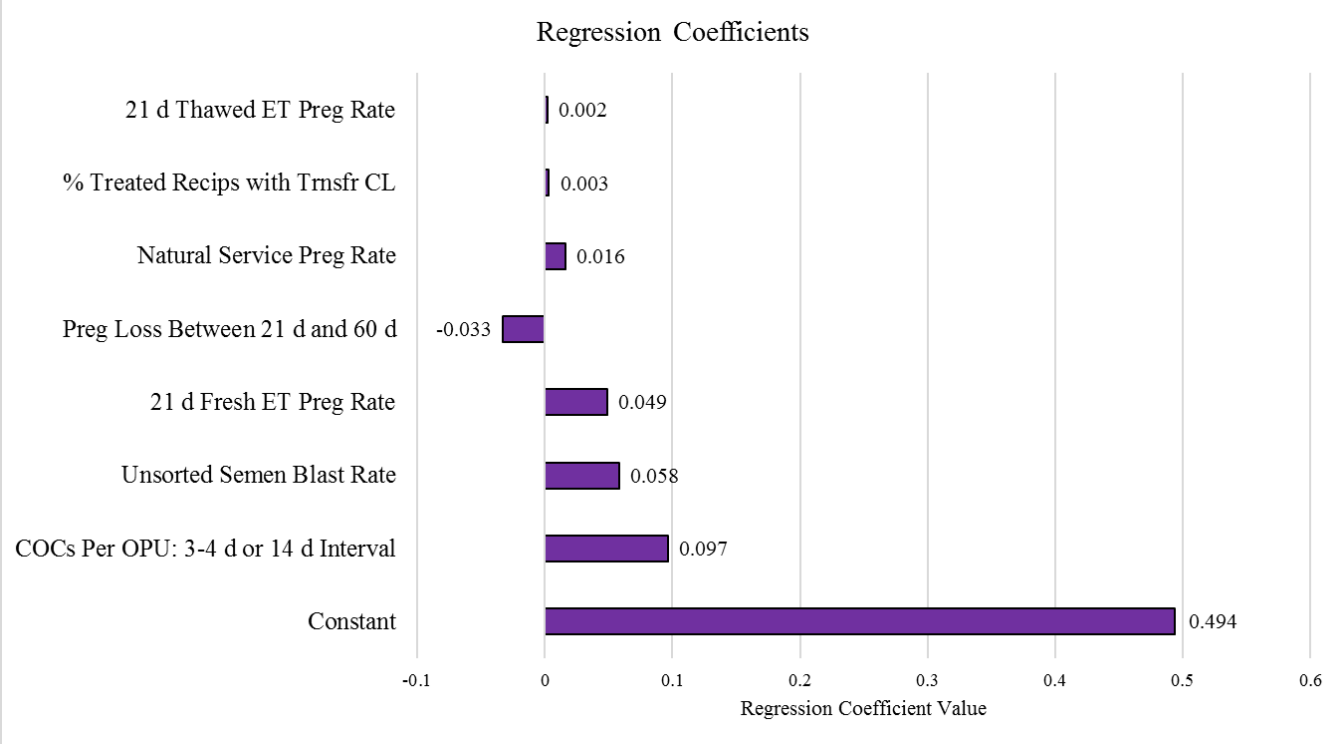
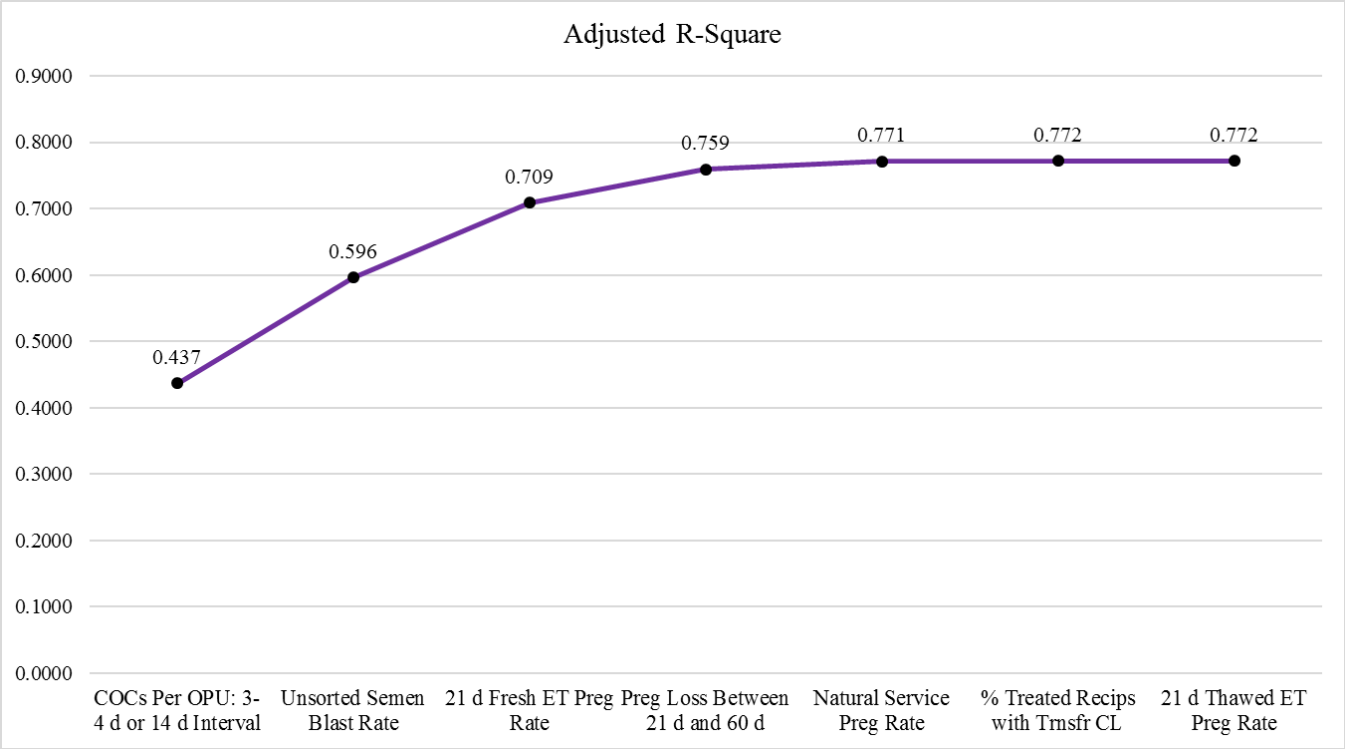


Figure 70. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of IVP NS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.



Preg Rate (pregnancy rate 21 post-ovulation). Trnsfr CL (CL qualified for recipient to receive an embryo). Blast Rate (blastocyst rate). COCs Per OPU (number of oocytes incubated per OPU).

Figure 71. Cumulative distribution of the adjusted R-squared value associated with the stochastic variables influencing the scenario of IVP NS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.



Preg Rate (pregnancy rate 21 post-ovulation). Trnsfr CL (CL qualified for recipient to receive an embryo). Blast Rate (blastocyst rate). COCs Per OPU (number of oocytes incubated per OPU).

Scenario C3: IVP SS

Figure 72. Probability distribution of the NPV resulting from the scenario of IVP SS- unsorted semen- custom recipients- market sexed pregnancies.

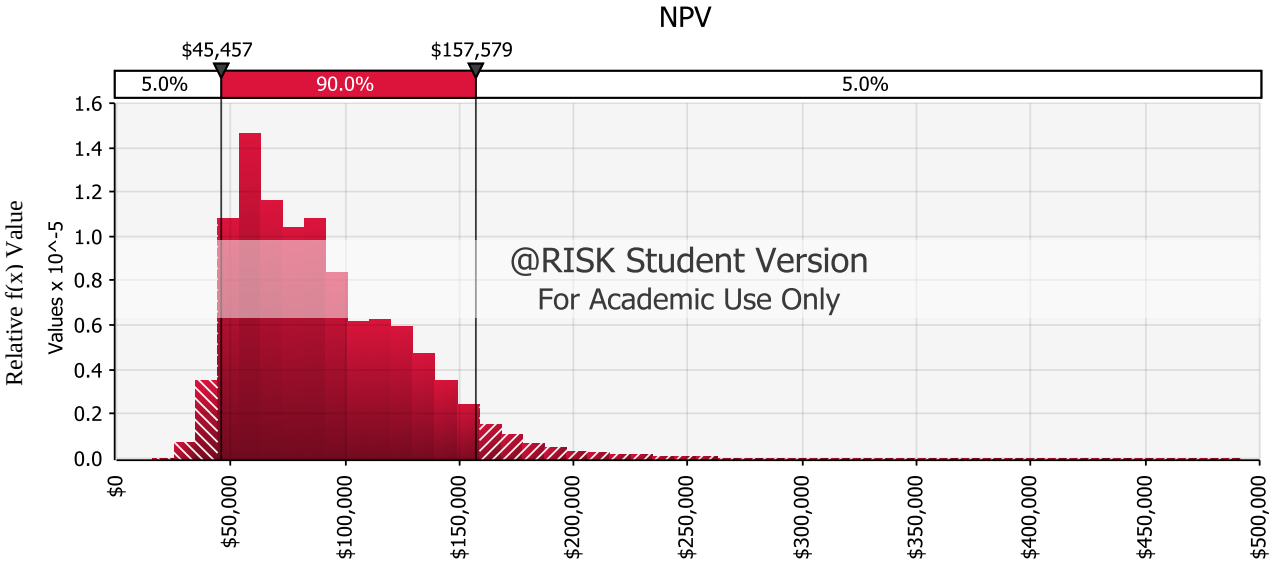


Figure 73. Probability distribution of the ANPV resulting from the scenario of IVP SS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

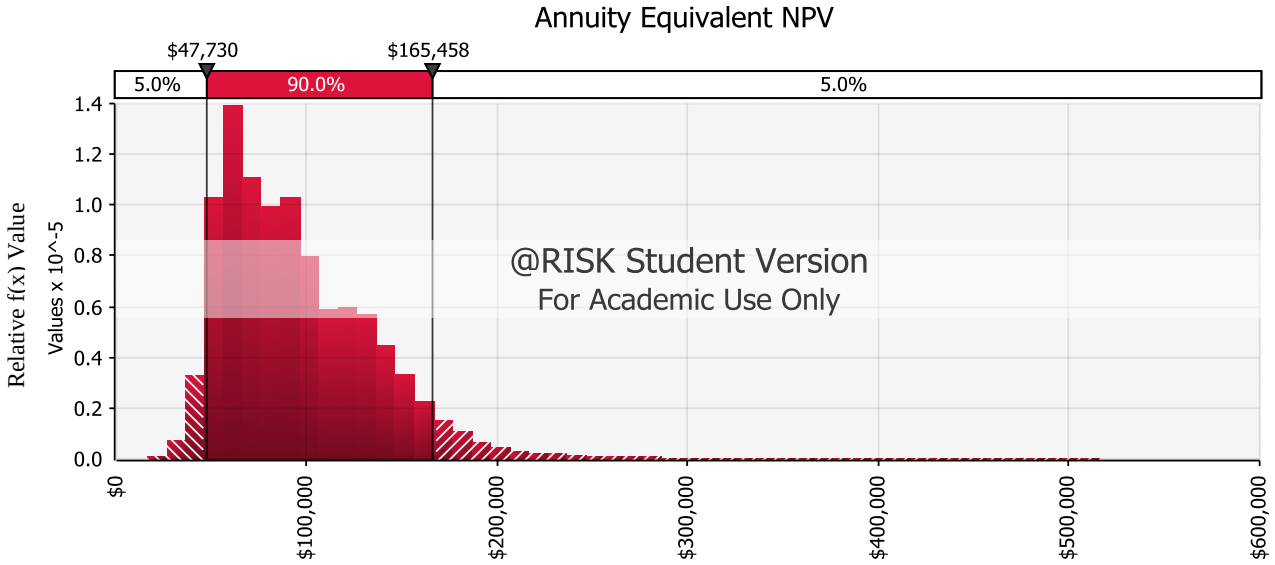


Figure 74. Probability distribution of the ROI resulting from the scenario of IVP SS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.

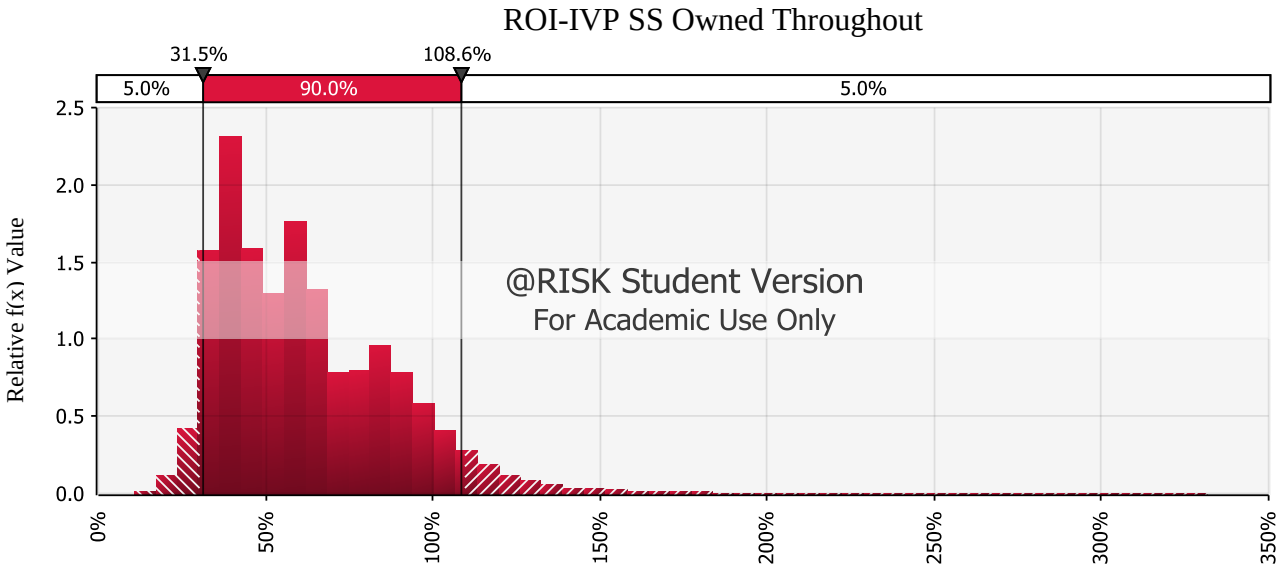
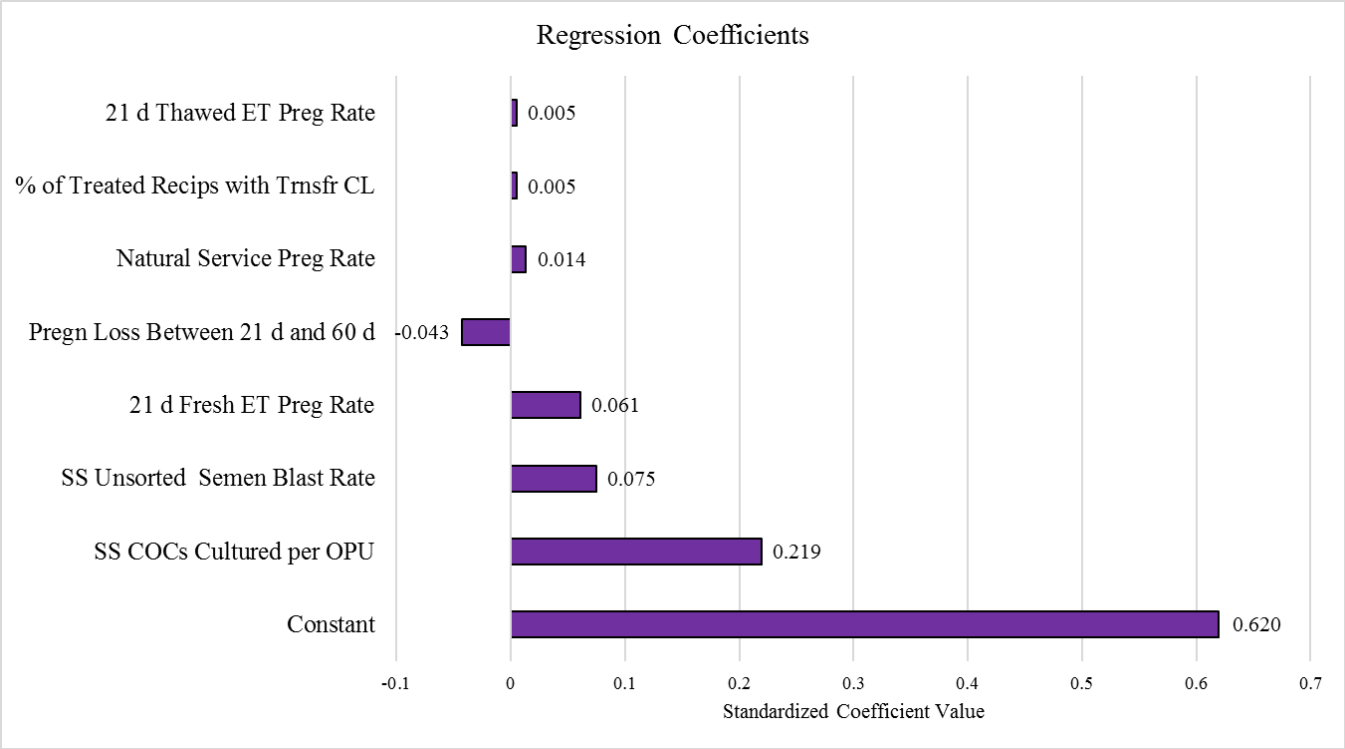
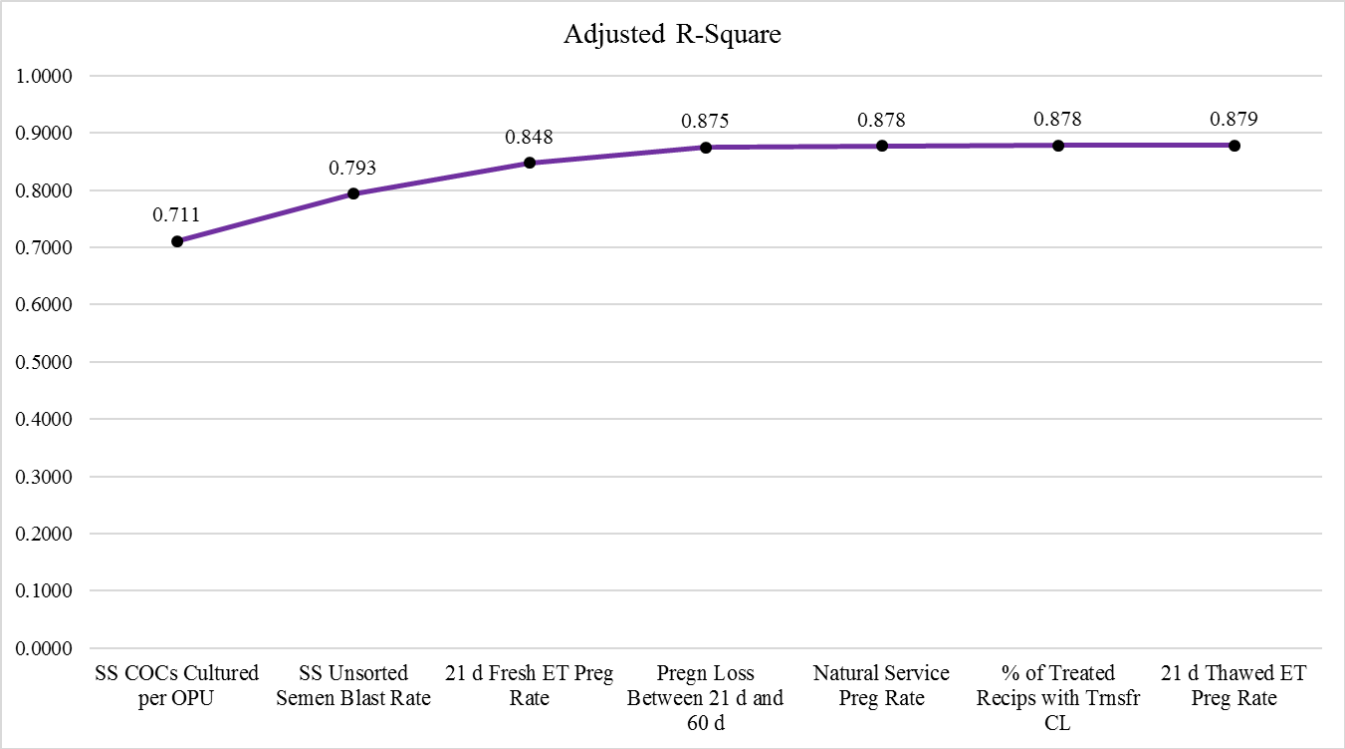


Figure 75. Standardized stepwise regression coefficients for the stochastic variables influencing the scenario of IVP SS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.



Preg Rate (pregnancy rate 21 post-ovulation). Trnsfr CL (CL qualified for recipient to receive an embryo). Blast Rate (blastocyst rate).

Figure 76. Cumulative distribution of the adjusted R-squared value associated with the stochastic variables influencing the scenario of IVP SS- unsorted semen- custom recipients- market ultrasound sexed pregnancies.



Preg Rate (pregnancy rate 21 post-ovulation). Trnsfr CL (CL qualified for recipient to receive an embryo). Blast Rate (blastocyst rate).

Table 129. Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI resulting from the scenario of unsorted semen- custom recipients- market ultrasound sexed pregnancies.

NPV ($)	MOET	IVP NS	IVP SS
Mode	23,936.51	50,315.81	83,806.19
5%	24,015.75	45,287.62	45,456.95
25%	37,657.56	56,630.00	60,878.29
Median	63,176.32	67,262.38	82,856.48
75%	110,523.27	84,102.29	113,467.33
95%	142,936.98	112,143.24	157,579.05
Mean ± 90% C.I.	73,634.22 ± 216.49	71,646.43 ± 105.97	90,144.24 ± 194.21
SD	41,621.06	20,373.38	37,336.75
ANPV ($)	**MOET**	**IVP NS**	**IVP SS**
Mode	25,133.33	52,831.60	87,996.50
5%	21,133.33	47,552.00	47,729.80
25%	39,540.43	59,461.50	63,922.20
Median	66,335.13	70,625.50	86,999.30
75%	116,049.43	88,307.40	119,140.70
95%	150,083.83	117,750.40	165,458.00
Mean ± 90% C.I.	77,315.93 ± 227.32	75,228.75 ± 111.27	94,651.46 ± 203.92
SD	43,702.12	21,392.05	39,203.59

(*cont.*)

Table 129 (*cont.*). Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI resulting from the scenario of unsorted semen- custom recipients- market ultrasound sexed pregnancies.

ROI (%)	MOET	IVP NS	IVP SS
Mode	16.1	40.0	37.5
5%	13.7	31.3	31.5
25%	26.0	38.5	41.2
Median	42.5	45.4	57.3
75%	75.3	58.3	79.1
95%	98.6	78.4	108.6
Mean ± 90% C.I.	50.5 ± 0.15	49.2 ± 0.075	62.0 ± 0.134
SD	28.9	14.4	25.9
Probability of Negative Return	0.0	0.0	0.0

Discussion

The distributions presented in Figures 62-64, Figures 67-69, and Figures 72-74 may seem rather irrational; however, they are result of the distribution of initial investment (Table 143). The distribution of initial investment is a direct result of the number of natural service sires required to cover the recipient females not pregnant to ET. The user-defined value for the cow to bull ratio is 30 to 1. If the number of open recipients at the end of the ET program reaches 1, 31, 61, or 91 an additional bull, with a user-defined cost of $6,000.00, is purchased. Scenario C only has a one-year investment life and the operation only owns the recipients; thus, the addition of a bull increases total expense by a greater percentage for Scenario C than Scenario A or Scenario B. Furthermore, the cost of a bull is only spread over one year for Scenario C, rather than several years for Scenario A and Scenario B, which have six-year investment lives for this simulation. All told, the distribution of initial investment expense has a more visible impact on the distribution of profit for Scenario C than Scenario A or Scenario B.

The mean ROI for MOET, IVP NS, and IVP SS for the custom recipient program described was 50.5%, 49.2%, and 62.0%, respectively, all significantly different (Table 129). The mode ROI and median ROI for each program were 16.1% and 42.5%; 40.0% and 45.4%; and 37.5% and 57.3%, respectively (Table 129). The primary driver of revenue is the number of pregnant recipients created and subsequently marketed. Although the mean number of ET bred recipients for MOET and IVP NS were relatively close in value at 38.6 and 33.9, respectively, (Table 140) the different input costs and the shape of the distribution of the number pregnant recipients, as described in preceding sections, produces a different ROI distribution for each of the respective scenarios. The mean number of pregnant recipients for IVP SS was 44.6 head (Table 140).

Scenario C has shifted the risk of involvement in an ET program to the owner of the donor females. If zero embryos are produced, the recipient herd essentially becomes a commercial female herd for which the user-defined variables are set at profitability. Different from the other scenarios that have already been described, in this scenario, the operation has also mitigated risk by establishing a contracted selling price for pregnant recipients. Market conditions undoubtedly have an influence on the realistic value of pregnant recipients; however, the operation still has more control over the price received than cattle sold at auction or sold by the pound. For these reasons, it seems rational that if the selling price is set high enough and demand is sufficient, the probability of negative return could be 0%, as seen in all the scenarios illustrated here.

The two largest regression coefficient values for MOET are the number of transferable embryos per collection and fresh ET pregnancy rate at 21 d post-ovulation. The three largest regression coefficient values for IVP NS and IVP SS are the number of incubated oocytes per OPU, blastocyst rate, and fresh ET pregnancy rate at 21 d post-ovulation. In this scenario, the operation contracts the use of its recipients. If there are no embryos, the recipients simply become

commercial females which leaves unused profit potential. The adjusted R-square value for each ET method is greater in Scenario C when compared to the corresponding method in Scenario A or Scenario B. This is because the number of binomial distributions that rely on a stochastic variable estimating a mean probability is reduced.

Chapter 5 - Conclusion

Since its inception as a commercial application, ET has had a profound impact on the cattle industry by creating a means to propagate the genetics of elite females. More recently, through sexed-semen technology, ET has allowed for progressive producers to respond to market signals by predetermining the sex of resulting progeny. While the adaptation of technology serves as a crucial mode of industry advancement and improvement, financial feasibility and risk must be assessed when developing a strategy for implementation. The potential inefficiencies and biological uncertainties associated with ET make such financial risk assessment a challenging prospect. To further complicate matters, cattle producers are now presented with a choice between two primary methods of ET. In-vivo derived (IVD) ET describes the traditional method of ET that involves follicular stimulation and insemination of a donor female followed by the collection of fertilized embryos from the uterus. In-vitro fertilization (IVF) commonly refers to the method of generating transferable embryos by collecting oocytes by ovarian aspiration; in-vitro fertilization of the collected oocytes; and incubated maturation of the fertilized oocytes. Encompassed within the two methods of ET exist several different sub-techniques, principally regarding the exception or inclusion of follicular synchronization and/or stimulation before ovum pick-up (OPU) in IVF procedures. Ultimately, operators must decide whether or not ET programs, of any type, serve as an economically viable means to increase rate of genetic improvement or take advantage of marketing opportunities.

Inherent to the identity of the beef industry is the variation of environment, cattle type, and management practices between operations. Thus, a critical aspect of the stochastic model described and applied in the preceding pages is the ability to incorporate user-defined variable values, specific to an individual operation, as parameters for the program in question. The stochastic

elements of the model create a more realistic outlook than the use of means in deterministic models, as distributions defining the biological uncertainty for a multitude of reproductive outcomes are incorporated into the model. Applying the LHS variation of Monte Carlo simulation, a sample value from the descriptive distribution associated with each stochastic variable is included in an iteration of the simulation. Through large numbers of iterations with dynamic combinations of variables, the process culminates in a distribution of possible values for the net present value (NPV), annuity equivalent net present value (ANPV), and return on investment (ROI) associated with the model described scenario of IVD or IVF. Finally, using the distributions of NPV, ANPV, and ROI a decision maker can assess the economic risk linked to a user-defined ET program.

This model does not account for the increased magnitude and rate of genetic gain that is possible through ET and the potential long-term impact those genetic improvements may have on a breeding program. Ideally, those genetic improvements would not only make resulting progeny more marketable, but allow for increased production efficiency in both the breeding herd and market bound progeny who trace their genetics to the nucleus herd. True increases in production efficiency should have a positive impact on an operation's profit margin. Accounting for the long term economic impact of accumulated improvements or changes in production efficiency is the next step in analyzing the economics of ET. This model could serve as a foundational template for that opportunity.

The IVP industry has seen rapid expansion in the U.S. with technological advancement frequently changing production protocols and improving expected outcomes. It is likely that several of the major IVP companies have implemented such advancements to improve production levels past what is reported in this thesis. The pace of change is rapid enough that many changes

are not reported in the scientific literature before being implemented in industry. Furthermore, it is likely that IVP companies may regard technological advancements as trade secrets that yield a competitive advantage in the marketplace. Thus, a challenge in the application of this model is creating and maintaining an accurate representation of expected production outcomes from the most current ET practices.

Primarily, this model is not intended to be distributed to the public for personal use because of the complexity of managing the variables and ensuring model integrity. Instead, the core function of this model should be as a consultative tool with the firm that is interested in the model output working through someone skilled in the navigation, operation, and interpretation of the model.

Regardless of one's method for measuring economic success and the risk associated with that success, the numerical and logical analysis afforded through the stochastic simulation of alternative scenarios through this model allows for in-depth assessment of ET programs not previously available. The caveat is that any model, no matter how robust, will never be completely accurate, as all are a simplified version of a complicated reality. That said, there is ample opportunity for the commercial application of this stochastic model to complement the deterministic, instinctive, and experience based elements of the decision-making process pertaining to the prediction of the economic outcome of an ET program, through methodology that the ET industry, as known to the author, has not fully exploited.

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Appendix A - Supplemental Information for the Scenarios of ET Using Unsorted Semen- Owned Donors- Owned Recipients- Market Developed Bulls and Heifers

Table 130. Distribution of the number of full term ET bred females, natural sire bred females, and open females associated with each scenario.

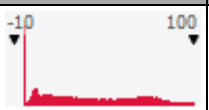
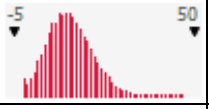
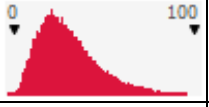
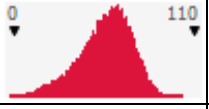
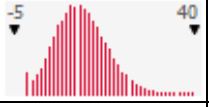
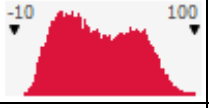
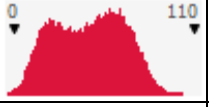
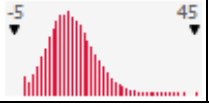
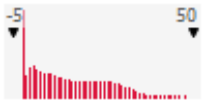
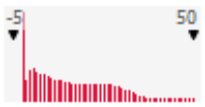
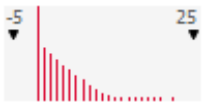
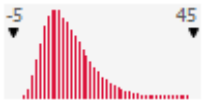
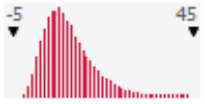
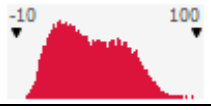
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of ET Bred Recipients	IVD Unsexed Embryo Production		0	34.38374	98	0	78
Number of Bull Bred Recipients	IVD Unsexed Embryo Production		0	52.53136	100	8	88
Number of Open Recipients	IVD Unsexed Embryo Production		0	13.0849	47	5	23
Number of ET Bred Recipients	IVF NS Unsexed Production		0	31.6839	94	12	61
Number of Bull Bred Recipients	IVF NS Unsexed Production		1	55.99705	95	28	76
Number of Open Recipients	IVF NS Unsexed Production		0	12.31905	42	5	21
Number of ET Bred Recipients	IVF SS Unsexed Production		0	41.70015	98	11	75
Number of Bull Bred Recipients	IVF SS Unsexed Production		1	46.58499	95	16	76
Number of Open Recipients	IVF SS Unsexed Production		0	11.71486	38	4	20

Table 131. Distribution of the number of ET calves, marketable ET bull calves, marketable ET heifer calves, cull ET bull calves, and cull ET heifer calves generated by each scenario- using unsorted semen and marketing developed bulls and heifers.

Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of ET Calves	IVD US Development		0	30.8592	94	0	71
Number of Marketable ET Bull Calves	IVD US Development		0	12.3437	49	0	30
Number of Marketable ET Heifer Calves	IVD US Development		0	12.3410	48	0	30
Number of Culls-ET Bull Calves	IVD US Development		0	3.08601	19	0	9
Number of Culls-ET Heifer Calves	IVD US Development		0	3.08841	18	0	9
Number of ET Calves	IVF NS US Development		0	27.6050	91	10	54
Number of Marketable ET Bull Calves	IVF NS US Development		0	11.0442	48	3	22
Number of Marketable ET Heifer Calves	IVF NS US Development		0	11.0400	42	3	22
Number of Culls-ET Bull Calves	IVF NS US Development		0	2.76103	16	0	7
Number of Culls-ET Heifer Calves	IVF NS US Development		0	2.75974	16	0	7
Number of ET Calves	IVF SS US Development		0	36.3286	96	10	67

(*cont.*)

Table 131 (*cont.*). Distribution of the number of ET calves, marketable ET bull calves, marketable ET heifer calves, cull ET bull calves, and cull ET heifer calves generated by each scenario- using unsorted semen and marketing developed bulls and heifers.

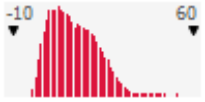
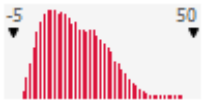
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of Marketable ET Bull Calves	IVF SS US Development		0	14.5265	50	3	28
Number of Marketable ET Heifer Calves	IVF SS US Development		0	14.5363	47	3	28
Number of Culls-ET Bull Calves	IVF SS US Development		0	3.63245	20	0	8
Number of Culls-ET Heifer Calves	IVF SS US Development		0	3.63329	18	0	8

Table 132. Distribution of the total annual revenue generated by the marketing of developed ET bulls and heifers produced via unsorted semen through MOET, IVP NS, and IVP SS.

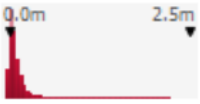
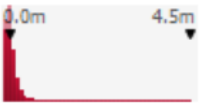
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD Revenue- US Development	Revenue		60,784.25	201,230.60	2,277,653.00	78,393.38	453,564.90
IVF NS Revenue- US Development	Revenue		64,594.05	185,656.30	2,183,872.00	99,131.44	351,792.70
IVF SS Revenue- US Development	Revenue		61,423.66	226,616.40	4,396,642.00	98,526.05	461,367.80

Table 133. Distribution of the total annual expense incurred by the production of developed ET calves via unsorted semen through MOET, IVP NS, and IVP SS.

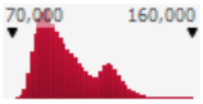
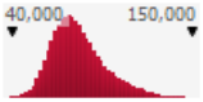
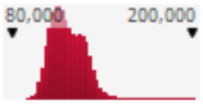
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD US	Total Expenses-Owned Donors		75,525.20	99,269.95	158,253.10	83,392.04	123,548.40
IVF NS US	Total Expenses-Owned Donors		43,731.47	82,134.23	143,447.30	61,876.77	109,533.50
IVF SS US	Total Expenses-Owned Donors		94,525.18	119,675.40	198,789.90	105,307.10	136,252.30

Table 134. Initial investment present value for MOET, IVP NS, and IVP SS embryo production methods- develop bulls/heifers.

Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
MOET Year 0 PV	NPV Owned Throughout		(219,000.00)	(210,239.80)	(195,000.00)	(219,000.00)	(201,000.00)
IVF NS Year 0 PV	NPV Owned Throughout		(219,000.00)	(210,188.80)	(201,000.00)	(213,000.00)	(201,000.00)
IVF SS Year 0 PV	NPV Owned Throughout		(219,000.00)	(207,625.40)	(195,000.00)	(213,000.00)	(201,000.00)

Appendix B - Supplemental Information for the Scenarios of ET Using Sex-Sorted Semen- Owned Donors- Owned Recipients- Market Developed Bulls and Heifers

Table 135. Distribution of the number of full term ET bred females, natural sire bred females, and open females associated with each sex-sorted semen scenario.

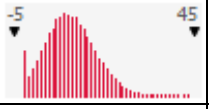
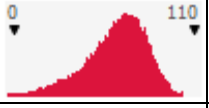
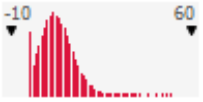
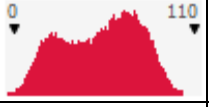
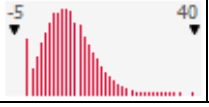
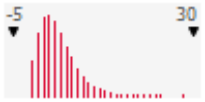
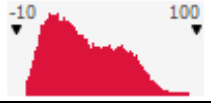
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of ET Bred Recipients	IVD Sexed Embryo Production		0	21.20253	96	0	68
Number of Bull Bred Recipients	IVD Sexed Embryo Production		0	67.14041	100	19	92
Number of Open Recipients	IVD Sexed Embryo Production		0	11.65706	45	4	21
Number of ET Bred Recipients	IVF NS Sexed Production		0	28.31706	94	7	60
Number of Bull Bred Recipients	IVF NS Sexed Production		2	62.43416	99	31	85
Number of Open Recipients	IVF NS Sexed Production		0	9.24878	37	3	17
Number of ET Bred Recipients	IVF SS Sexed Production		0	37.64002	97	7	74
Number of Bull Bred Recipients	IVF SS Sexed Production		0	53.16721	99	18	84
Number of Open Recipients	IVF SS Sexed Production		0	9.196549	33	3	17

Table 136. Distribution of the number of ET calves, marketable ET bull calves, marketable ET heifer calves, cull ET bull calves, and cull ET heifer calves generated by each sex-sorted scenario- marketing developed bulls and heifers.

Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of ET Calves	IVD SB Development		0	19.0342	92	0	61
Number of Marketable ET Bull Calves	IVD SB Development		0	13.8450	72	0	44
Number of Marketable ET Heifer Calves	IVD SB Development		0	1.37969	18	0	5
Number of Culls-ET Bull Calves	IVD SB Development		0	3.46239	27	0	12
Number of Culls-ET Heifer Calves	IVD SB Development		0	0.34719	8	0	2
Number of ET Calves	IVF NS SB Development		0	24.6738	90	6	53
Number of Marketable ET Bull Calves	IVF NS SB Development		0	17.9472	70	4	39
Number of Marketable ET Heifer Calves	IVF NS SB Development		0	1.7951	19	0	5
Number of Culls-ET Bull Calves	IVF NS SB Development		0	4.48465	26	0	11
Number of Culls-ET Heifer Calves	IVF NS SB Development		0	0.44678	7	0	2
Number of ET Calves	IVF SS SB Development		0	32.797	94	6	66

(*cont.*)

Table 136 (*cont.*). Distribution of the number of ET calves, marketable ET bull calves, marketable ET heifer calves, cull ET bull calves, and cull ET heifer calves generated by each sex-sorted scenario- marketing developed bulls and heifers.

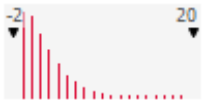
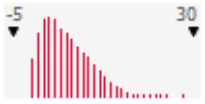
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of Marketable ET Bull Calves	IVF SS SB Development		0	23.8519	78	4	49
Number of Marketable ET Heifer Calves	IVF SS SB Development		0	2.38389	21	0	7
Number of Culls-ET Bull Calves	IVF SS SB Development		0	5.96483	26	1	14
Number of Culls-ET Heifer Calves	IVF SS SB Development		0	0.59636	8	0	2

Table 137. Distribution of the total annual revenue generated by the marketing of developed ET bulls and heifers produced via sex-sorted semen through MOET, IVP NS, and IVP SS.

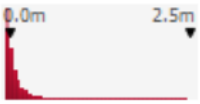
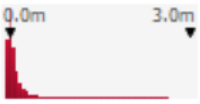
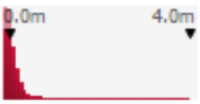
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD Revenue- SB Development	Revenue		57,707.49	163,280.70	2,404,984.00	76,167.69	387,270.00
IVF NS Revenue- SB Development	Revenue		63,284.20	186,238.90	2,587,811.00	90,667.02	384,670.60
IVF SS Revenue- SB Development	Revenue		62,850.87	230,144.50	3,886,047.00	92,372.08	509,080.30

Table 138. Distribution of the total annual expense incurred by the production of developed ET calves via sex-sorted semen through MOET, IVP NS, and IVP SS.

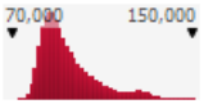
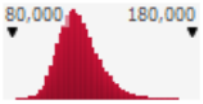
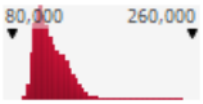
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD Sexed	Total Expenses- Owned Donors		76,162.13	95,959.56	149,513.70	83,810.02	120,144.90
IVF NS Sexed	Total Expenses- Owned Donors		86,328.50	118,899.90	170,057.30	104,087.40	137,192.10
IVF SS US	Total Expenses- Owned Donors		97,935.94	123,470.50	246,684.90	106,040.00	148,577.30

Table 139. Initial investment present value for MOET, IVP NS, and IVP SS embryo production methods- sex-sorted semen- develop bulls/heifers

Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
MOET Sexed Year 0 PV	NPV SB Owned Throughout		(219,000.00)	(213,448.70)	(195,000.00)	(219,000.00)	(201,000.00)
IVF NS Sexed Year 0 PV	NPV SB Owned Throughout		(219,000.00)	(210,853.50)	(201,000.00)	(219,000.00)	(201,000.00)
IVF SS Sexed Year 0 PV	NPV SB Owned Throughout		(219,000.00)	(208,603.20)	(189,000.00)	(213,000.00)	(201,000.00)

Appendix C - Supplemental Information for the Scenarios of Custom Recipients- Market Ultrasound Sex Determined Pregnancies

Table 140. Distribution of the number of 60 d ET pregnancies and open females at the conclusion of all ET rounds before naturally sired pregnancy determination generated by each scenario- marketing ultrasound sexed pregnancies.

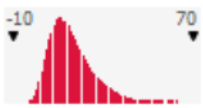
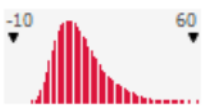
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Total Number of 60 d ET Bred Recipients	IVD Unsexed Embryo Production		0	38.59176	100	0	90
Number of 60 d ET Bred Recipients- Bull Calf	IVD Unsexed Embryo Production		0	19.29788	65	0	47
Number of 60 d ET Bred Recipients- Heifer Calf	IVD Unsexed Embryo Production		0	19.29388	62	0	47
Number of Open Recipients in Herd	IVD Unsexed Embryo Production		0	61.40824	100	10	100
Total Number of 60 d ET Bred Recipients	IVF NS Unsexed Production		0	33.90469	98	13	65
Number of 60 d ET Bred Recipients- Bull Calf	IVF NS Unsexed Production		0	16.95195	59	6	33
Number of 60 d ET Bred Recipients- Heifer Calf	IVF NS Unsexed Production		0	16.95274	61	6	33
Number of Open Recipients in Herd	IVF NS Unsexed Production		2	66.09531	100	35	87
Total Number of 60 d ET Bred Recipients	IVF SS Unsexed Production		0	44.6277	99	12	80

Table 140 (*cont.*). Distribution of the number of 60 d ET pregnancies and open females at the conclusion of all ET rounds before naturally sired pregnancy determination generated by each scenario- marketing ultrasound sexed pregnancies.

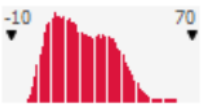
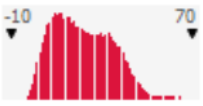
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of 60 d ET Bred Recipients-Bull Calf	IVF SS Unsexed Production		0	22.30886	61	6	41
Number of 60 ET Bred Recipients-Heifer Calf	IVF SS Unsexed Production		0	22.31884	63	6	42
Number of Open Recipients in Herd	IVF SS Unsexed Production		1	55.3723	100	20	88

Table 141. Distribution of the annual revenue generated by each scenario- marketing ultrasound sexed pregnancies.

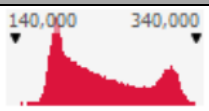
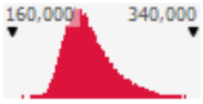
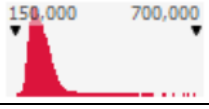
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD Revenue	Revenue		154,000.00	240,465.00	331,000.00	186,000.00	312,000.00
IVF NS Revenue	Revenue		178,000.00	237,628.60	327,500.00	209,500.00	279,000.00
IVF SS Revenue	Revenue		180,000.00	256,967.80	679,000.00	209,500.00	327,300.00

Table 142. Distribution of the annual expense generated by each scenario- marketing ultrasound sexed pregnancies.

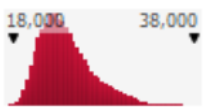
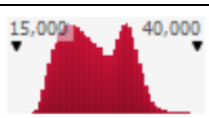
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD / Total Annual Expense	Total Expenses-Custom Recip		19,666.67	24,957.21	38,701.17	19,666.67	32,196.17
IVF NS / Total Annual Expense	Total Expenses-Custom Recip		18,333.40	24,248.81	36,337.50	20,770.20	29,270.80
IVF SS / Total Annual Expense	Total Expenses-Custom Recip		18,109.80	26,216.01	38,361.20	20,545.70	32,107.80

Table 143. Initial investment present value for MOET, IVP NS, and IVP SS embryo production methods- market ultrasound sexed pregnancies.

Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
MOET Year 0 PV	NPV Custom Bred Recip Sexed Pg		(144,000.00)	(135,239.80)	(120,000.00)	(144,000.00)	(126,000.00)
IVF NS Year 0 PV	NPV Custom Bred Recip Sexed Pg		(144,000.00)	(135,188.80)	(126,000.00)	(138,000.00)	(126,000.00)
IVF SS Year 0 PV	NPV Custom Bred Recip Sexed Pg		(144,000.00)	(132,625.40)	(120,000.00)	(138,000.00)	(126,000.00)

Appendix D - Biopsied Embryos

In response to an industry based question regarding the feasibility of subjecting embryos to biopsy to determine important genetic information prior to transfer and as a means of model validation, the following scenarios were simulated using the stochastic model described in this thesis. The biopsy simulation exercise also created an opportunity to demonstrate the flexibility and adaptability of the stochastic model in question.

Model Adaptations

Several adaptations are incorporated as additions to the model previously described. The adaptations are described in the following tables.

Table 144. Biopsy procedure cost structure

Number of Embryos	Biopsy Procedure Cost/Embryo ($)
1	100.00
9	100.00
10	95.00
19	95.00
20	90.00
29	90.00
30	85.00
39	85.00
40	80.00
49	80.00
50	75.00
Minimum Biopsy Procedure Cost ($)	400.00
Genetics Lab Fee/Sample ($)	52.00

In addition to adding the cost of biopsied embryos, a 10 percent reduction in pregnancy rate at full term is built into the model for recipients receiving a biopsied embryo. It is assumed

that the mating used to create potential carriers is a non-carrier mated to a carrier. Details specific to each of the four scenarios tested that may differ from the original model are described in the following pages.

Scenario D1:

- 60 available recipients
- All embryos are frozen before transfer
- 25 non-carrier embryos
- 50 potential carrier embryos
- Non-carrier embryos always put in before potential carrier embryos
- No biopsy of embryos
- True probability of carrier status: 50%
- 20% cull rate on non-carrier ET calves
- Non-carrier ET calves are marketed as seedstock
- Carrier calves, culls, and natural service sired calves are marketed as feeders

Figure 77. NPV of an ET program with potential carrier embryos and a fixed number of available recipients- no biopsy.

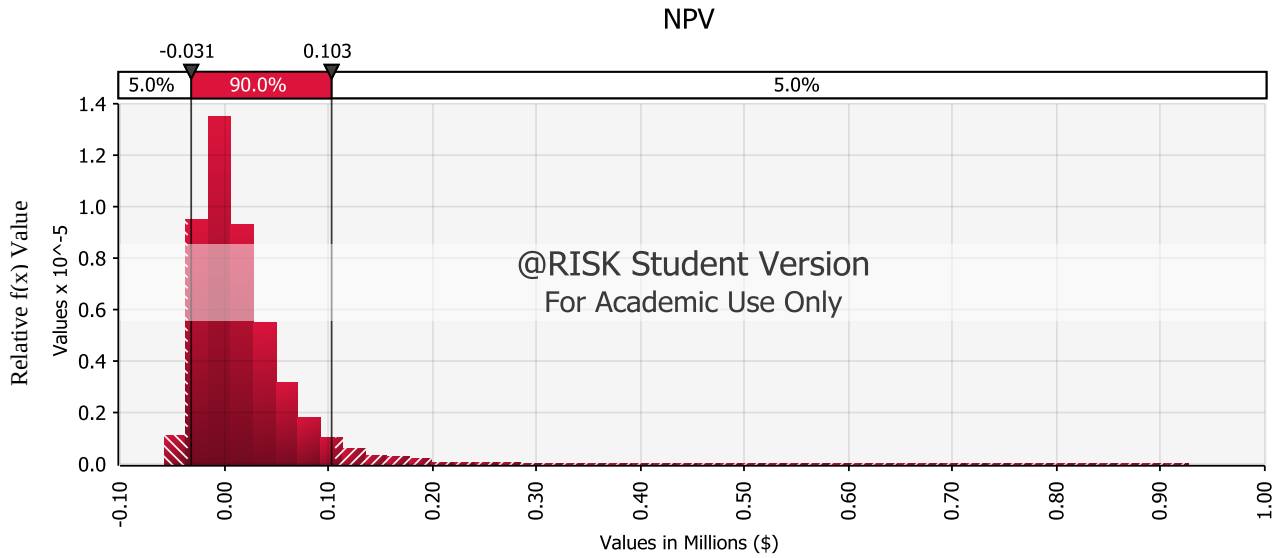


Figure 78. ANPV of an ET program with potential carrier embryos and a fixed number of available recipients- no biopsy.

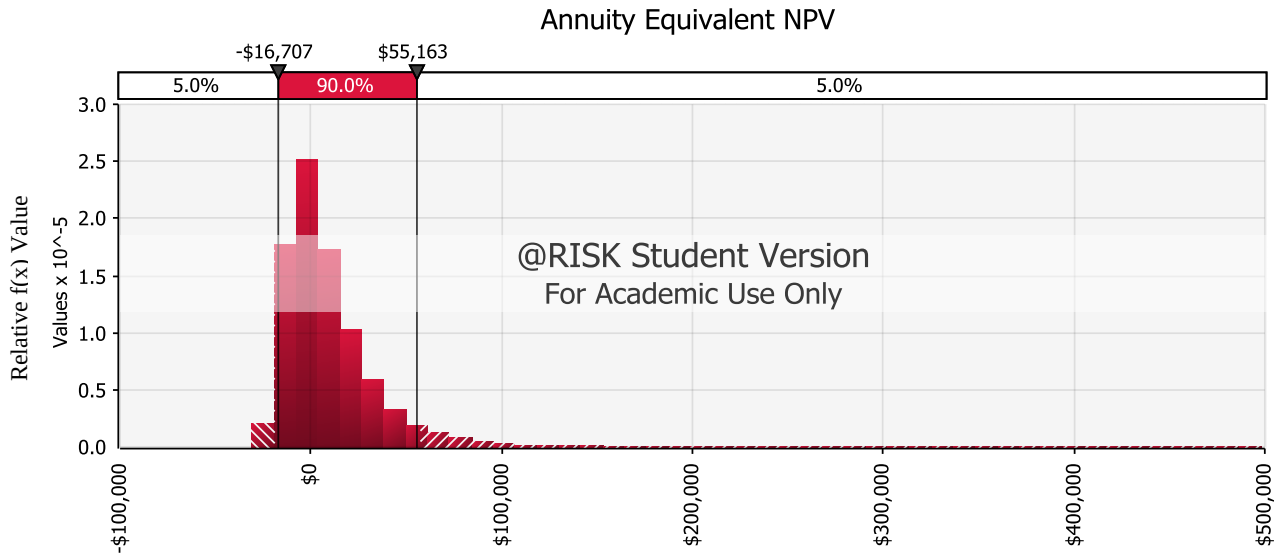
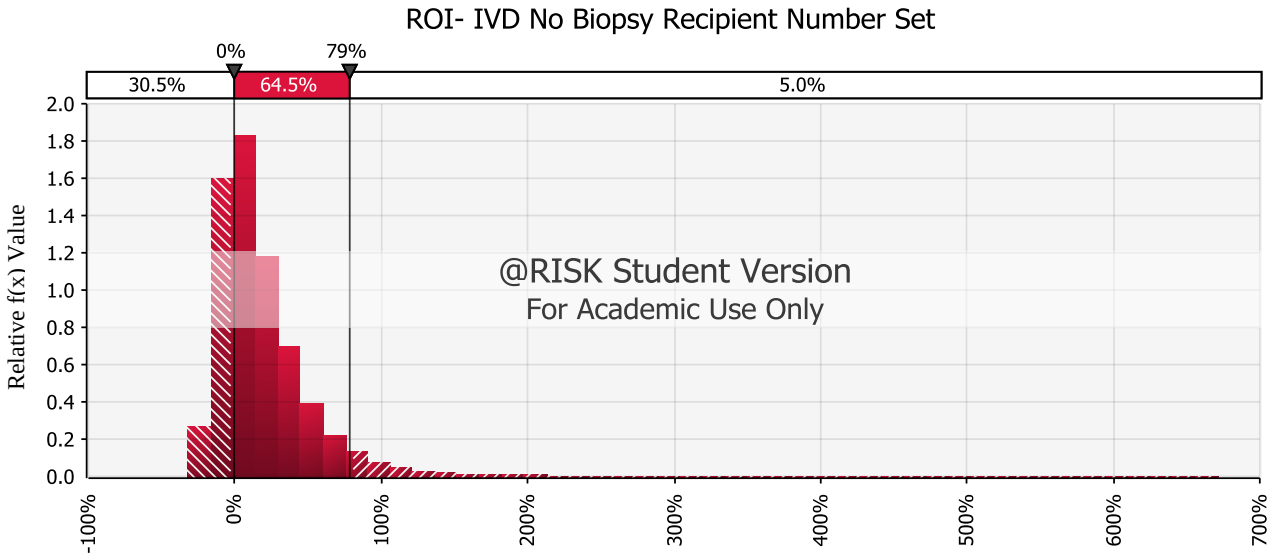


Figure 79. ROI of an ET program with potential carrier embryos and fixed number of available recipients- no biopsy.



Scenario D2:

- 60 available recipients
- All embryos are frozen before transfer
- 25 non-carrier embryos
- 50 Potential Carrier Embryos
- Biopsy of embryos
- True probability of carrier status: 50%
- Only non-carrier embryos transferred
- 20% cull rate for ET calves
- Non-carrier ET calves are marketed as seedstock
- Cull ET calves and natural service sired calves are marketed as feeders
- Carrier embryos are sold for $50

Figure 80. NPV of an ET program with potential carrier embryos and a fixed number of available recipients- biopsy.

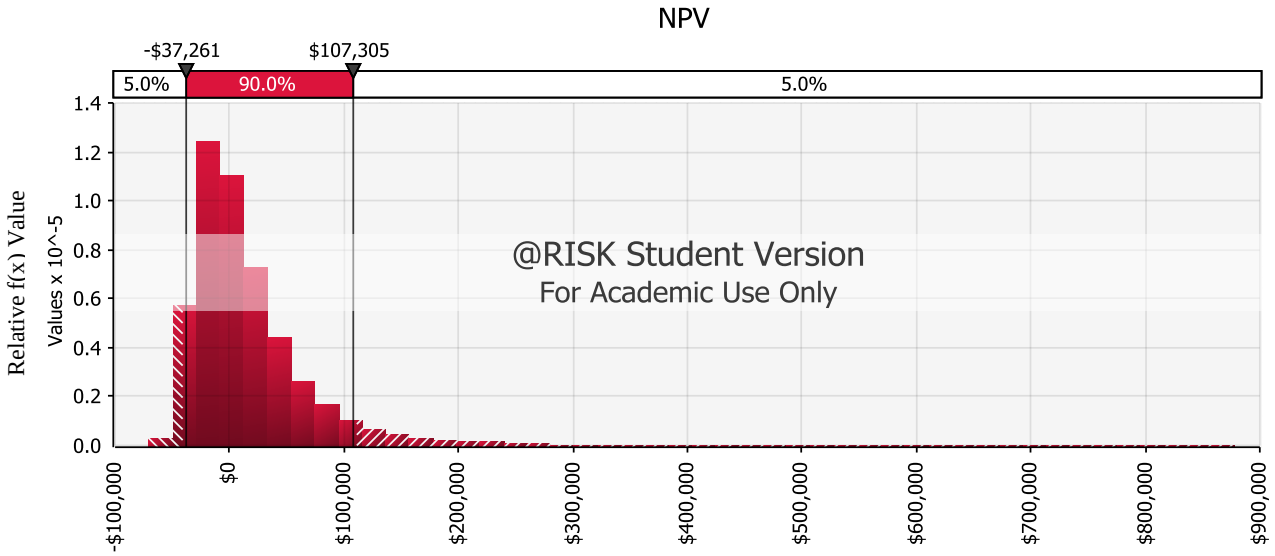


Figure 81. ANPV of an ET program with potential carrier embryos and a fixed number of available recipients- biopsy.

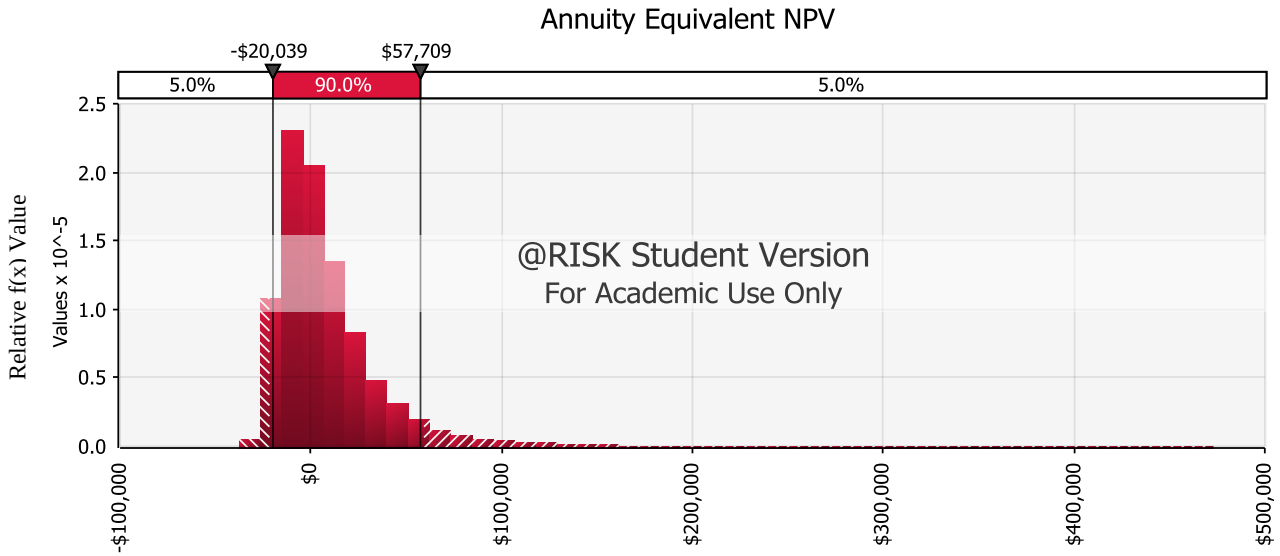


Figure 82. ROI of an ET program with potential carrier embryos and fixed number of available recipients- biopsy.

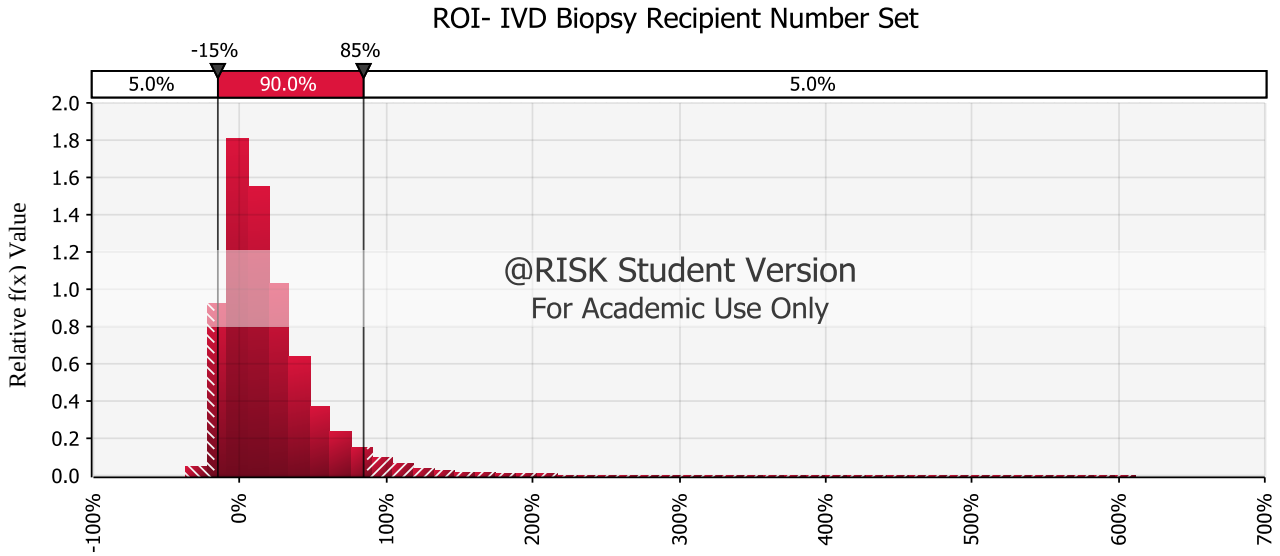


Table 145. Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI of an ET program with potential carrier embryos and a fixed number of available recipients.

NPV ($)	**NO BIOPSY**	**BIOPSY**
Mode	(10,943.31)	(17,014.21)
5%	(31,311.04)	(36,959.55)
25%	(13,444.42)	(18,131.98)
Median	4,918.95	1,633.80
75%	32,592.10	31,880.62
95%	101,516.67	107,421.82
Mean ± 90% C.I.	16,403.58 ± 243.78	14,410.47 ± 264.76
SD	46,862.28	50,875.52
ANPV ($)	**NO BIOPSY**	**BIOPSY**
Mode	(5,885.37)	(9,150.33)
5%	(16,707.33)	(19,877.03)
25%	(6,954.00)	(9,751.47)
Median	2,645.43	878.67
75%	17,528.19	17,145.56
95%	54,596.16	57,771.98
Mean ± 90% C.I.	8,821.93 ± 131.10	7,750.02 ± 142.39
SD	25,202.76	27,361.10

(*cont.*)

Table 145 (*cont.*). Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI of an ET program with potential carrier embryos and a fixed number of available recipients.

ROI (%)	NO BIOPSY	BIOPSY
Mode	0.03	-2.77
5%	-15.3	-14.9
25%	-2.7	-2.0
Median	10.3	11.8
75%	30.0	32.9
95%	78.7	85.7
Mean ± 90% C.I.	18.5 ± 0.173	20.7 ± 0.184
SD	33.2	35.4
Probability of Negative Return	30.4	28.9

Scenario D3:

- Number of recipients dependent on the number of embryos available to be transferred (1.5 times the number of embryos to be transferred)
- 50 potential carrier embryos
- No biopsy of embryos
- All embryos available for transfer
- True probability of carrier status: 50%
- 20% cull rate on non-carrier ET calves
- Non-carrier ET calves are marketed as seedstock
- Carrier calves, culls, and natural service sired calves are marketed as feeders

Figure 83. NPV of an ET Program with potential carrier embryos and a variable number of available recipients- no biopsy.

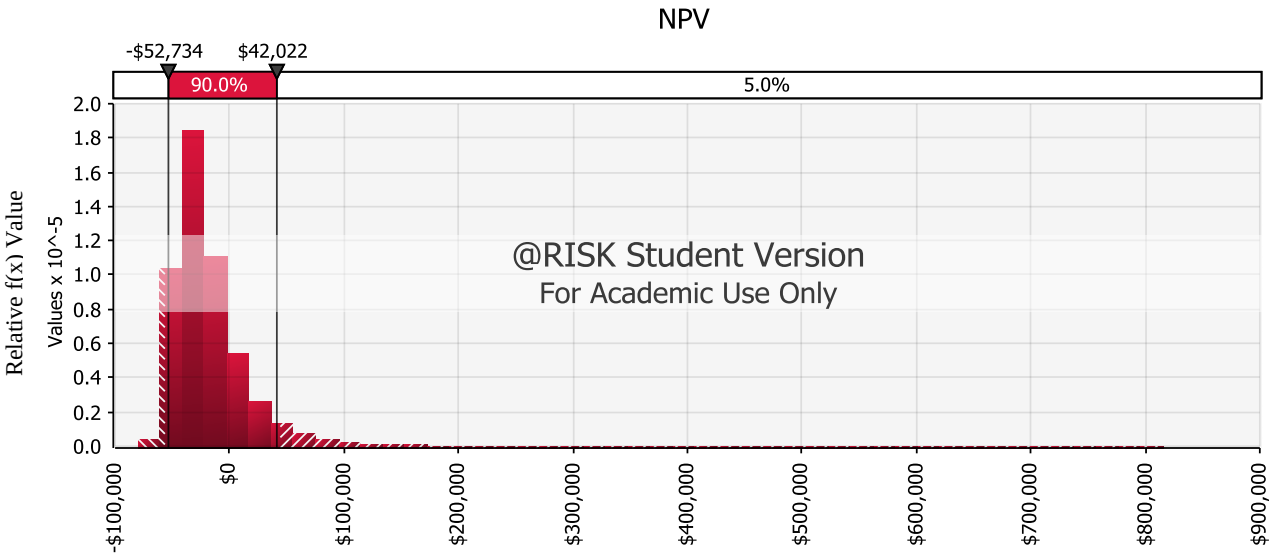


Figure 84. ANPV of an ET program with potential carrier embryos and a variable number of available recipients- no biopsy.

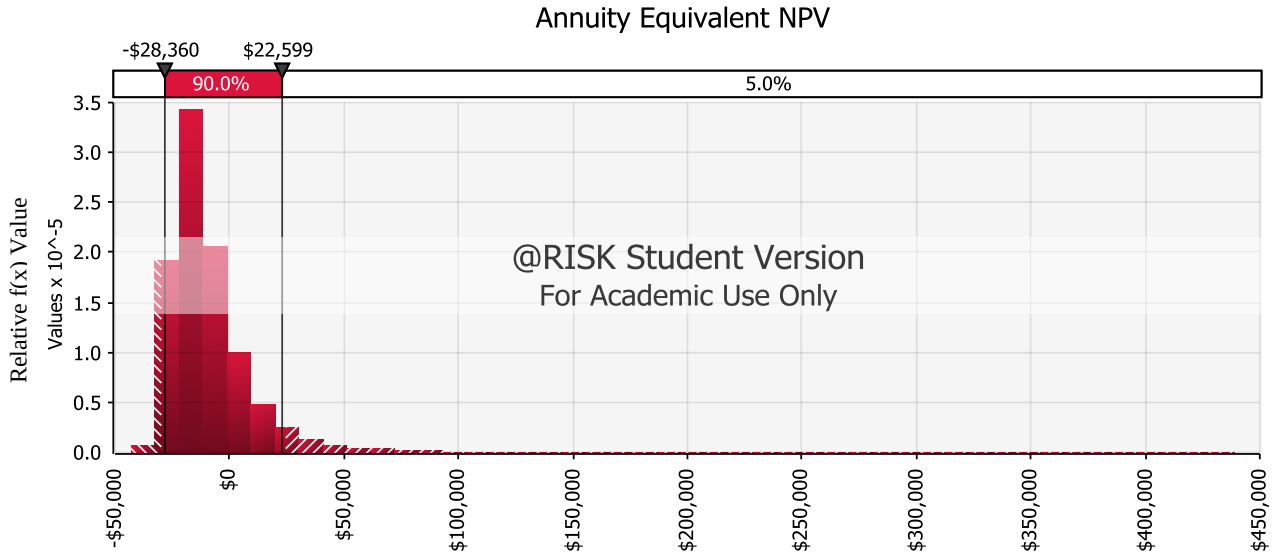
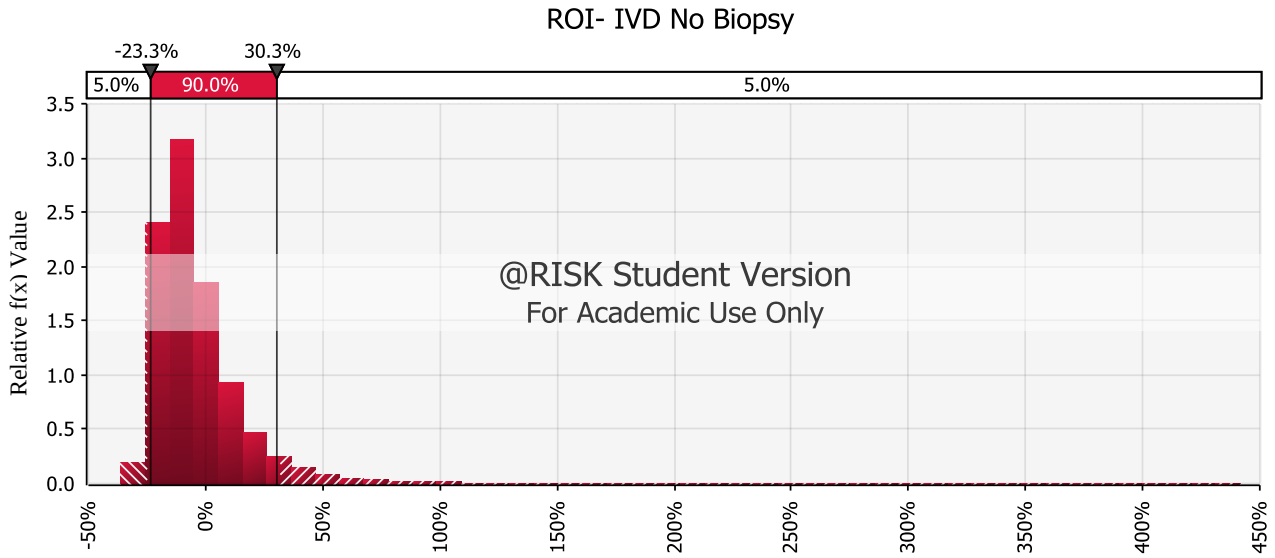


Figure 85. ROI of an ET program with potential carrier embryos and variable number of available recipients- no biopsy.



Scenario D4:

- Number of recipients dependent on the number of embryos available to be transferred (1.5 times the number of embryos to transfer)
- 50 Potential Carrier embryos
- Biopsy of embryos
- All non-carrier embryos after biopsy are available for transfer
- True probability of carrier status: 50%
- 20% cull rate on non-carrier ET calves
- Non-carrier ET calves are marketed as seedstock
- Culls and natural service sired calves are marketed as feeders
- Carrier embryos sold for $50

Figure 86. NPV of an ET program with potential carrier embryos and a variable number of available recipients- biopsy.

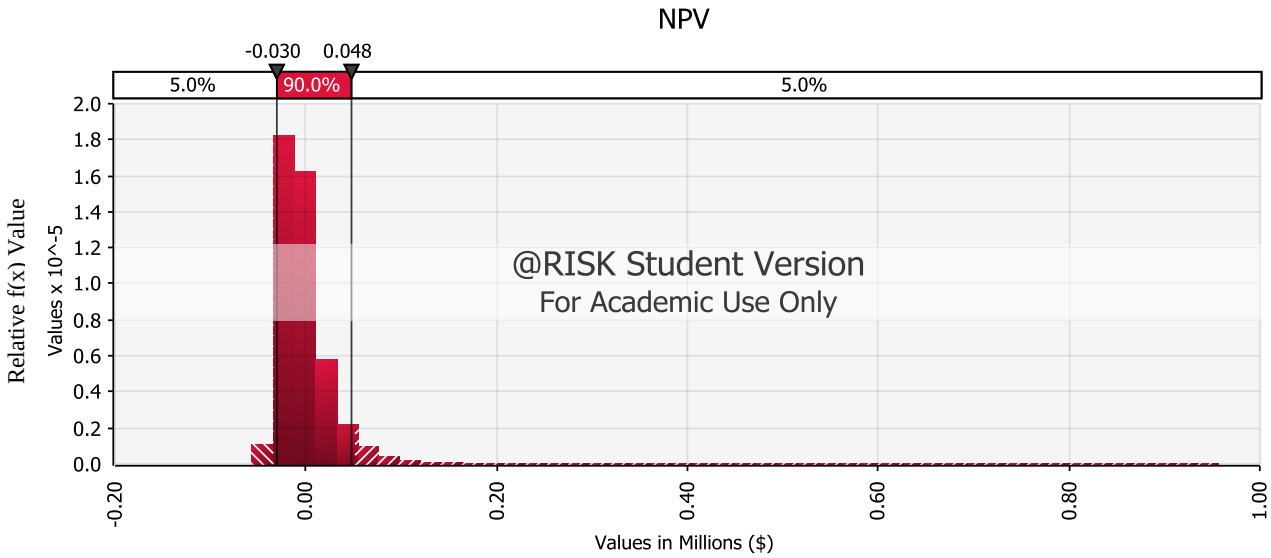


Figure 87. ANPV of an ET program with potential carrier embryos and a variable number of available recipients- biopsy.

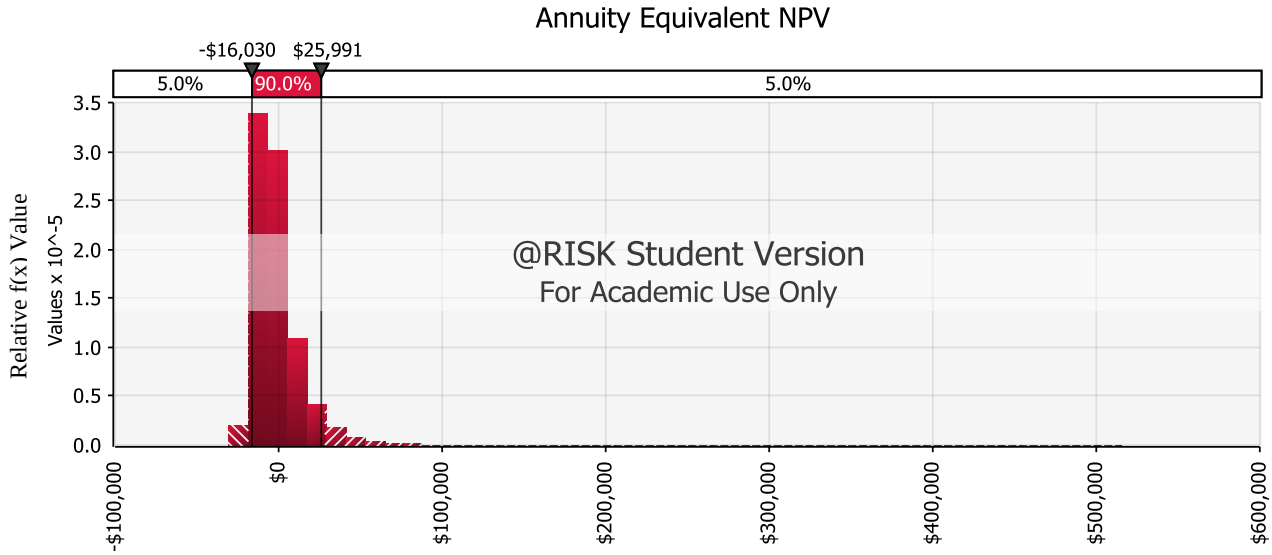


Figure 88. ROI of an ET program with potential carrier embryos and variable number of available recipients- biopsy.

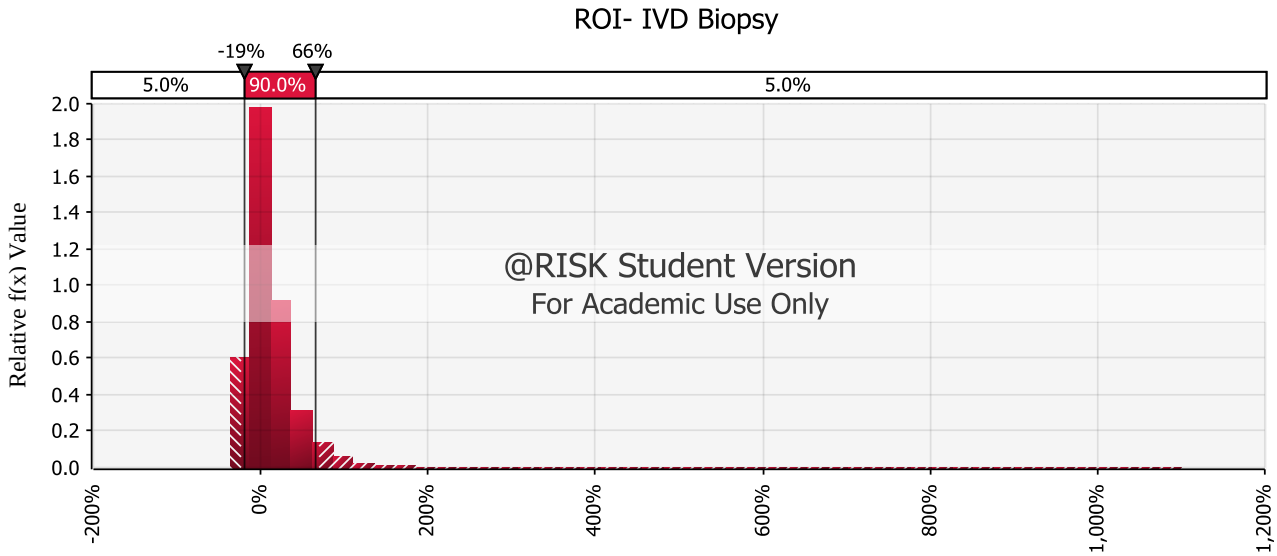


Table 146. Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI of an ET program with potential carrier embryos and a variable number of available recipients.

NPV ($)	**NO BIOPSY**	**BIOPSY**
Mode	(37,274.23)	(16,851.17)
5%	(52,949.87)	(29,309.52)
25%	(40,447.90)	(18,623.08)
Median	(27,616.05)	(8,552.54)
75%	(8,502.19)	6,950.01
95%	40,765.76	46,596.80
Mean ± 90% C.I.	(19,541.70) ± 172.46	(1,909.28) ± 142.72
SD	33,152.55	27,227.31
ANPV ($)	**NO BIOPSY**	**BIOPSY**
Mode	(20,046.26)	(9,062.64
5%	(28,476.70)	(15,762.80)
25%	(21,753.08)	(10,015.58)
Median	(14,852.05)	(4,599.60)
75%	(4,572.52)	3,737.75
95%	21,924.02	25,059.98
Mean ± 90% C.I.	(10,509.62) ± 92.75	(1,026.82) ± 76.75
SD	17,829.60	14,642.98

(*cont.*)

Table 146 (*cont.*). Mode, 5th percentile, 25th percentile, median, 75th percentile, 95th percentile, mean, and standard deviation of the NPV, ANPV, and ROI of an ET program with potential carrier embryos and a variable number of available recipients.

ROI (%)	NO BIOPSY	BIOPSY
Mode	-14.4	-5.3
5%	-23.0	-18.8
25%	-15.9	-7.7
Median	-8.6	3.6
75%	2.4	20.8
95%	30.4	64.3
Mean ± 90% C.I.	-4.0 ± 0.1	10.9 ± 0.155
SD	18.9	29.6
Probability of Negative Return	70.9	42.5

Scenario D1 and Scenario D2- Supporting Tables

Table 147. Distribution of the number of full term ET bred females, natural sire bred females, and open females associated with Scenario D1 and Scenario D2.

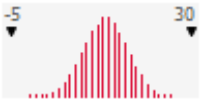
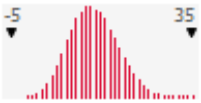
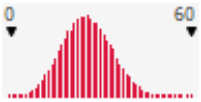
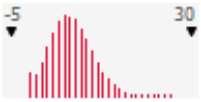
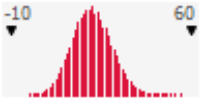
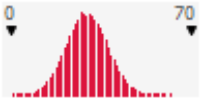
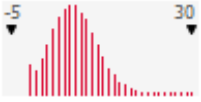
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
Number of ET Bred Recipients-Clean	No Biopsy IVD Embryo Production		0	13.37944	25	7	20
Number of ET Bred Recipients-PC	No Biopsy IVD Embryo Production		0	13.62407	34	6	22
Number of Bull Bred Recipients	No Biopsy IVD Embryo Production		2	25.77448	58	14	38
Number of Open Recipients	No Biopsy IVD Embryo Production		0	7.247525	25	1	14
Number of ET Bred Recipients	IVD Biopsied Embryo Production		0	22.00482	54	11	34
Number of Bull Bred Recipients	IVD Biopsied Embryo Production		4	30.21978	60	18	43
Number of Open Recipients	IVD Biopsied Embryo Production		0	7.80584	29	1	15
Number of Saleable IVDC Embryos	IVD Biopsied Embryo Production		8	24.99996	40	19	31

Table 148. Distribution of the number of ET calves, marketable et bull calves, and marketable ET heifer calves, generated by Scenario D1 and Scenario D2.

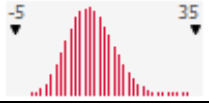
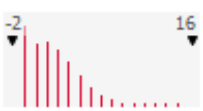
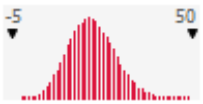
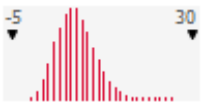
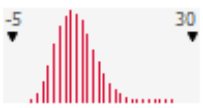
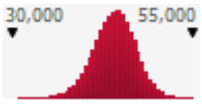
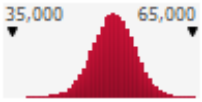
Name	Worksheet	Graph	Min	Mean	Max	5%	95%
ET Calves-Clean	No Biopsy Embr US Develop		0	12.01166	25	6	18
ET Calves-PC	No Biopsy Embr US Develop		0	12.23158	32	5	20
Marketable ET Bull Calves-Clean	No Biopsy Embr US Develop		0	4.8063	16	1	9
Marketable ET Heifer Calves-Clean	No Biopsy Embr US Develop		0	4.8065	15	1	9
Marketable ET Bull Calves-PC (Clean)	No Biopsy Embr US Develop		0	2.472689	12	0	6
Marketable ET Bull Calves- PC (Clean)	No Biopsy Embr US Develop		0	2.565951	14	0	7
ET Calves	Biopsied IVD Embryo US Develop		0	19.75538	47	9	31
Marketable ET Bull Calves	Biopsied IVD Embryo US Develop		0	7.90096	25	3	14
Marketable ET Heifer Calves	Biopsied IVD Embryo US Develop		0	7.89988	25	3	14

Table 149. Distribution of the total annual expense, total annual revenue, and annual cash flow associated with the production of developed ET calves via Scenario D1 and Scenario D2.

Name	Worksheet	Graph	Min	Mean	Max	5%	95%
IVD Unsexed Embryos-Frozen / Total Annual Expense	Total Expenses-Biopsied Embryo		31,977.82	44,297.99	54,544.65	39,825.81	48,512.29
IVD Unsexed Embryos-Biopsied-Frozen / Total Annual Expense	Total Expenses-Biopsied Embryo		38,558.50	52,042.03	64,706.40	46,780.76	57,196.59
IVD Unsexed Embryos-Frozen / Total Annual Revenue	Cash Flow-Biopsied Embryos		39,968.38	122,607.50	1,025,847.00	69,799.05	218,987.30
IVD Unsexed Embryos-Biopsied-Frozen / Total Annual Revenue	Cash Flow-Biopsied Embryos		43,678.26	128,278.20	1,000,104.00	71,422.89	232,036.50
IVD Unsexed Embryos-Frozen / Annual Cash Flow	Cash Flow-Biopsied Embryos		640.59	78,308.80	978,955.40	26,704.48	173,428.10
IVD Unsexed Embryos-Biopsied-Frozen / Annual Cash Flow	Cash Flow-Biopsied Embryos		(3,062.64)	76,231.64	944,212.40	21,503.89	178,069.70

Scenario D3 and Scenario D4- Supplemental Tables

Table 150. Distribution of the number of full term ET bred females, natural sire bred females, and open females associated with Scenario D3 and Scenario D4.

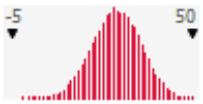
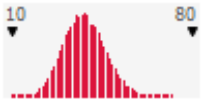
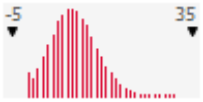
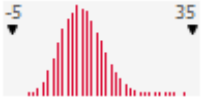
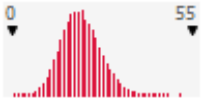
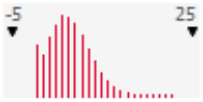
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
Number of ET Bred Recipients	No Biopsy Embryo Production		0	26.76442	48	15	38
Number of Bull Bred Recipients	No Biopsy Embryo Production		13	38.91462	70	27	52
Number of Open Recipients	No Biopsy Embryo Production		0	9.34408	30	2	17
Number of ET Bred Recipients	IVD Biopsied Embryo Production		0	11.00178	32	5	18
Number of Bull Bred Recipients	IVD Biopsied Embryo Production		3	21.732	50	13	31
Number of Open Recipients	IVD Biopsied Embryo Production		0	5.08658	21	0	11

Table 151. Distribution of the number of ET calves, marketable ET bull calves, and marketable ET heifer calves generated by Scenario D3 and Scenario D4.

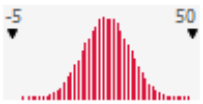
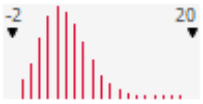
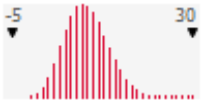
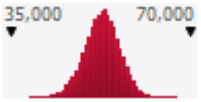
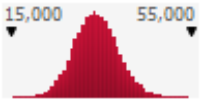
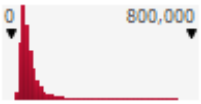
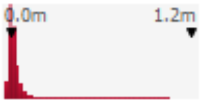
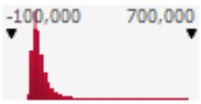
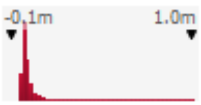
Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
ET Calves	No Biopsy Embr US Develop		0	24.02204	47	13	35
Marketable ET Bull Calves	No Biopsy Embr US Develop		0	4.81574	18	1	9
Marketable ET Heifer Calves	No Biopsy Embr US Develop		0	4.81884	18	1	9
ET Calves	Biopsied IVD Embryo US Develop		0	9.8756	29	4	17
Marketable ET Bull Calves	Biopsied IVD Embryo US Develop		0	3.94888	18	1	8
Marketable ET Heifer Calves	Biopsied IVD Embryo US Develop		0	3.9523	19	1	8

Table 152. Distribution of the total annual expense, total annual revenue, and annual cash flow associated with the production of developed ET calves via Scenario D3 and Scenario D4.

Name	Worksheet	Graph	Min ($)	Mean ($)	Max ($)	5% ($)	95% ($)
IVD Unsexed Embryos-Frozen / Total Annual Expense	Total Expenses-Biopsied Embryo		39,909.92	53,079.17	66,642.56	47,838.77	58,084.00
IVD Unsexed Embryos-Biopsied-Frozen / Total Annual Expense	Total Expenses-Biopsied Embryo		17,104.94	34,272.01	53,144.80	27,119.58	41,686.20
IVD Unsexed Embryos-Frozen / Total Annual Revenue	Cash Flow-Biopsied Embryos		51,242.01	108,023.50	720,438.90	70,522.75	175,400.70
IVD Unsexed Embryos-Biopsied-Frozen / Total Annual Revenue	Cash Flow-Biopsied Embryos		17,645.59	71,613.38	1,032,128.00	38,186.09	129,788.70
IVD Unsexed Embryos-Frozen / Annual Cash Flow	Cash Flow-Biopsied Embryos		(1,686.49)	54,943.56	665,326.60	18,680.47	121,215.90
IVD Unsexed Embryos-Biopsied-Frozen / Annual Cash Flow	Cash Flow-Biopsied Embryos		(8,563.00)	37,287.13	987,443.40	7,175.79	93,052.59