From oocyte to calf: practical aspects of bovine in vitro embryo production

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Abstract

Commercial use of sex-sorted semen and genomic evaluations have revolutionized cattle breeding, and demand for sex-sorted embryos produced from very young heifers has increased over time. Worldwide, in vitro produced embryos accounted for 76.2% of all transferrable cattle embryos in 2020, probably because in vitro embryo production is a more efficient way to supply this demand. Several factors need to be coordinated to obtain a live calf from an in vitro produced embryo. Aspects related to oocyte quality, laboratory quality control, embryo quality, recipient selection, and pregnancy loss are addressed here, based on the measures that the RuAnn Genetics Laboratory (Riverdale, San Joaquin Valley, CA) adopted in the last 14 years, with the goal of improving production of live, healthy calves from in vitro produced embryos. Follicular wave synchronization and stimulation with follicular stimulating hormone is necessary to improve oocyte quality and consequently embryo production. Laboratory quality control and the use of high-quality supplies are essential to reduce variability in production and facilitate identification of other factors that might interfere with embryo production. High pregnancy rates, similar to in vivo produced embryos, can be achieved with good quality embryos selected at optimal time and stage of development, transferred by an experienced embryo transfer technician, to well managed recipients 7 or 8 days after estrus. Attention to detail at every step of the process is crucial to success.

Keywords: Cattle, embryo, in vitro production, pregnancy, recipient

Introduction

Embryo production has proven to be a powerful technology for genetic improvement of dairy animals, primarily to propagate the genes of females with superior genetic values and lineage. Despite the Covid Pandemic, 2020 was a good year for the embryo transfer industry worldwide.[1] Number of embryos recorded increased in most countries and for all of the most representative species (cattle, horses, sheep, and goats). In cattle, overall embryo production (in vivo and in vitro) increased 7.0% compared to 2019, with more than 1.5 x 10^6 embryos recorded. Worldwide, in vitro produced (IVP) embryos accounted for 76.2% of all transferrable cattle embryos in 2020.[2] In US, since 2016, the number of IVP embryos transferred is higher than in vivo derived (IVD) embryos for dairy probably due to increased commercial use of sex-sorted semen, use of genomic evaluations, and improved success of commercial IVP (Figure 1).[3] IVP is more efficient than IVD to supply the demand for sex-sorted embryos produced from very young heifers with high genetic merit. A similar trend has started to be observed in beef breeds in 2020 (Figure 2), probably also due to increase of beef in cattle programs.

Several factors need to be coordinated to obtain a live calf from an IVP embryo. Donor must provide a good quality oocyte that can be matured and be successfully fertilized. Embryo needs to develop in vitro until day 7 postfertilization and be carefully selected, loaded, and transferred by a qualified practitioner. Semen, laboratory equipment and supplies, IVP media, quality control protocols, and laboratory technicians will determine the success of the process. Care taken before and after the embryo is produced will determine pregnancy outcome.[4-6] Goal is to have a pregnant recipient that can successfully carry a healthy calf to term. Over the past decade, IVP commercial success has substantially improved, as higher blastocyst rates, better cryotolerance, higher pregnancy rates, lower pregnancy loss, and decreased incidence of large offspring syndrome have been reported. Nevertheless, embryos generated in vitro still differ from their in vivo produced counterparts.[5] Approximately 80 - 90% of immature bovine oocytes undergo nuclear maturation in vitro, ~ 80% undergo fertilization, 30 - 40% develop to blastocyst stage, and ~ 50% of transferred embryos establish a pregnancy.[7]

This manuscript describes the measures that the RuAnn Genetics Laboratory (Riverdale) adopted in the last 14 years to improve success of IVP systems in dairy breeds. RuAnn Genetics produces ~ 10,000 Holstein, Jersey, and Angus embryos per year (90% IVP) for in-house use, to other local farmers and exported worldwide. Ovum pick-up (OPU), in vitro maturation (IVM), fertilization (IVF), culture (IVC), embryo transfer (ET) and cryopreservation for direct transfer are performed in-house.

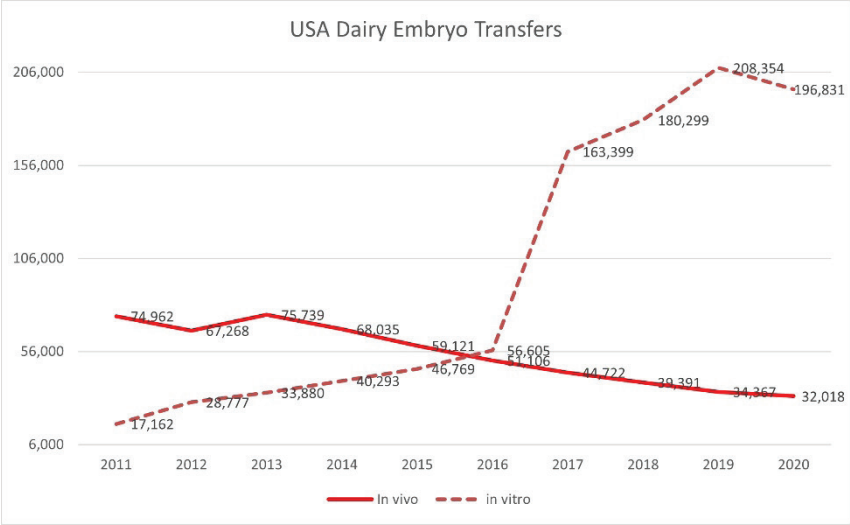

Figure 1. Number in vivo and in vitro dairy embryo transfers in last 10 years in US

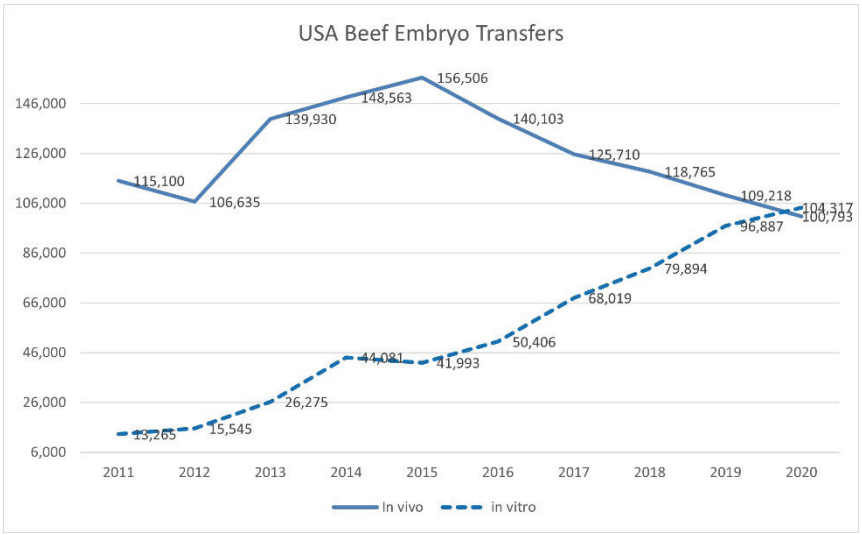

Figure 2. Number of in vivo and in vitro beef embryo transfers in last 10 years in US

Our data are collected and analyzed frequently to monitor performance with the intent of improving results. Since we established the in-house IVP laboratory (2008), our main goal was to make in vivo-like IVP embryos (Figure 3), with similar pregnancy outcomes. Protocols described herein were developed to improve embryo production and pregnancy results focusing on oocyte quality, laboratory quality control, embryo quality, and recipient selection.

Donor preparation

According to data collected by the American Embryo Transfer Association Statistical Committee in 2020,[3] in US, dairy cows produced an average of 17.1 oocytes and 3.8 viable embryos per ovum pick up (OPU) session whereas beef cows had 24.9 oocytes and 6.6 embryos. Number of viable embryos was higher for donors stimulated with FSH: 4.7 embryos for dairy and 7.7 for beef. Based on our previous studies,[6,8,9] synchronization of follicular wave emergence and stimulation of FSH has a positive impact on oocyte quality and embryo production.

We start OPU in genetically superior genomic tested heifers at 8 months of age, and some will be aspirated every 2 weeks until 100 days of pregnancy. Lactating and dry cows are also used as oocyte donors. Currently, donors are synchronized with gonadorelin (GnRH, Fertagyl®, Merck Animal Health, Madison, NJ) and stimulated with FSH (Folltropin®, Vetoquinol, Fort Worth, TX) prior to OPU and are not selected by quantity of oocytes, but by the goal of multiplying desired genetics. Gonadorelin (GnRH 129 µg) is given intramuscularly to synchronize the follicular recruitment and a single intramuscular injection of FSH is given 36 hours after GnRH treatment. One vial of Folltropin (700 UI) is diluted in 10 ml of saline and 3.5 ml (245 UI) is given to lactating Holstein cows and mature Angus donors, and 2.5 ml (175 UI) to Holstein heifers and lactating Jersey donors. Oocyte Pick-Up is performed 48 - 52 hours after FSH injection (Figure 4). Donor FSH dosages

and coasting times (time from FSH injection to OPU) will be adjusted based on previous results. Our goal is to have the majority of the follicles between 6 - 8 mm for OPU (Figure 5).

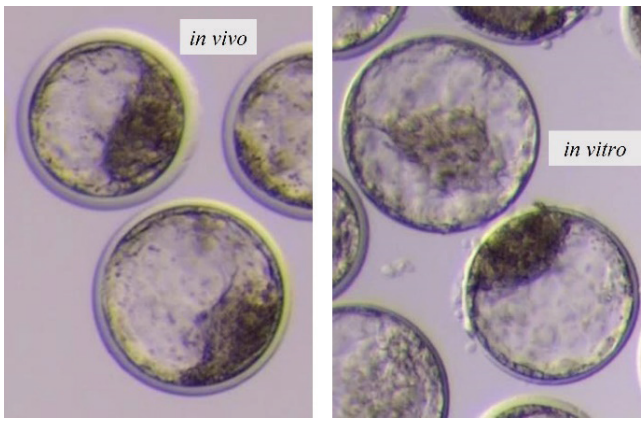

Figure 3. In vivo and in vitro Grade 1 expanded blastocysts

WEEK	TIME	DONOR PROTOCOL - 1 FSH injection						
		Sun	Mon	Tues	Wed	Thu	Fri	Sat
1	9:00 AM							FSH
	4:00 PM					GnRH		
2	9:00 AM		OPU					
	4:00 PM							

Figure 4. Example of a protocol to synchronize and stimulate follicular emergence

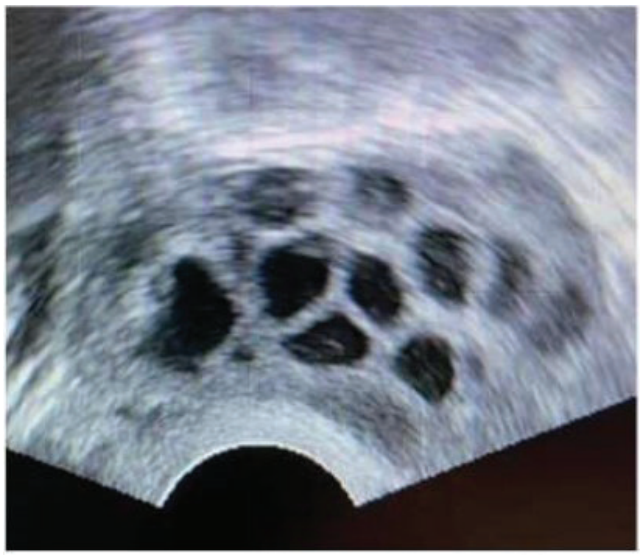

Figure 5. Ultrasonogram of a bovine ovary with 6 - 8 mm follicles

Ovum pick-up

At RuAnn, donors are brought to the laboratory, properly restrained, and receive an epidural with 2% lidocaine prior to OPU. Cumulus oocyte complexes (COCs) are retrieved using a medical ultrasound equipment with a 6.5 MHz micro-convex transvaginal transductor (Mindray DP50, Shenzhen, China) adapted for bovine ovary aspiration. A 2 mm Teflon tubing system is attached to an aluminum stopper on top of a 50 ml tube on 1 side and a needle on the other. A negative pressure produced by a vacuum pump (COOK®, Brisbane, Australia) is applied to the stopper at 20 - 30 ml/minute flow rate. All visible follicles (> 3 mm) are aspirated using a 20 gauge disposable hypodermic needle. Modified Dulbecco's phosphate buffered saline (DPBS, ABT Complete Flush, ABT 360®, Pullman, WA) with 10 IU/ml heparin is used to flush the system to avoid coagulation. The medium and tube are kept at 37°C. This process is timed and should not take more than 15 minutes. Oocyte Pick-Up technical skills have an important part in oocyte quality. We have noticed that inexperienced practitioners tend to retrieve lower quality COCs.

After aspiration, the tube containing the follicular fluid is rinsed and placed into a cell strainer (100 µm) to be filtered and washed with modified DPBS and placed into a grided 60 mm dish to be searched under a stereomicroscope. Retrieved COCs are placed in a 6-well dish with 1,000 µl of wash medium (TCM 199 medium supplemented with HEPES) and washed at least 3 times. This process is timed and should not take more than 15 minutes.

Recovered COCs are classified as Grade 1 - 4 (viable) or degenerated based on the International Embryo Technology Society (IETS) oocyte classification and takes into consideration the number and presentation of cumulus cell layers and the ooplasm homogeneity (Figure 6).[10] The degenerated oocytes are not included with the viable oocytes for IVM but are accounted for to determine the oocyte recovery rate.

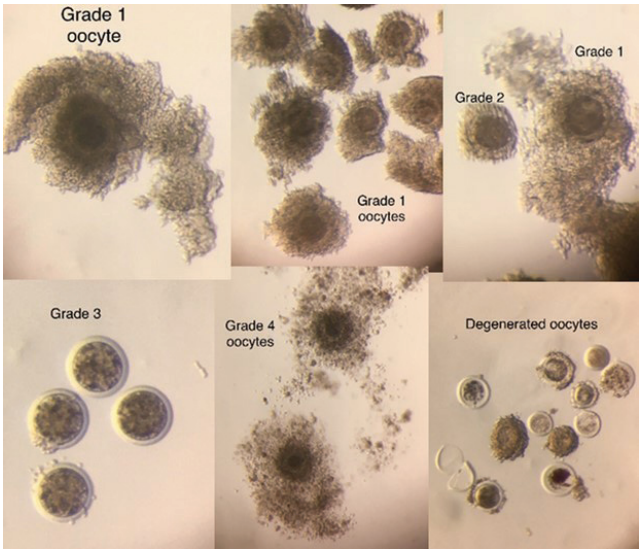

Figure 6. IETS cumulus oocytes complexes classification

In vitro maturation, fertilization, and culture

After OPU, the COCs undergo IVM for 18 - 24 hours to become competent for fertilization. The IVM is conducted in tubes inside a portable incubator (38.5°C) or in 4-well dishes with supplemented TCM199, inside an incubator (38.7°C, 5% CO_2 in a humidified atmosphere) with a maximum of 50 COCs per tube or drop. Matured COCs are removed from the IVM tube or dish, washed twice in IVF medium (supplemented TALP), and moved to the fertilization dish (a drop of

50 µl medium overlaid with 1,000 µl oil at 38.7°C, 5% CO_2 in a humidified atmosphere, in 4-well dishes). A straw of semen is thawed, and its contents are placed on top of 2 layers (40 and 80%) of silica-based colloidal medium (Puresperm®, Nidacon International, Healdsburg, CA) and centrifuged to separate live sperm from the cryoprotectant, seminal plasma, and dead sperm. Supernatant is removed, and remaining sperm are washed with IVF media and centrifuged once again. Motility and concentration on the final pellet are evaluated by the iSperm® (Aidmics Biotechnology Co., LTB, Taipei, Taiwan) analyzer to determine the final insemination volume. Sex-sorted (female) semen is used in 90% of our procedures. Oocytes are usually inseminated with 2 - 15 µl sperm solution to achieve a final concentration of 2 x 10^6 sperm/ml; they undergo IVF for 7 - 12 hours. After fertilization, the cumulus cells surrounding the zygotes are removed by pipetting (also known as stripping). Zygotes are washed and placed into droplets of culture medium 970 µl drop of supplemented synthetic oviduct fluid [SOF] medium overlaid with 1,000 µl oil at 38.7°C, 6% CO_2 and 5% O_2 in a humidified atmosphere). Some Grade 3 and 4 oocytes placed in maturation initially are shrunken or degenerated and are removed from culture. There is usually a 5 - 10% reduction in oocyte numbers from IVM to IVC. On day 3 of culture, morulae with 8 - 16 cells should be present and the cleavage rate is calculated (total cleaved divided by total oocytes in IVC). Unfertilized oocytes (UFO) and zygotes with < 4 cells are removed from the drop. Medium from the drop is removed (65 µl) and replaced by a new IVC-day 3 medium containing different nutrients necessary for this phase. On day 5 of culture, compact morulae and early blastocysts should be present and an estimate of the final embryo production is made. Medium from the drop is removed once more (65 µl) and replaced with new medium (IVC-day 5) containing different nutrients necessary for this phase. On day 6 of culture, compact morulae and blastocysts should be present. Day 6 blastocysts are removed from culture for transfer depending on recipient availability. On day 7 of culture, remaining embryos are transferred fresh to recipients or cryopreserved for direct transfer (15% of the viable embryos). A good quality day 7 IVF expanded, or hatched blastocyst should have a defined inner cell mass and clear trophoblast cells (Figures 3 and 7). Day 6 and 7 embryos are manipulated at 36.5°C in holding media (HEPES-SOF) and it should not take > 10 minutes, to avoid medium pH changes that can damage the embryo. Embryos that do not reach the early blastocyst stage on day 7 are discarded. Embryos are placed in a portable straw incubator at 35 - 36.5°C. Interval from loading the first embryo to ET of the last embryo do not exceed 7 hours. Embryo development rates (EDR) are calculated by the number of embryos produced divided by the number of oocytes in IVC (EDR-T). We also divide the number of embryos by the sum of Grade 1 and 2 oocytes recovered (EDR-1,2), and use it as a quality control check, expecting an embryo production rate higher than 60%. Only embryos that are transferred fresh or cryopreserved are included in this calculation.

Laboratory quality control and assurance

Improvements in the success of IVP can be attributed to the continued efforts of laboratories around the world to optimize media formulations. However, what is evident is that media are but 1 aspect of the embryo culture system.[11] To support the development of a viable and healthy embryo capable of resulting in a healthy offspring, one needs to look There is an absolute necessity for an effective quality management and assurance system to ensure that variables such as equipment, laboratory air quality, oil overlay, and lot numbers of consumables do not negatively impact embryo health.[12] far beyond the formulation of the culture media employed.

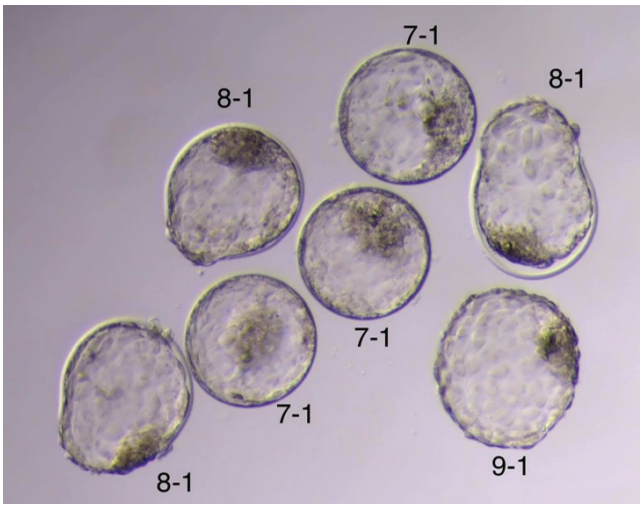

Figure 7. Grade 1 day 7 IVP Embryos - stage 7 (expanded blastocyst), 8 (hatching blastocyst) and 9 (hatched blastocyst)

With the intention to avoid chemical and physical stress factors that might affect the oocyte and embryo, we adopted several measures based on information interchanged with other IVP bovine and human practitioners, research papers, and most of all, trial and error.[6] Establishment of a standard operating procedure is an important part of a successful quality system, as it allows maintaining consistency and decreasing the chances of error during the execution of procedures. We keep written records of all changes that have been made in the laboratory (e.g., dates of changes of batches of media and oil, cleaning and gas exchange of the incubators and any other minor or major changes in the procedure) that could affect embryo production. Incubators in the IVF laboratory have a pivotal role in providing a stable and appropriate culture environment required for optimizing embryo development outcome. In vitro culture should be performed under low oxygen (5%) atmosphere to avoid oxidative stress and preferably in benchtop incubators with rapid recovery times for atmosphere and temperature that can improve our embryo quality. Perfect oil for IVP should do nothing but protect the culture medium from evaporation. It should not have any negative influence on the gametes, embryos, or medium by adding unwanted or removing essential components. We have had several issues with different batches of oil in the laboratory, and in 2016 we decided to purchase oil used in human IVP (Ovoil™, Vitrolife, Englewood, CO) and the variability in our embryo production results decreased dramatically. An important factor that can have a detrimental impact on the IVF treatment outcome, but is sometimes overlooked, is the quality of the plastics used. It is also worth considering that effects of suboptimal utensils are cumulative; the negative impact will increase if more than 1 toxic item is used. We purchase IVP certified utensils (e.g., dishes, tips, and ET straws) wherever possible, that have lower risk of toxic molecules from the plastic entering the medium or oil. High quality supplies and precise equipment have be-

come essential throughout our IVP process, enabling the necessary laboratory stability that allows us to be more accurate when measuring other critical aspects of production such as individual donor performance, mating interactions and others. Overall production costs increase; however, it is usually compensated by higher yield of blastocysts and pregnancies.

Oocyte quality and embryo production

Culture conditions throughout IVP may influence developmental potential of the early embryo; however, the quality of the oocyte at the start of the process is the key factor determining the proportion of oocytes developing to the blastocyst stage.[6,10,13] Grades 1 and 2 oocytes are expected to have a higher EDR than 3 and 4. We conducted a small study (unpublished) to evaluate the relationship between oocyte quality and EDR. Angus donors (n = 16), synchronized and stimulated with FSH were aspirated and the oocytes were pooled and grouped into 4 Grades (1, 2, 3, and 4) according to the IETS oocyte classification.[10] Results (Table 1) confirmed that Grades 1 and 2 had higher EDRs (50.4%) than 3 and 4 (23.0%).

Table 1. Angus embryo production according to oocyte grade

Oocyte Grade	Number of oocytes	Cleavage rate (%)	Number of blastocysts	EDR (%)
1	52	87	28	53.8
2	59	78	28	47.5
3	57	72	12	21.1
4	43	47	11	25.6
Total	**211**	**72**	**79**	**37.4**

Data from 3,233 Holstein OPUs performed in our laboratory from January 2017 to March 2020 are summarized (Table 2). The donor cows were grouped into 1 of 4 groups: heifers younger than 10 months, heifers older than 10 months, lactating cows, and dry cows. We also placed them in 3 groups based on oocyte production: < 10 oocytes (low), 11 - 20 oocytes (medium) and > 20 oocytes (high).

Table 2. Oocyte recovery and embryo production from Holstein OPUs performed at RuAnn Holstein donors from January 2017 to March 2020

Donor status/no. oocytes	OOPUs	Total oocytes per OPU	IVC oocytes per OPU	High quality oocytes (%)	Cleavage rate (%)	Embryos per OPU	EDR-T (%)	EDR-G1,2 (%)
> 20 oocytes per OPU	**517**	**34.1**	**29.3**	**35**	**78**	**7.7**	**26**	**64**
< 10 mo heifers	65	34.3	28.0	33	69	4.8	17	42
> 10 mo heifers	77	29.4	26.2	41	76	7.0	27	57
Lactating cows	117	32.1	27.5	30	77	6.7	24	70
Dry cows	258	36.4	31.3	37	81	9.1	29	68
> 10 - 20 oocytes per OPU	**1192**	**17.0**	**14.5**	**35**	**78**	**4.1**	**28**	**69**
< 10 mo heifers	157	16.5	14.3	34	74	3.2	22	56
> 10 mo heifers	385	16.2	14.2	39	81	4.6	32	71
Lactating cows	257	17.3	14.7	27	77	3.5	24	77
Dry cows	393	17.9	14.9	37	79	4.5	30	68
< 10 oocytes per OPU	**1524**	**8.2**	**6.3**	**31**	**78**	**1.7**	**27**	**67**
< 10 mo heifers	177	8.2	6.5	30	76	1.5	23	59
> 10 mo heifers	710	7.9	6.2	34	80	1.9	30	71
Lactating cows	371	8.0	6.3	23	74	1.5	23	77
Dry cows	266	9.0	6.5	35	79	1.7	26	55
TOTAL	**3233**	**15.6**	**13.0**	**34**	**78**	**3.6**	**27**	**67**
< 10 mo heifers	399	15.7	13.1	33	73	2.7	21	52
> 10 mo heifers	1172	12.0	10.2	37	80	3.1	30	69
Lactating cows	745	15.0	12.5	27	76	3.0	24	74
Dry cows	917	20.5	17.1	36	80	5.0	29	66

Data from 261 Jersey OPUs from December 2019 to April 2021 are summarized (Table 3). The donor cows were grouped into 1 of 4 groups, namely heifers or lactating cows and oocyte production (> or < 15 oocytes).

According to the results from Tables 2 and 3, higher embryo production was achieved by donors that start with more oocytes.[6,14] Dry donors produced more embryos than heifers and lactating cows, probably because they do not have any metabolic challenges, whereas lactating cows are producing milk and the heifers are still growing. Older heifers (> 10 months), have higher EDRs than younger heifers. On our previous studies, we included donor dam and sire in our statistical models, and they are always the main causes for variability.

As mentioned before, we do not select our donor cows by the number of oocytes, but if we did, we would increase the average of embryos per cow and reduce the cost of embryo production. As long as we do not mind foregoing producing offspring from highest genetic merit cows, of course! Another way to improve embryo production results would be increasing numbers of Grades 1 and 2 oocytes recovered per donor.

Table 3. Oocyte recovery and embryo production from Jersey OPUs performed from December 2019 to April 2021

Donor status / no. oocytes	OPUs	Total oocytes per OPU	High quality oocytes (%)	IVC Oocytes per OPU	Cleavage rate (%)	Embryos per OPU	EDR-T (%)	EDR-G1,2 (%)
More than 15 oocytes	**121**	**22.8**	**46**	**19.4**	**73**	**6.0**	**30.9**	**57.1**
Heifer	5	18.6	42	15.2	79	5.4	35.5	69.2
Lactating cow	116	23.0	46	19.6	73	6.0	30.8	56.7
Less than 15 oocytes	**140**	**11.1**	**48**	**9.0**	**76**	**2.8**	**31.3**	**53.2**
Heifer	30	11.1	48	8.7	82	3.1	35.6	57.8
Lactating cow	110	11.1	48	9.1	75	2.8	30.2	52.0
Total	**261**	**16.5**	**47**	**13.8**	**74**	**4.3**	**31.1**	**55.6**

Embryo quality, recipient selection, and pregnancy rates

Morphological evaluation has been the method of embryo selection for many years and remains the primary approach of embryo assessment. However, this evaluation method poses limitations, not only arising from the subjectivity of the embryologist, but also because of the evaluation system itself that views embryo development statically. We have incorporated the observation of the embryo during various phases as another way to determine embryo viability. Based on our observations and data, embryos that reach compact morula stage on day 5 of IVC, blastocyst stage on day 6, and expanded blastocyst or greater stage on day 7, have a much higher chance of making a pregnancy than those that do not. Day 7 IVP embryos at various stages of development and quality grades are presented (Figure 8).

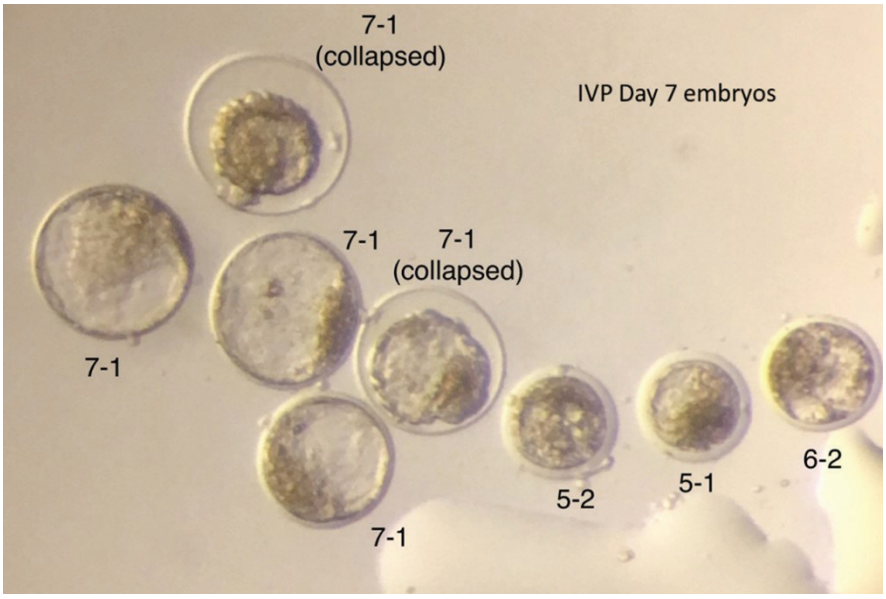

Figure 8. Day 7 IVP embryos with expanded blastocysts (7) and some that are behind in development (5 and 6) and disorganized. First number refers to stage and second number refers to grade.

Differences in pregnancy rates obtained in Holstein heifers and lactating cow recipients from Grades 1 - 3 embryos (January 2019 - December 2021) are presented (Table 4). There was a significant decrease in pregnancy rates when Grade 2 embryos were used and that is why we avoid using them in lactating cow recipients since these animals must get pregnant soon after calving to optimize economic returns. Experienced ET practitioners have a substantial positive impact on the results as evident in our previous study and it is also observed as we train new veterinarians.[6]

Table 4. Holstein pregnancy rates for Grades 1 - 3 embryos transferred fresh to heifer or lactating cow recipients (January 2019 - December 2021)

Recipient type	Heifers		Lactating cows		All recipients	
	ET	Preg (%)	ET	Preg (%)	ET	Preg (%)
Grade 1 embryo	4481	54	838	50	5319	5
Grade 2 embryo	2168	39	52	40	2220	39
Grade 3 embryo	144	28	-	-	144	28
Total	6793	49	890	50	7683	49

To better identify factors that affect pregnancy rates, we analyzed Grade 1 embryo pregnancy results separately. Since all our work is done in-house, and we have natural estrus on heifer recipients all week long, we can select some of the embryos on day 6 of culture to be transferred. Differences between Grade 1 day 6 and day 7 IVP embryos transferred to heifer recipients at various days from estrus are summarized (Table 5). Day 6 embryos have higher pregnancy rates than day 7 embryos. Embryos that do not reach blastocyst stage by day 6 or expanded blastocyst stage by day 7 have lower pregnancy rates, especially if transferred 6 or 9 days after estrus. Embryos transferred 7 and 8 days after estrus have higher pregnancy rates. If we only transferred Grade 1 embryos, that reach blastocyst stage by day 6 or expanded blastocyst by day 7, to recipients 7 and 8 days after estrus, we can achieve high pregnancy rates (58%, n = 1,613, selection from Table 5), similar to our IVD embryos rates.[15]

Table 5. Pregnancy rates for Grade 1, days 6 or 7 IVP Holstein embryos in various stages, transferred fresh to Holstein heifers and lactating recipients at various days after estrus (January 2019 to December 2021)

Stage / time of year	Day 6 Grade 1 IVP embryos						Day 7 Grade 1 IVP embryos					
	Stage < 6		Stage ≥ 6		Total		Stage < 7		Stage ≥ 7		Total	
Estrus	ET	Preg (%)	ET	Preg (%)	ET	Preg (%)	ET	Preg (%)	ET	Preg (%)	ET	Preg (%)
66	10	40	19	68	29	59	243	46	585	45	828	45
77	136	63	167	60	303	62	527	47	1092	57	1619	54
88	688	53	535	60	1223	56	324	52	521	58	845	56
99	175	43	189	61	364	53	39	31	69	57	108	47
Total	1009	53	910	61	1919	56	1133	48	2267	54	3400	52

Jersey embryos transferred to lactating Jersey cows (December 2019 - January 2021) that reached the blastocyst stage or older had higher pregnancy rates than younger embryos (56.7 versus 45.3%).[16] Cows that received an embryo after 65 days in milk (n = 639) had higher pregnancy rates (54.8 versus 46.7%). First and second lactation recipients (n = 537) had higher pregnancy rates than third and fourth (56.6 versus 46.1%).

Embryo transfer can also be an important tool to increase pregnancy rates in lactating cows because it can bypass the negative effects of high milk production, low concentrations of P_4, and heat stress on the early embryo.[17] To confirm this, data from Holstein artificial inseminations (AI) and ET at Maddox Dairy between June 2017 to May 2019 were analyzed.[18] Embryo transfer pregnancy rates were superior to AI, especially during the critical months. Fresh IVD embryos had the most impact. Besides producing higher genetic merit animals, ET can also increase fertility in lactating Holstein cows, especially during the critical months. Another benefit of using ET, is that cows that do not ovulate are resynchronized right away, which is not the case for cows that were bred by AI.

Lactating cows and heifers can be used as recipients, with higher pregnancy rates when a Grade 1, day 6 early blastocyst (or older) or day 7 expanded blastocyst (or older) is transferred by an experienced ET practitioner, 7 or 8 days after estrus.

Pregnancy loss and live calf

Based on literature reports from the past 25 years, only 27% of cattle receiving IVP embryos will produce a live calf. Approximately 60% of these pregnancies fail during the first 6 weeks of pregnancy.[19]

In 2020, 4,088 Holstein embryos (IVP and IVD, fresh and frozen) were transferred at RuAnn and Maddox dairies, resulting in 1,952 pregnancies (48%) examined before 40 days of pregnancy and resulted in 1,578 live calves (81%). Most of the losses occurred in the first trimester (83%) and there were some specific donors, that had a very high pregnancy loss (> 50%), suggesting a very strong individual influence, despite the technique used (IVP or IVD), bringing the overall rate up. These data were similar to our data from 2019.[20]

One Jersey herd had 8% pregnancy loss from AI versus 12% for IVP Jersey embryos transferred to lactating Jersey cows, and 98% of the ET calves were born alive in 2021 (n = 299). Another Jersey herd had 2.9% pregnancy loss from AI versus 5.8% from IVP Angus embryo pregnancies transferred to Jersey lactating cows and reported 95% of live ET births (n = 564) in 2021.

In vitro pregnancy loss rates and livability are much better than they used to be. Further data analysis is necessary to evaluate pregnancy loss from newer IVP systems and compare it to AI and/or IVD embryos to isolate other factors (e.g., infectious, management, nutritional). More studies are necessary to clarify some of the reasons of higher pregnancy losses in embryos from younger donors and sires or from certain individuals.

Roles and opportunities for veterinarians in embryo transfer and production

In vitro embryo production is a multifactorial procedure that depends on several carefully and correctly performed sequential steps. Poor performance in any step directly affects the success rate of the final results, e.g., pregnancy rates and/or the number of live calves. As the embryo transfer industry continues to evolve, especially due to the rapid improvement and new applications of biotechnologies, veterinarians will continue to have a critical role not only in the health and reproductive management of elite donor animals, but also in the production and transfer of both IVF and IVD embryos (OPU, flush, ET and embryo export). Demand for quality service will continue to increase as the demand for elite genetics continues to grow. As ET veterinarians, we fulfill our oath to use our scientific knowledge and skills for the benefit of society, through protection of animal health and welfare and advancement of medical knowledge.

Conclusion

There are many factors involved to obtain a live calf from an IVP embryo. Attention to detail in every step of the process is crucial to success. Quality of the oocyte at the start of IVP is the key factor to determine the rest of the process. Follicular wave emergence synchronization and stimulation with FSH improve oocyte quality and consequently embryo production. Laboratory quality control and the use of high-quality supplies are essential to have less variability in embryo production and identifying other factors that might interfere with embryo production. Good quality embryos, selected at the right time and stage of development, transferred by an experienced ET practitioner, to well managed recipients, 7 or 8 days after estrus will result in in vivo-like pregnancy rates. Good record keeping and data analysis are good measures to identify problems and to take prompt action if necessary. Results and management recommendations presented here are based on the data collection, analysis and the actions taken to improve the IVP embryo production program at RuAnn Genetics. We always try to learn from good and poor results alike.

Conflict of interest

None to declare.

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