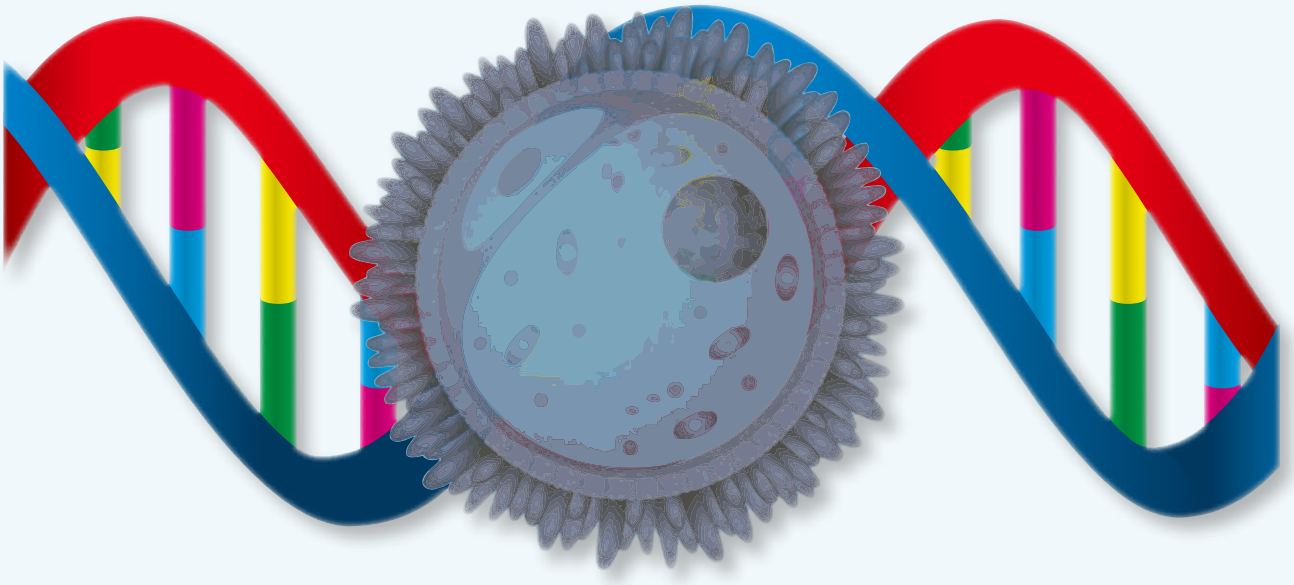
2017年度　海外農業技術セミナー

2017 FY Overseas Agricultural Technique Seminar

カナダにおける乳牛のゲノム評価とOPU−IVFの現状

“The present situation of Genomic evaluation and OPU - IVF in dairy cattle in Canada.”

パトリック・ブロンディン博士
Dr. Patrick Blondin



2017年11月21日（火）14:00〜17:00　開場13:30

[Date] November 21, 2017　14:00〜17:00　OPEN:13:30

酪農学園大学　黒澤記念講堂（江別市文京台緑町582番地）

[Place] Rakuno Gakuen University Kurosawa Memorial Auditorium

主催　北海道アルバータ酪農科学技術交流協会

Hosted by Hokkaido-Alberta Dairy Science and Technique Exchange Association

後援　酪農学園大学エクステンションセンター

Supported by Rakuno Gakuen University Extension Center

講師紹介 ［Guest Speaker］

パトリック・ブロンディン博士

（SEMEX生殖研究及び経営部門副責任者）

Dr. Patrick Blondin

VP of Reproductive Operations and Research, The Semex Alliance

パトリック･ブロンディン博士はラバル大学で内分泌生理学の修士号（1993年）、畜産学の博士号（1997年）をそれぞれ取得する。その後、ノースカロライナ州立大学で博士研究員（ポスドク）として2年間のインターンシップを終える（1999年）。そしてBoviteq社に入り産業研究特別研究員として新しい生殖技術の開発に従事する。さらにヒトの体外受精研究所の臨床研究部長に就任する。2003年に研究開発部門の責任者としてBoviteq（Semex）社に戻る。

2014年にSemex社の胚操作研究室の責任者となり、カナダおよびアメリカの研究開発部門の統括責任者に就任する。2017年、Semex社の生殖研究および経営部門の副責任者に就任する。

ブロンディン博士は多くの研究機関の科学者、個人そして公的機関の研究所と共同研究を行っている。また、ケベックの生殖研究ネットワークの科学委員会のメンバーであり、ラバル大学とモントリオール大学の准教授として大学院の多くの若手研究者を指導している。

1995年から国際胚移植学会のメンバーとして活動し、2011年1月に理事会のメンバーとなり、2015年には会長、2016年には前会長職に就任している。

Dr. Patrick Blondin obtained an MSc in Physiology-Endocrinology (1993) and a PhD in Animal Science (1997) at Laval University and then went on to complete a 2-year postdoctoral internship at North Carolina State University (1999). He then joined Boviteq as an Industrial Research Fellow to assist in the development of novel reproductive technologies. Dr. Blondin became Director of Clinical Research in a human IVF laboratory to advance the field of reproductive biology in humans and finally returned with Semex and Boviteq in 2003 as Director of R&D. In 2014, Dr. Blondin also became Director of Embryo Operations and manages IVF laboratories in Canada and the USA. And in 2017, Dr. Blondin became Semex's VP of Reproductive Research and Operations. Dr. Blondin collaborates with many scientists from academic, private and government laboratories encouraging industry support of various research projects.

Dr. Blondin is a member of the Scientific Committee of the Quebec Research Network on Reproduction. Dr. Blondin is an associate professor with Université Laval and Université de Montreal, co-directing many young scientists undergoing graduate studies. Member of IETS since 1995, Dr. Blondin is a member of the IETS Board of Governors since January 2011 and acted as President in 2015 and Past-President in 2016.

プログラム ［Program］

司会進行：北海道アルバータ酪農科学技術交流協会　事務局長　堂地　修
MC： Osamu Dochi, Secretary General
Hokkaido-Alberta Dairy Science and Technique Exchange Association

14:00　開　　会 Opening

開会あいさつ：北海道アルバータ酪農科学技術交流協会　　会長　谷山　弘行
Opening Address：Hiroyuki Taniyama, Chairperson
Hokkaido-Alberta Dairy Science and Technique Exchange Association

14:05　セミナー Seminar　カナダにおける乳牛のゲノム評価とOPU-IVFの現状
"The present situation of Genomic evaluation and OPU - IVF in dairy cattle in Canada."

パトリック・ブロンディン博士
Dr. Patrick Blondin

通訳：加藤　和代 氏
Interpreter：Ms. Kazuyo Kato

途中休憩有
Break

16:30　質疑応答 Q & A

17:00　閉　　会 Closing

閉会あいさつ：北海道アルバータ酪農科学技術交流協会　副会長　町村　　均
Closing Address：Hitoshi Machimura, Vice-Chairperson
Hokkaido-Alberta Dairy Science and Technique Exchange Association

カナダにおける乳牛のゲノミック評価とOPU-IVFの現状

パトリック　ブロンディン
ボビテック・シーメックス　アライアンス

要旨

カナダおよび世界において体外受精技術（IVF技術）の利用が増えている。これは、より良いIVF用の培地および技術の開発、性選別精液の開発、および最近の農家におけるゲノミック評価の導入などをみても説明できる。農場のOPUを行う多くの専門家や大量のIVF操作を行う研究所が、着実で持続可能な方法で、このことを大規模に行うことができるようになるには、まだ多くの課題が残っている。成功のためには、農場の獣医、実験室の培養士（胚培養士）、家畜の所有者の間のパートナーシップが必要である。農家は、異なる条件下で、IVFで何ができるのか、あるいは何ができないのかその限界を理解しなければならない。獣医師は、見込みのある供卵牛について（IVFの）計画を立てたら農家の期待を管理しなければならない。培養士は予算内で目的が達成されることを確認するために、獣医師と農家の円滑なコミュニケーションを維持しなければならない。このような事業計画は壮大であるが、正しく実施されれば多くの成果が得られる可能性がある。本稿では、どのようにすればこれを実現できるか、すべての関係者が考慮しなければならないさまざまな側面に重点をおいて解説する。

はじめに

IVF技術は、世界中のさまざまな家畜の遺伝子の取引を現実のものにする重要な革新の一つである。図１は国際胚移植学会（IETS）の統計分科会（Perry、2016）によって回収されたデータに基づいて、1997年から2015年までのウシの体内受精卵および体外受精卵の生産の推移を示している。この図では体内受精卵の生産は、2006年に横ばい状態になるまで多くの生産者が使用した重要な技術であることを明示している。そして、2015年までわずかに減少しているように見える。その一方で、1990年代後半にはほとんど使用されていなかったが、IVF由来受精卵（IVF受精卵）の生産は年々増加しており、2015年に生産されたIVF受精卵の数は600,000個を超え、その年に生産されたすべての受精卵の48％に相当する（図１）。 2015年には、北米でIVF卵の34％の受精卵が生産された一方で、60％以上が南米で生産されたことに注目することが重要である。さらに、図２は2008年から2015年の間に北米の生産者による受精卵の使用が、世界の受精卵生産量のうちの５％から34％に増えていることを示している。図２は北米がより多くのIVF卵を生産する一方で、2008年から2015年までの南米におけるIVF卵の生産割合は87％から62％に減少したことを示している。

IVF技術は1970年代に発展し、1978年に初めて体外受精児が生まれた。つづいてウシ（1981）、ブタ（1983）、ヒツジ（1984）の最初のIVF産子がそれぞれ生まれた。ウシのETビジネスにおいて、IVF技術はいくつかの理由により活発に利用されている。第一に、初期のIVF用培養液および技術は導入以来大きく改善した。最初のIVFシステムは体細胞の培養法を基本とし、血清添加と共培養による１段階法（培養液を交換しない方法）であった。このような初期の方法（今日の培養方法の基盤となった方法）で生産されたIVF卵は、MOET（過剰排卵誘処置法による受精卵の生産）で生産された受精卵と異なっていた。その違いは、IVF

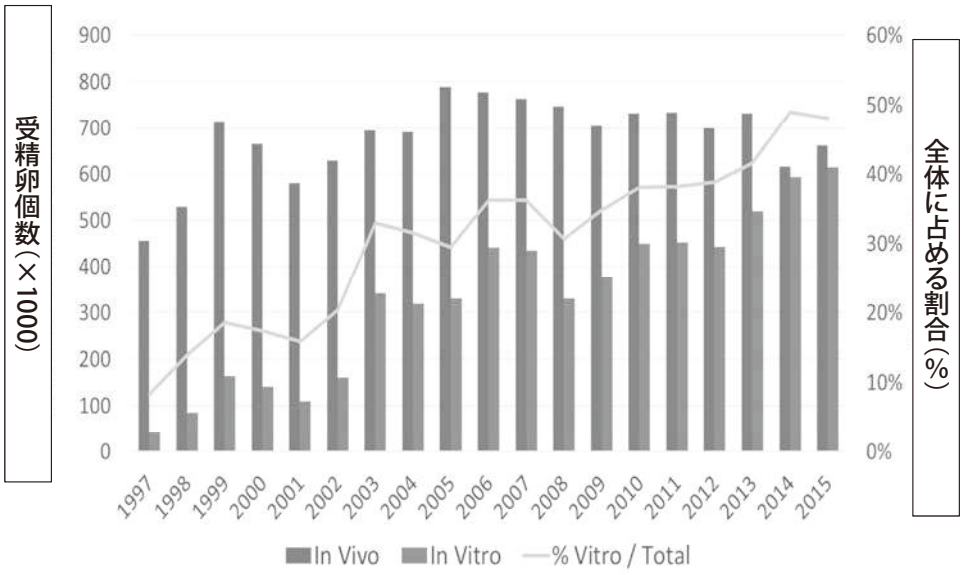


図１．世界における体内および体外牛受精卵の生産（1997年～2015年）

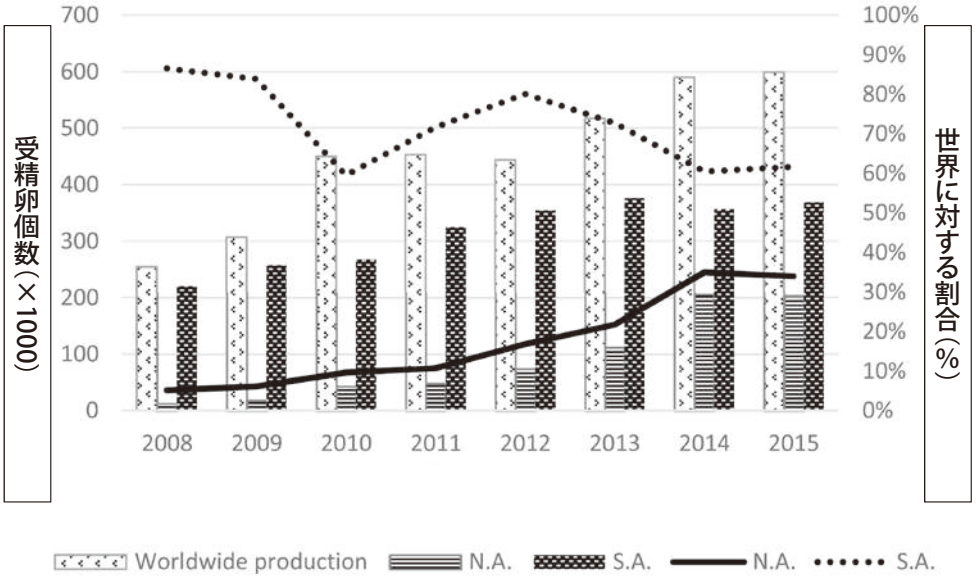


図２．北米、南米、世界における体外牛受精卵の生産（2008年～2015年）

卵は多くの脂質を細胞に有していたため色彩が暗く、緩慢凍結法における耐凍性が低く（Abeら、2002)、内細胞塊（ICM）と栄養膜細胞が明瞭に識別できなかった。さらに、他の研究ではIVF卵から異常な子牛の誕生が報告されている（AOS；Farinら、2006)。この分野の研究が進展するにつれて、体内受精卵が発育する最初の7日間の卵管や子宮の環境の特徴に関する研究に基づいた完全合成または半合成の培養液がIVFラボで利用されるようになった。これらの新しい培養溶液を用いたことによりIVF卵の品質は良くなり、緩慢凍結法による凍結融解後の生存性は有意に向上し、異常子牛の誕生は有意に減少した。耐凍性の向

上により体内受精卵と同様にダイレクト法による凍結卵の移植が可能になった。この点を考慮に入れることで、IVF卵は体内受精卵と同じように容易に輸出できるようになった。凍結IVF卵の輸出入は依然として限定的であるが、国際的なウシの遺伝子需要に対応するため民間、大学、政府のどの組織もそれぞれの立場において市場を開放するために適切に規制を行う機関と協力して実施されている。しかし、IVF技術における一つの懸念は、世界中の商業ビジネスによって使用されるさまざまな（個々に異なる）IVFシステム（培養組成と技術）があるということだ。これは、IVFラボごとに受精卵の品質に差があり、受胎率同様、高品質なIVF卵の有効個数、受精卵の耐凍性にも差が生じる、という結果を生む。

IVF技術がET産業でより多く使用されているもう一つの理由は、IVF技術は性選別精液（Garner and Seidel、2008）と逆ソート（RS）精液（Morottiaら、2014）が使いやすいためである。酪農家にとって、一回のIVFサイクルで90%以上の雌受精卵を生産するということは、たとえ受精卵あたりのコストが高くてもIVF技術が魅力的であるということをアピールするという付加価値を生んでいる。肉牛市場では、雄性選別精液を使用し90%以上の確率で雄受精卵を生産できる。MOETプログラムでRS精液を使用することは考えられないのだが、IVFの利点を提供すると、すべてではないが多くの雄牛にRS精液を使うことができる。したがって、生産者は供卵牛と彼らの経営に必要な種雄牛を交配し、望む性別の受精卵の大部分を生産できる。

ゲノミックスは、選抜精度の向上、世代間隔の短縮、選抜強度の増加によって、ウシの遺伝子産業（Shojaei Saadiら、2014）に変革をもたらした。生産者が彼らの農場で生まれた子牛の新しい遺伝子から利益を得るための時間的な間隔はより短い。IVF技術は短期間に多くの受精卵を生産することができるため、農業ビジネスにゲノム情報を利用するための発生工学的手法を提供することが可能である。IVF技術の大きな利点の一つは、40～60日以内に従来法（過剰排卵誘処置）では一回の採卵しかできないのに対して、過剰排卵誘処置による四回のIVFを行うことができることである。したがって、IVF技術を使用すると、期間あたりに得られる受精卵数が多くなることが大きな利点である。

IVFプログラムの成功は、受胎頭数で評価されるものであり、一回の生体卵子吸引（OPU）における受精卵の生産個数で評価されるものではない。つまり、受精卵の量よりも質が重要なのである。したがって、自然現象では体内受精が上手くいくのだが、それを体外で行うことには大きな課題がある。そして、持続的で大規模なIVFビジネスを確立しようとすると多くの課題が発生する。

商業的なIVFの方向性

成功するIVFプログラムの計画を単純化してみると、受胎率の高い高品質のIVF卵を生産するためには取り組むべき三つの側面がある。第一にIVFのための理想的なドナーの特定、IVFのために発生能の高い卵子を採取する供卵牛の同期化と過剰排卵誘処置を確定することである。残念なことにIVFシステムでは奇跡は起こらない。最良の結果を得るためには、高品質の卵子を採取してIVFに使用することが不可欠である。ここでの経験則は「低品質の卵子からは低品質の受精卵しか採取できない」ということだ。 卵子の採取前に供卵牛の準備に必要な検査を行わなければ、IVFで生産される高品質の受精卵の平均個数が大幅に減少する。このことについては後段で詳しく述べる。

第二に、毎週安定的で持続的なIVF技術を樹立するためには、最高品質の胚盤胞を生産できる最高の品質管理（QC）と培養液を用いたIVFシステムの確立が必要不可欠である。「悪魔は細部に潜む」という言

葉は、IVFでは実際に相当によく当てはまる。IVFでは、卵子をドナーから吸引採取する瞬間から受精卵が受卵牛に新鮮卵移植するまでまたは凍結するまでを考えると配偶子/受精卵は体外で９日間培養されていることになる。人工授精によるMOETでは、受精卵は体内で受精および培養された後８日後に回収され、一般に同期化した受卵牛に新鮮卵移植するかまたは凍結するまでに２～３時間体外培養される。したがって、IVFにおける課題は、体内条件を体外で９日間再現し、そして可能な限り受精卵へのストレスをなくすことである。多大なる努力がたちまち無駄になってしまう可能性がある。このことについては、本稿の後半でさらに詳しく解説する。

第三に、これらの受精卵で受胎させるためには、受精卵を新鮮卵または凍結卵を同期した繁殖が正常な受卵牛に移植することが重要である。この最後の局面は、IVFであれ、MOETであれ変わることはないが、受精卵あたりの費用がIVF卵は高くなる可能性があるため、IVF受精卵にとってさらに重要である。高い遺伝的能力牛とより多くの投資により生産される受精卵の場合、このことは先述の二つのことと同様に重要であると思われる。受精卵移植において正常な繁殖性で正常周期の受卵牛が重要であるが本稿ではそのことについて言及しないが、いくつかの優れた論文を見つけることができる（Haslerら、1987・Lonergan、2007）。

IVFのためのドナーの特定と管理

ウシの品種によって卵胞発育ウェーブが異なり、ウェーブごとの卵子数も大きく異なる。多くのボス・インディカス（Bos Indicus）種は、ボス・タウルス（Bos Taurus）種より卵胞数が有意に多く、OPUあたりの受精卵の生産個数も有意に多いことが良く知られている（Pontesら、2010）。また、同じ品種内でも、供卵牛の個体によって大きな差がある。 2006年に著者らは94頭のホルスタイン供卵牛の５年間の成績のなかで、これらの供卵牛の90％が能力の低いことを確認している。即ち、これらの供卵牛はFSH処置開始時点で限られた卵胞しかなかったため､限られた受精卵しか生産できなかったことを確認している（Durocherら、2006）。この報告は、受精卵移植の実務者は、このようなタイプの受卵牛とこのような特徴の低反応牛（多数の卵胞を有するが、過剰排卵誘処置に反応しないウシ）は、卵胞発育処置法を変更しても移植可能な受精卵の数が増えることがほとんどないことを認識しなければならないことを示している。超音波診断技術を用いると、低反応牛は多くの小卵胞が存在するにも関わらずFSH処置中に卵胞が発育しない様子を観察できる。低反応の未経産牛や経産牛は潜在的な卵胞数が少なく（少ない二次卵胞数）、この表現型は繁殖期間を通して変わらないことが報告されている（Abdullah 2008、Ireland 2011、Mossa 2012）。これらの研究は、胎子発育中の母親の環境が不十分であるために二次卵胞数が低いことを示唆している。ここで大切なことは、農家が自ら所有する供卵牛でIVFを実施する場合にはその農家に対して慎重に助言しなければならないということである。低反応牛は、生理学的な限界があるため高品質なIVF卵の生産個数は少なく、そのためIVF料金は決まった料金体系であるので、受精卵あたりの費用は自動的に高くなる。IVFラボが５または25個の卵子を受精させるとすると、９日間以上の同じ作業であるため料金は同じであるが、受精卵あたりの料金は前者（５個の卵子）の方が高くなる。責任あるサービスプロバイダー（技術提供者）として、これを潜在顧客に伝えて、彼らの期待を管理しなければならない。

卵子の発生能については、多くの文献（Sirard MAら、2006; Husseinら、2006; Moussaら、2015;

Labrecque and Sirard、2014）に豊富に解説されている。それらのすべての報告において、発生能の低い卵子は、標準的なIVFでは受精卵への発生能が低いことを指摘している。卵子の発生能力は、たとえば卵胞サイズ、卵胞閉鎖の程度、卵胞の主要構成子の有無などのさまざまな生物学的および生理学的因子と関係している。現在のIVF技術を利用した商業ビジネスは、発生能力のある卵子を受精させて受精卵を生産することを可能にしている。これらの同じシステムでは、体外成熟中に発生能を獲得するために必要な分子シグナルまたは分子を卵子に誘導または伝達することができない。したがって、IVFのための発生能の高い卵子を生産するための最善の方法で供卵牛を準備することはOPUの専門家に託されている。商業的には、同期化および過剰排卵誘処置は、この目的を達成するために最も使用される方法である。しかし、OPUによるIVF卵の生産は過剰排卵誘処置していない供卵牛から可能である。したがって、一長一短である。発生能の高い卵子数の増加のための既存の薬物を使用した供卵牛の同期化および過剰排卵誘処置は、高い受精卵の発生率につながるが、受精卵あたりのコストは高くなる。または、自然周期を無視して少数の発生能の高い卵子を生産すると受精卵の発生率は低くなるが受精卵あたりの生産コストは安くなる。おそらく、ボス・インディカス（Bos Indicus）種のような外因性ホルモンなしに自然に多くの卵胞を産生するウシを所有する農家は、受精卵あたりのコストを可能な限り低く保ちたいために、供卵牛の同期化および過剰排卵誘処置は使いたくないと考えるはずである。

しかし、自然周期中に中程度の卵胞数を持つウシは、IVFにより高品質の受精卵を数多く生産するために同期化および過剰排卵誘処置を検討したいと考える。しかし、実に多くの専門家が忘れてしまう一つの傾向がある。*卵子の品質は、結果的に受精卵の品質に密接に関係する（Sirardら、2006）*。これは、同期化と過剰排卵誘処置が受精卵の発生率を向上させるだけでなく、耐凍性があり高い受胎率の得られる高品質の受精卵を多数生産するということである。このことは、IVFビジネスにおいて非常に重要であり、農家が生産した受精卵から受胎を望むためしばしば過小評価される。農場で顧客と商談する場合、獣医師は多くの受精卵を得たいという期待があるため最大限に多くの卵子を生産しなければならないというプレッシャーを受ける。しかし、OPU後の卵子数の多さが、必ずしもIVF後に高品質受精卵の数の多さを意味するというわけではないことを農家に伝える必要がある。残念なことに、これが起こると農家はIVFが効果的な技術でないと性急に誤解する。成功するために、OPUを行う獣医師は、農家に透明性の高い情報を提供するIVFラボと連携しなければならない！即ち最高品質の受精卵を生産することがすべてである。

IVFラボ（laboratory, 実験室）運営を成功させるための戦略と課題

IVFラボ運営を成功させるための課題は、一週、また一週と持続的で再現性の高いシステムを確立して卵子や受精卵が受けるストレスを管理することである。幸いに卵子と受精卵は、体外培養中に起こりうるさまざまなストレスに対してかなり抵抗性がある。しかし、それには限界もある。胚培養士（embryologist）は、Laneら（2008）によって丁寧に示された“許容される”ストレスを認識していなければならない。彼らは、受精卵は実体顕微鏡下の形態観察では明らかにならない一定のストレスに適応できるが、受胎率は有意に低下することを示した。他の研究者は、受精卵が不十分な体外培養条件にどのように影響され、その影響は胎子発育中または出生時にしか見られないことを示している（Sinclair 1999; Zander 2006）。成功するためにモニターされなければならないIVFのすべての項目を詳しく述べることは非常に難しいが、ここでは考え

なければならない重要点を示す。OPUと卵子検索中の時間とコンディションは体外成熟する高品質な配偶子（gamete）の生産に非常に重要である。不適切なOPUポンプまたは技術のために起こるOPU中の卵丘細胞の剥離は、卵子の品質に影響する可能性がある。卵丘細胞が少ないか、ほとんどない卵丘細胞－卵子複合体（COC）は、体外成熟培養下で成熟せず、受精卵がほとんど発生しないことが示されている（Wardら、2000）。

さらに、卵子は温度変化に対して非常に敏感である。卵子は約30℃の培養液で吸引しなければならない（Gordon、2004）。最後に卵子は温かいプレート（ホットプレート）上ですばやく検索しなければならない。インキュベーターのように制御されていない環境に卵子が曝される時間が長くなればなるほど、不必要なストレスが生じるリスクが高くなる。受精卵にも同じことが言える。培養液は、卵管および子宮の環境を再現する理想的な状態が維持できるように開発されているため、卵子が受けるストレスを最小限に抑えることができる（Laneら、2008）。受精卵の操作および培養中は温度、浸透圧およびpHを維持することが重要である。大気中では移植用液へのpH緩衝剤の添加が強く推奨される。さまざまなタイプの培養器があり、適切なものを選択することが重要である。もっとも一般的な細胞培養のために開発された培養器はより安価で多くのディッシュを用いて培養することができ、また多くのブランドの物が利用できる。

しかし、実験室のスペースをより多く必要とすること、内部のガス組成の調整が困難であること（停滞＝精度が低い）、停電の場合に備えて長時間のバックアップを取ることが難しいこと、低O_2条件でガスを多く消費すること、大気および温度条件が、ドアを開けた際に気相と温度の回復に長時間を要することが欠点として挙げられる。最近のベンチトップ型（実験台に設置できるタイプ）培養器は、温度と気相条件の回復時間が短く、スペースが少なくて済み、調整不要の３種の混合ガス（マルチガスインキュベーター）、２日間の自動バッテリー内蔵、陽圧（バイオセキュリティーの向上）、温度とガス流量のリアルタイム記録、問題発生時のアラームと電子メール通知が可能である、などの利点がある。このような装置の欠点は、培養器あたりの収納可能なディッシュの数が少なく、高価であり、チャンバーが小さいために問題が生じた場合には「バッファ」（緩衝できる余裕）がないことである。移植または凍結する前に受精卵を評価するための最善のツールを持つことも重要である。従来の実体顕微鏡は、多くの胚培養士にとって十分であると思えるかもしれない。しかし、適切な倍率および最高の光学品質を有する実体顕微鏡が不可欠である。胚盤胞の品質は、受精卵の生産数よりも重要である。受精卵の品質、細胞の質感および形態の微妙な変化を発見することは、IVFシステムを改善するため、または生産過程の中で起こる問題を検出するために不可欠である。IVFラボの空気の品質も非常に重要である。IVFラボでは陽圧のHEPAろ過は最善である。しかし、毒性化合物（VOC）は除去されない。

Mertonら（2007）は、炭素活性化フィルターを用いたガスろ過がウシIVF卵の受胎率を向上させることを示した。しかし、このことよりも卵子/受精卵の「体外での生命」の99.5％以上が培養器内にあることを覚えておく必要がある。高品質のガスが重要であるが、このようなガスの品質はミニインキュベーターでも十分に得られる。VOCレベルが極めて低い高純度のガスも利用可能である。注意：医療用ガスのグレードは純度の高さを意味するものではない。IVFラボで使用される消耗品やガラス製品にも注意すべきである。ある種のプラスチックが配偶子や受精卵に有毒であることが多く報告されている。グレード１（Milli-Q PF）の水のみを使用してすべてのIVF培養液を調整すべきであり、技術者は厳格な保守管理スケジュール

を実行しなければならない。可能であれば、最高品質の試薬のみを使用すべきである（可能であれば組換え型および合成型）。 BSA（牛血清アルブミン）やFBS（牛胎児血清）、ホルモンなどの生物由来の成分は、IVFに使用する前に検査する必要がある。受精卵を培養する場合、オイルで覆うことは水分の蒸発を制限し、浸透圧の変動を制限し、培養器からディッシュを取り出す際に重要となる。使用するオイルの種類は非常に重要であり、おそらくIVFで最も危険を伴う。ヒトIVFで使用可能な製品など広範囲に渡って検査されたオイルを使用することが推奨される。全体として、品質管理（QC）は非常に時間がかかる可能性があるが、同時に平均的なIVFラボと高品質なIVFサービスを提供するラボの違いを意味している。

結論

われわれの業界において、IVF技術はますます普及している生殖補助技術であることは明らかである。この技術を世界中の農家が利用できるようにするには、獣医師と胚培養士の継続的な関係が必須である。農家が自身の農場および経営において何を達成したいのか理解することが重要である。この分野の専門家としての責務は、農家が彼らの経営のために達成したいことに対して生殖補助技術が何であるか正確で適切な助言を行うことである。 IVFか、MOETか？過剰排卵誘処置か、自然発情か？通常精液か、性選別精液か、逆ソート精液か？ゲノミクスか？農家が経営を発展させるのを援助するためには、これらすべてを考慮する必要がある。さらに、農家の期待に応えたい場合は、各技術の限界と可能性を理解することが重要である。これは非常に重要なことであるのだが、一方で一部の農家は技術がもたらすことができる以上の期待を持っている。このようなことは、農家、獣医師、またはIVFラボにとって有益でない。農家がIVF技術の利用を決意する時、それは即ち供卵牛ごとの必要事項を決定するために獣医師とIVFラボとのパートナーシップに農家が参加することを意味している。「IVFが簡単だ」と言う人はいない。したがって、もし農家や獣医師が受精卵生産の技術を検討する際に、限られた時間しかなく努力できないならIVFは考えるべきでなくMOETを利用すべきである。しかし、十分な時間があり努力できるのであれば、IVFは非常に価値がある。

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（和訳：北海道アルバータ酪農科学技術交流協会　事務局　堂地　修・柿﨑　明子）

The present situation of genomic evaluation and OPU-IVF in dairy cattle in Canada

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Abstract

There is a growing utilization of in vitro fertilization (IVF) in Canada and the world. This can be explained by the development of better IVF media and techniques, development of sexed semen and recent introduction of bovine genomics on farms. Being able to perform this on a large scale with multiple on-farm experts to perform ovum pick-up and IVF laboratories capable of handling large volumes in a consistent and sustainable way remains a huge challenge. To be successful, there must be a partnership between the veterinarians on farms, embryologists in the laboratory and animal owners. Farmers must understand the limits of what IVF can or cannot do under different conditions. Veterinarians must manage expectations of farmers once strategies have been developed on potential donors. Embryologists must maintain fluent communications with both groups to make sure that objectives are met within predetermined budgets. The logistics of such operations can be very overwhelming, but the return can be considerable if done right. This paper will describe how such operations can become a reality with emphasis on the different aspects that must be considered by all parties.

Introduction

IVF is one of these important innovations that will assure the trade of genetics of various species across the globe. Figure 1 illustrates the evolution of both in vivo and in vitro bovine embryo production between 1997 and 2015 based on data recovered by IETS' Data Retrieval Committee (Perry, 2016). In this figure, it is apparent that in vivo embryo production was an important technology used by many producers until this production leveled off in 2006 and seemed to decrease slightly with the following years up to 2015. On the other hand, although hardly used in the late 1990s, IVF embryo production has increased significantly year after year with an all-time high of over 600,000 IVF embryos produced in 2015, which represents 48% of the total embryos produced that year (Fig. 1). It is important to note that in 2015, South America alone produced over 60% of the IVF embryos while North America produced 34% of these embryos. Furthermore, Figure 2 illustrates how North American producers are using more and more IVF embryos between 2008 and 2015 increasing from 5% to 34% of worldwide embryo production, respectively. As North America is producing more IVF embryos, Figure 2 illustrates that South America went from 87% to 62% of worldwide embryo production between 2008 and 2015, respectively.

IVF was developed in the 1970s with the first human IVF baby born in 1978, followed by the first

IVF calf (1981), IVF pigs (1983) and IVF lambs (1984). For the bovine ET business, IVF is being used more and more for a few reasons. Firstly, initial IVF media and techniques have evolved significantly since its introduction. The first IVF systems were based more on somatic cell culture protocols and were 1-step systems that used serum supplementation and/or cell co-culture. IVF embryos resulting from such platforms were different than embryos produced using MOET. Examples of differences were that IVF embryos possessed more lipids and thus were darker, possessed low cryotolerance to slow-step freezing protocols (Abe et al., 2002), and ICM and trophoblastic cells not as defined. Furthermore, other studies reported higher incidences of calves resulting from these IVF embryos that exhibited abnormal offspring syndrome (AOS; Farin et al., 2006). As research progressed in this field, laboratories introduced sequential defined or semi-defined media that were based on studies that characterized the oviduct and uterine environments during the first 7 days of life of an in vivo embryo. With these new media, IVF embryos were of higher quality, survived significantly more slow-freezing protocols and reduced significantly the incidences of AOS. This increase in cryotolerance made it possible to apply Direct Transfer (DT) techniques just like in vivo produced embryos. Considering this advantage, this opens up opportunities to export IVF embryos as easily as in vivo embryos. Although the import/export of frozen IVF embryos is still limited, different players, whether from the private sector, the universities, or the government, are working with appropriate regulatory agencies to open these markets to meet the global demand for bovine genetics. However, the one caveat of IVF is that there are different IVF systems (media suite and techniques) used by commercial businesses worldwide. This will result in variations in embryo quality from one IVF unit to another and therefore impact number of high quality IVF embryos produced, the cryosensitivty of these embryos as well as resulting gestation rates.

Another reason why IVF is being used more in the ET industry is that IVF works very well with sexed (Garner and Seidel, 2008) and Reverse Sorted (RS) semen (Morottia et al., 2014). Producing over 90% of female embryos for dairy producers following an IVF cycle provides an added-value that makes IVF appealing even at a higher cost per embryo. The beef market can use male sexed semen and produce over 90% male embryos. It is unthinkable to use RS semen in MOET programs thus, offering IVF with a net advantage as most, not to say all, bulls can be RS. Therefore, producers have the opportunity to mate their female donors with the males they need for their businesses and produce a majority of embryos of the desired sex.

Genomics has changed the bovine genetic industry (Shojaei Saadi et al., 2014) by increasing the accuracy of animal selection, reducing the generation interval and increasing selection intensity. The time interval producers may have to profit from the new genetics they calved on their farms is shorter. IVF can produce many embryos in a short period of time and therefore offer agricultural businesses a biotechnological tool to take advantage of genomic information. One of IVF's major advantage is that within 40 to 60 days, a producer would have time to perform 1 conventional flush vs 4 IVF cycles using superovulated donors. So, the gain in higher numbers of embryos per time period becomes significantly advantageous when using IVF.

A successful IVF program is evaluated on the number of **pregnancies**, and not on the total number

of embryos, that result following one ovum pick-up (OPU) session. So, embryo quality is as important, not to say more important, than embryo quantity. Reproducing in vitro what nature does so very well in vivo is therefore a major challenge. And trying to establish a **sustainable** IVF business on a large scale brings with it many challenges to be successful.

Logistics of commercial IVF

Looking at the logistics of a successful IVF program at a simple level, there are 3 aspects of an IVF business that need to work in synchrony to produce high quality IVF embryos that will result in high gestation rates. Firstly, identifying the ideal donors for IVF and determine the best synchronisation and superovulation protocol for these donors to produce developmentally competent oocytes for IVF. Unfortunately, IVF systems do not perform miracles. It is imperative that oocytes of high quality are collected and used in IVF to get the best results. The rule of thumb here is garbage in (poor quality oocytes), garbage out (poor quality embryos). Not taking the necessary measures to prepare donors before oocyte collection will reduce significantly the averages of high quality embryos produced with IVF. This aspect will be analyzed in more detail later in this paper.

Secondly, establishment of IVF techniques with the highest quality control (QC) and media to result in top quality blastocyst embryos are essential if one hopes to establish an IVF system that is reproducible and sustainable week after week. The idiom 'the devil is in the details' certainly applies very well to IVF. In IVF, when we consider the moment the oocytes are aspirated from a donor to the time the embryos are transferred fresh into a recipient animal or frozen, the gametes/embryos have incubated in vitro for 9 days. For MOET, following AI, the embryos are collected 8 days later, after being inseminated and cultured in vivo, and will be cultured in vitro generally for a few hours before they are either transferred fresh in synchronized recipients or frozen. So, the challenge for IVF is to reproduce in vitro for 9 days in vivo conditions and avoid as much as possible any stresses to the embryos. A colossal endeavor that can go wrong quickly. This aspect will also be analyzed in more detail later in this paper.

Thirdly, transferring these fresh or frozen embryos in synchronized, fertile recipients is crucial if the objective is to produce pregnancies from these embryos. This last aspect is no different for embryos resulting from IVF or MOET but seems even more pivotal for IVF embryos as the cost per embryo may be higher. For embryos that result from high genetics and more investment, it seems crucial that this aspect should be as important as the two other aspects. The importance of using fertile, cycling recipients in any embryo transfer program will not be covered in this paper, but a quick search of the literature will result in several excellent reviews on this topic (Hasler et al., 1987, Lonergan 2007).

Identification and management of donors for IVF

Follicular waves of different cattle species will result in a significantly different number of oocytes per wave. It is well known that many Bos Indicus breeds will produce significantly more follicles and

embryos per OPU when compared to Bos Taurus breeds (Pontes et al. 2010). And within a breed, there will be significant differences between individual donors. In 2006, we followed 94 Holstein donors over 5 years and reported that 90% of these donors were lower potential animals which were donors producing a limited number of embryos because of the limited population of small antral follicles present in the ovaries at initiation of FSH treatment (Durocher et al., 2006). This paper recommended that ET practitioners must distinguish between these types of donors and those characterized as low responders (donor with large number of follicles but that does not respond to superovulation protocols) as modifications to the stimulation protocol for the low responders is unlikely to result in a higher number of transferable embryos. Using ultrasound technology, it is possible to follow follicle growth during FSH treatment such that low responders will have many small follicles that do not grow during the treatment. It has been reported that low potential heifers and cows with ovaries with inherent low ovarian reserves (low antral follicle count) will have this phenotype for their entire reproductive life (Abdullah 2008, Ireland 2011, Mossa 2012). These studies even suggest that low antral follicle count may be the result of inadequate maternal environment during foetal development. So, the take-home message here is that farmers must be advised carefully when they would like to perform IVF on their donors. Low potential cows will result in low numbers of viable IVF embryos because of inherent physiological limits and therefore automatically result in a higher cost per embryo as the IVF fee structure is a fixed cost model. Whether an IVF lab sets up to fertilize 5 or 25 oocytes, it is the same work over 9 days and therefore the fees will be the same but the fee per embryo will be higher for the former. As responsible service providers, this must be communicated to potential clients so we can manage their expectations.

Oocyte developmental competence has been reviewed abundantly in the scientific literature (Sirard MA et al 2006; Hussein et al 2006; Moussa et al 2015; Labrecque and Sirard 2014). All of them agree that an oocyte with low developmental competence has very small chances to produce an embryo using standard IVF. Oocyte developmental competence has been correlated with various biological and physiological factors such as follicle size, degree of follicle atresia, presence or absence of key follicular molecules, to name a few. Current commercial businesses use IVF systems that can permit a developmentally competent oocyte to be fertilized and produce an embryo. These same systems are not capable to induce or convey the necessary molecular signals or molecules to oocytes to acquire developmental competence during in vitro maturation. Therefore, it is up to experts performing OPUs to use the best strategies to prepare donors to produce developmentally competent oocytes for IVF. Commercially, synchronization and superovulation are the most used approaches to attain this goal. But it is possible to produce IVF embryos following OPU of donors that have not been superovulated. Therefore, there is a trade off. Use existing drugs to synchronize and superovulate a donor to increase the number of developmentally competent oocytes and thus result in a higher embryonic developmental rate but at a higher fee per embryo. Or work off the natural cycle and produce a lower number of competent oocytes and thus a lower embryonic developmental rate but at a lower fee per embryo. Perhaps animals that produce many follicles naturally without exogenous hormones such as certain Bos Indicus breeds owned by farmers that wish to keep costs per embryo lowest as possible may prefer not

using donor synchronisation and superovulation.

However, animals that produce a moderate number of follicles naturally may want to consider synchronisation and superovulation to increase the number of embryos of higher quality following an IVF cycle. However, there is one aspect too many experts out there tend to forget. *Oocyte quality will be closely correlated to the resultant embryo quality* (Sirard et al., 2006). This is where synchronisation and superovulation will not only increase embryonic development rates, but produce a higher number of high quality embryos that will be freezable and result in higher pregnancy rates. This aspect is extremely crucial in an IVF business and is underestimated too many times as a farmer hopes to produce pregnancies from the embryos produced. When dealing with clients at the farm, veterinarians are pressured to produce the highest number of oocytes in hopes of obtaining many embryos. But there needs to be education of farmers so they understand that a high number of oocytes following an OPU does not necessarily signify a high number of quality embryos after IVF. And unfortunately, when this happens, the farmer will conclude too soon, and wrongfully, that IVF is an inefficient technology. Veterinarians performing OPUs must partner with IVF laboratories to provide transparent information to farmers if this is to be successful! It is all about producing the highest number of Quality 1 embryos.

Logistics and challenges of running a successful IVF laboratory

The challenge of running a successful IVF laboratory is to set-up a system that will be sustainable and reproducible week after week and control any stresses that oocytes or embryos can be subjected to. The good news is that oocytes and embryos can be quite resistant to the different stresses that can occur during in vitro culture but there is a limit to this. An embryologist must be aware of 'permissive' stresses that was elegantly reviewed by Lane et al. (2008). They demonstrated that embryos can adapt to certain stresses that will not be apparent morphologically under stereomicroscope analysis, but result in significant reduction of pregnancy rates. Others have also demonstrated how embryos can be affected by inadequate in vitro conditions and these impacts are only seen during foetal development, or at birth (Sinclair 1999; Zander 2006). It would be very difficult to detail all aspects of IVF that must be monitored to be successful, but here are a few key examples that should be considered. The time and conditions during OPU and oocyte searching are very important to produce quality gametes that will mature in vitro. Stripping oocytes of their cumulus cells when performing OPUs due to inadequate OPU pumps or techniques can affect the quality of the oocytes. It has been shown that cumulus-oocyte complexes that have little, or no, cumulus layers will not respond to in vitro maturation conditions and thus produce very few embryos (Ward et al., 2000). Additionally, oocytes are very sensitive to temperature variations. Oocytes must be aspirated in a medium that is at approximately 30℃ (Gordon, 2004). Finally, oocytes should be searched quickly on warm plates. The longer the oocytes are not in a controlled environment such as the incubator, the higher the risks of creating unnecessary stresses to the oocytes. The same applies to embryos. Culture media have been developed to maintain ideal conditions that mimic oviduct and uterine environments and thus minimize any stresses they are subjected to (Lane et al, 2008).

Maintaining temperature, osmolarity and pH during manipulations and incubation of the embryos is critical. Using pH buffering agents for atmospheric conditions is strongly recommended in transfer solutions. Different models of incubators exist and choosing the right one is critical. The more traditional incubators originally developed for cell culture have advantages such as being less expensive, possible to culture many dishes per incubator and there are many brands available. However, the disadvantages are that they require more lab space, adjustment of internal gas composition is difficult (fyrite = low precision), difficult to get backup for many hours in case of power failure, high gas consumption in low O_2 conditions and the atmospheric and temperature conditions require long recovery times when the door is opened. The recent benchtop incubators offer advantages such as shorter recovery time for temperature and atmospheric conditions, less lab space required, use of tri-Gas mixes (no adjustments required), built-in battery with 2 days of autonomy, positive gas pressure (increased biosecurity), real-time recording of temperature and gas flow with alarm emails in case of problems. The cons of such devices are that they can hold less dishes per incubator, they are more expensive and there are no « buffer » in case of problems because of the small chambers. Having the best tools to evaluate embryos before transfer or freezing is also critical. Traditional stereomicroscopes might look sufficient for many embryologists. However, very high quality stereomicroscopes with a good magnification and very good optical quality are essential. Blastocyst quality is more important than the number of embryos produced. Detecting subtle changes in embryo quality, cellular texture and morphology is essential to improve IVF systems or detect problems that could have occurred during the procedure. The IVF laboratory air quality is also very important. HEPA filtration of the IVF lab with a positive pressure is a good start. However, it does not remove toxic compounds (VOCs). Merton et al. (2007) showed that gas filtration with a carbon-activated filter increases pregnancy rates in bovine IVF. But beyond this, we must remember that >99.5% of the « in vitro life » of an oocyte/embryo is inside an incubator. High quality gas is critical and such gas quality is affordable with mini-incubators. Very high purity gas is available, with very low levels of VOCs. Beware: medical grade does not mean high purity grade. The consumables and glassware used in IVF laboratories must also be monitored. There have been many reported cases where certain plastics are toxic for gametes and embryos. Only Grade I (Milli-Q PF) water should be used to make up all IVF media and technicians should follow a strict maintenance schedule. Only the highest quality reagents should be used (Recombinant and synthetic forms whenever possible). Any components of animal origin, such as BSA, FBS, and hormones should be tested before using in IVF. When culturing embryos in media, oil overlay of the media will limit water evaporation and thus osmolarity shifts and becomes important when dishes are taken out of incubators. The type of oil is very critical and probably is the riskiest product in IVF. It is recommended to use extensively tested oils used such as products available for human IVF. Overall, QC can be extremely time consuming, but can mean the difference between an average IVF laboratory and a laboratory offering high quality IVF services.

Conclusion

It is clear that IVF is an assisted reproductive technology that is becoming more and more prevalent in our industry. Making this technology accessible to farmers worldwide will require sustainable partnerships between veterinarians and embryologists. Understanding what farmers wish to accomplish on their farms and within their businesses becomes important. As experts in this field, it is our responsibility to clearly identify what a farmer wishes to accomplish to make the correct recommendation as to what assisted reproductive technologies they should consider for their businesses. IVF or MOET? Superovulation or natural cycle? Conventional, sexed or reverse sorted semen? Genomics? All we must be considered when assisting farmers to develop their businesses. Furthermore, understanding the limits and potential of each technology is crucial if we want to manage expectations of farmers. This is key and too often have some farmers have higher expectations that what the technology can deliver. This certainly does not help the farmer, the veterinarian or the IVF laboratory. When farmers decide to use IVF, this means they are willing to embark in a partnership with the veterinarian and IVF laboratory to make all necessary decisions for each donor on a program. No one said IVF is easy. So, if farmers or veterinarians are looking for technologies to produce embryos that require limited time and effort, do not attempt IVF and stick to MOET. But with the right amount of time and effort, IVF can be very rewarding.

Acknowledgments

I'd like to thank the Semex research and development team for compiling most of the data included in this paper. Special thanks to Dr Christian Vigneault, Dr Francois-Xavier Grand and Valerie Fournier for discussions on this topic.

I would also thank IETS (International Embryo Technology Society) for permitting the reprint of parts of the original paper that was presented at the 2017 International Meeting. The original reference for the IETS paper is: *Logistics of large scale commercial IVF embryo production. P. Blondin. Reproduction, Fertility and Development, 2017, 29, p32-36.*

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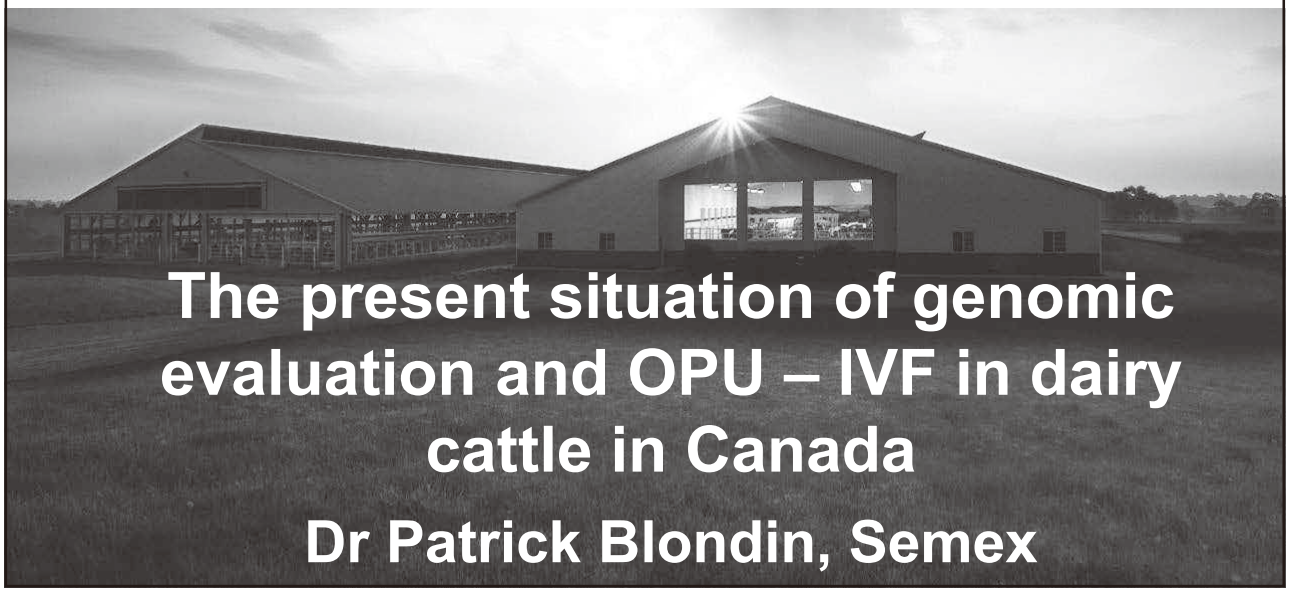
2017 Overseas Agricultural Technique Seminar



The present situation of genomic evaluation and OPU – IVF in dairy cattle in Canada

Dr Patrick Blondin, Semex

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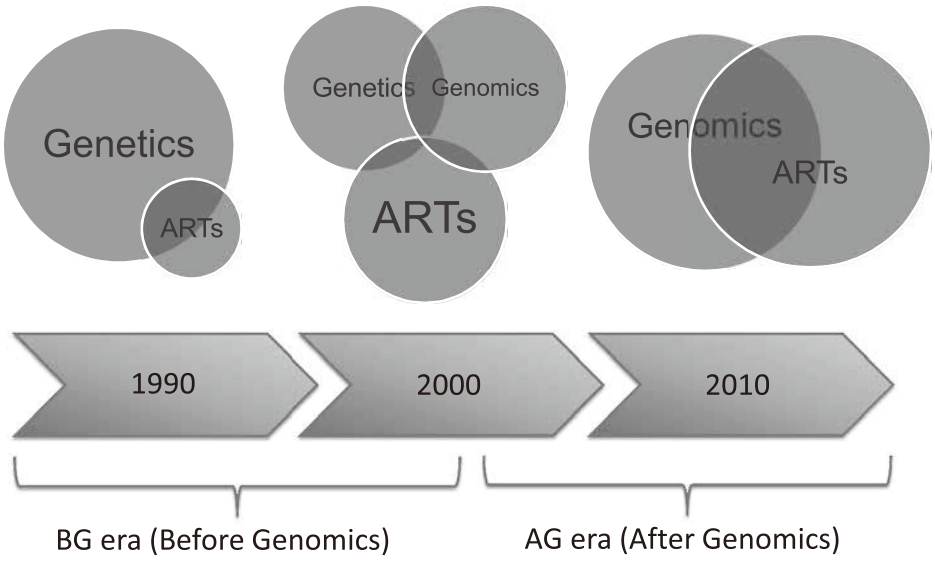
Our industry is changing



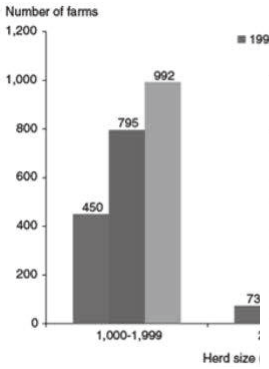
Status of dairy farms in the US

Farms with < 100 cows

Year	Farms (number)
1992	134.931
1997	97,134
2002	73,725
2007	53,324
2012	49,683

Source: Census of Agriculture.

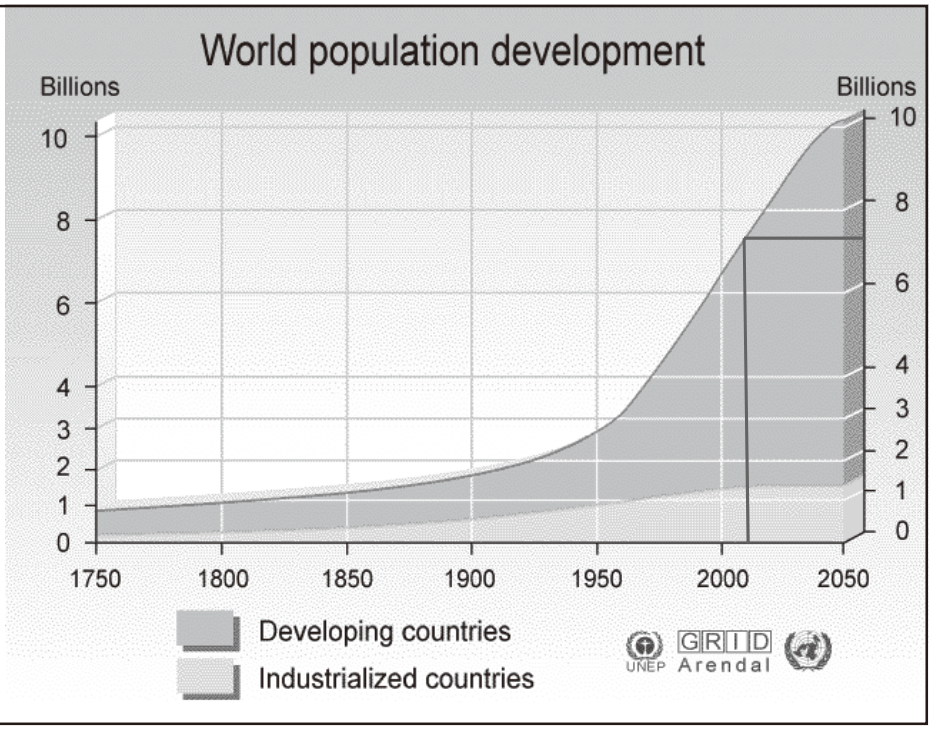
The number of very large dairy farms has grown rapidly



U.S. dairy industry 15-year overview

Year	Total milk production (in millions of lbs)	Number of cows (in thousands)	Milk per cow (lbs per year)	Licensed dairy herds	Average herd size
2002	170,063	9,1	18,608	74,110	123
2003	170,394		18,760	70,375	129
2004	170,832	9,010	18,960	66,830	135
2005	176,931	9,050	19,550	64,540	140
2006	181,782	9,137	19,895	62,070	147
2007	185,654	9,189	20,204	59,130	155
2008	189,982		20,395	57,127	163
2009	189,334	+22%	20,573	54,942	168
2010	192,848	9,119	21,148	53,132	172
2011	196,164	9,194	21,336	51,291	179
2012	200,537	9,233	21,720	49,281	187
2013	201,231	9,224	21,816	46,975	196
2014	206,054	9,257	22,259	44,809	207
2015	208,597		22,396	43,534	214
2016	212,436	9,3	22,774	41,809	223

- World supply and demand will grow by an average of 2.3% / year
- Supply growth will be driven more by yield than cow no.
- Approx. 17.5M (15%) farms will be lost in next 10 years



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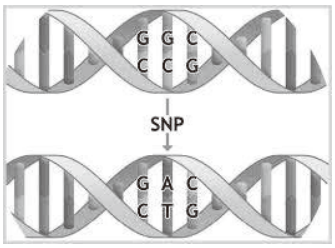
Genomics

Bovine Genome
Sequencing Consortium
Science Vol 324 2009

- SNPs, or **S**ingle **N**ucleotide **P**olymorphisms, are the most common type of genetic variation between individuals.
- SNPs can cause silent, harmless, harmful or latent effects



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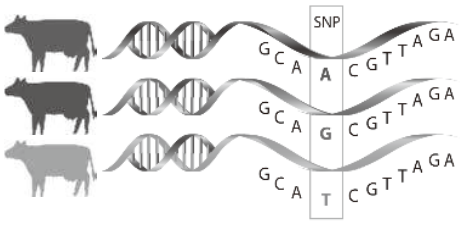
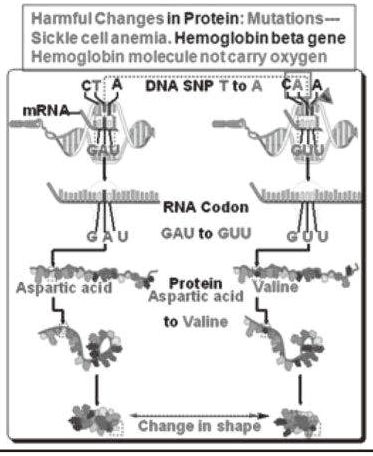
Genomics

- Most SNPs occur in noncoding regions and do not alter genes
  - Become markers for important genes related to phenotypes of interest



- SNPs can occur in coding regions and could alter the protein made by the that coding region, which in turn could influence a phenotype.



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Development of genomic tools

Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps

T. H. E. Meuwissen,* B. J. Hayes† and M. E. Goddard†,‡

*Research Institute of Animal Science and Health, 8200 AB Lelystad, The Netherlands, †Victorian Institute of Animal Science, Attwood 3049, Victoria, Australia and ‡Institute of Land and Food Resources, University of Melbourne, Parkville 3052, Victoria, Australia

Manuscript received August 17, 2000
Accepted for publication January 17, 2001

BovineSNP50 Genotyping BeadChip

Featuring 53,714 evenly spaced and strategically placed SNP probes that span the bovir genome.

Highlights

- **Excellent Call Rates and Accuracy**
  > 99% average call rates and 99.9% reproducibility
- **Comprehensive and Uniform Coverage**
  Evenly distributed polymorphic SNPs with a median spacing of 37.4 kb
- **Simple Workflow**
  PCR- and ligation-free protocol
- **High-Throughput Format**
  Up to 24 samples can be interrogated in parallel

BovineLD v2.0 Genotyping BeadChip

Extend genomic selection to the entire herd with scalable content at an economical price.

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Featuring 2,900 SNPs that provide high capacity for prediction of the genetic merit of cattle.

BovineHD Genotyping BeadChip

More than 777,000 SNPs that deliver the densest coverage available for the bovine genome.

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Impact of genomic tools

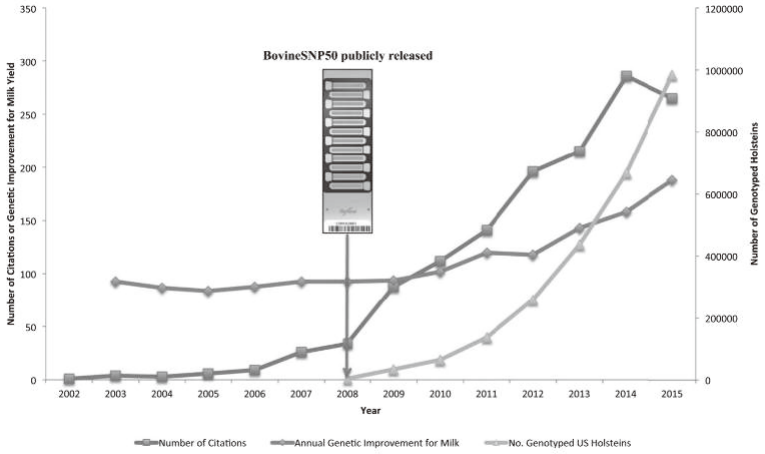

Fig. 1. Annual numbers of citations of Meuwissen et al. (1), rate of genetic improvement in milk production from Garcia-Ruiz et al. (3), and numbers of Holstein cows chip-genotyped by December of each year from the Council for Dairy Cattle Breeding database (https://www.cdcb.us/Genotype/cur_density.html).

Holsteins are the genomic selection poster cows

Jeremy F. Taylor[a,1], Kristen H. Taylor[b], and Jared E. Decker[a]

7690–7692 | PNAS | July 12, 2016 | vol. 113 | no. 28

SEMEX
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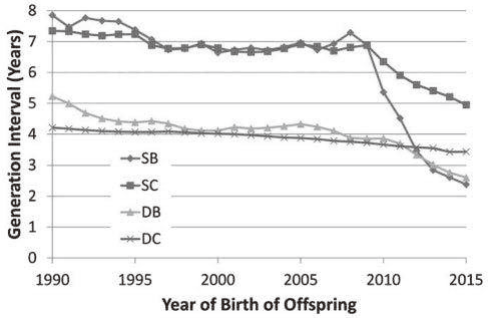
Impact of genomic tools

Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection

Adriana García-Ruiz[a,b], John B. Cole[b], Paul M. VanRaden[b], George R. Wiggans[b], Felipe J. Ruiz-López[a], and Curtis P. Van Tassell[b,1]



Significance

The introduction of genomic selection in dairy cattle improvement programs in 2008 was expected to increase rates of genetic gain, particularly for traits with low heritabilities, such as fertility and longevity. Our analysis of the US national dairy database found that generation intervals have decreased dramatically over the past 6 y, and selection intensity for lowly heritable traits has increased considerably. Genetic trends rapidly increased for fertility, lifespan, and udder health. These results clearly demonstrate the positive impact of genomic selection in US dairy cattle, even though this technology has only been in use for a short time. This progress in US Holsteins will have a favorable impact on other populations worldwide due to the widespread dissemination of US germplasm.

sire(s) of bulls (SB), sire(s) of cows (SC), dam(s) of bulls (DB), and dam(s) of cows (DC)

PNAS | Published online June 27, 2016 |

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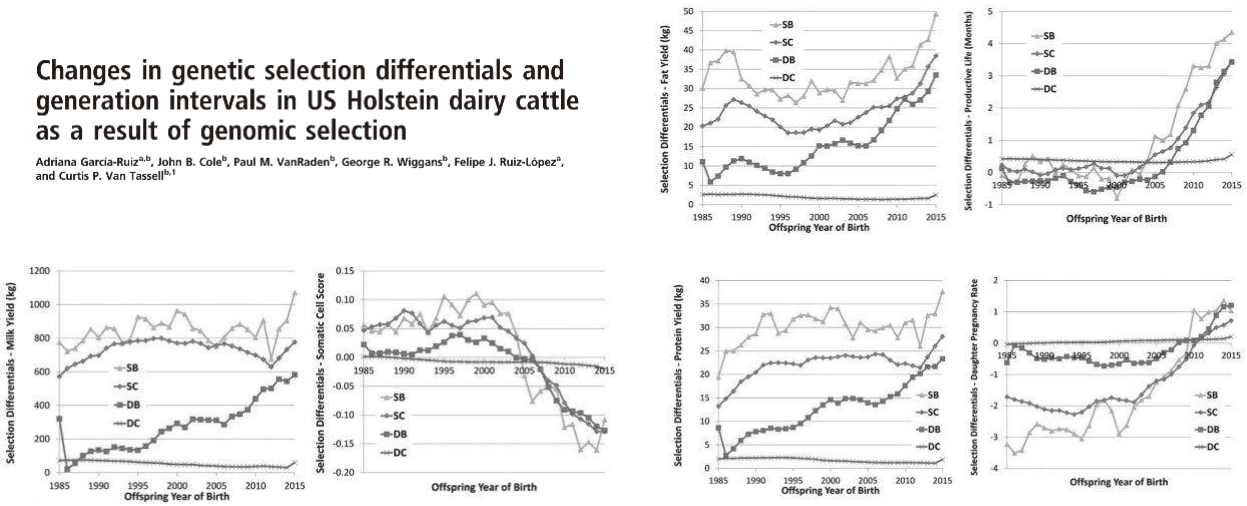

sire(s) of bulls (SB), sire(s) of cows (SC), dam(s) of bulls (DB), and dam(s) of cows (DC)

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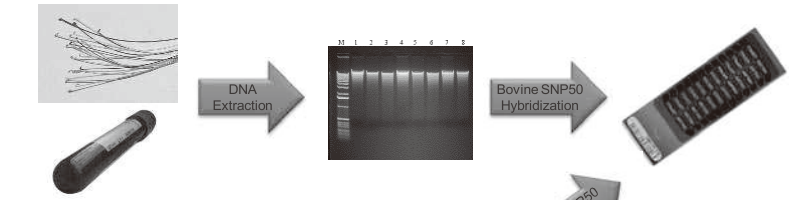
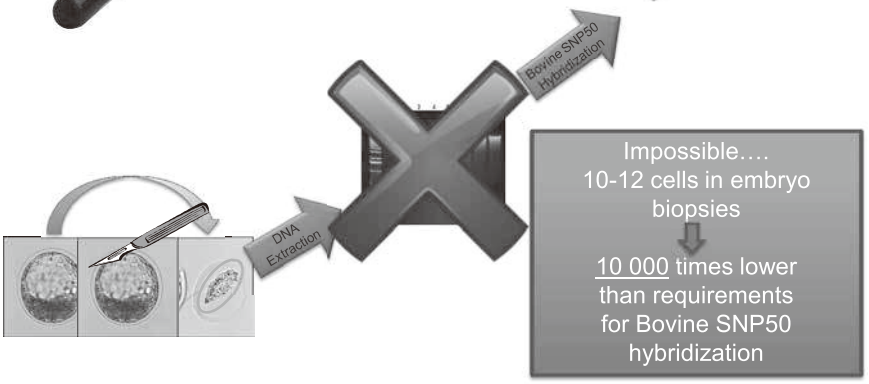
Getting DNA for genotyping





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DNA amplification is necessary but validate the technique you use

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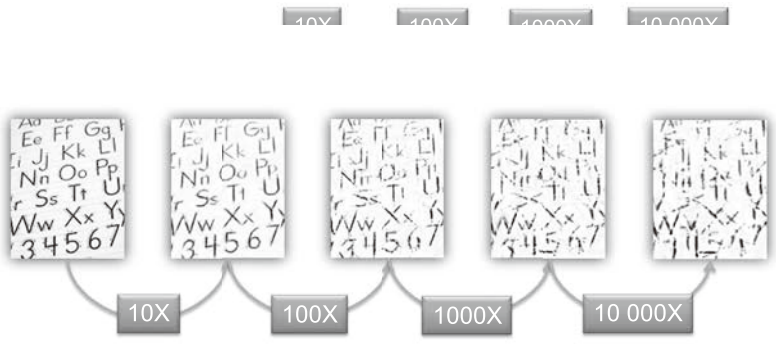

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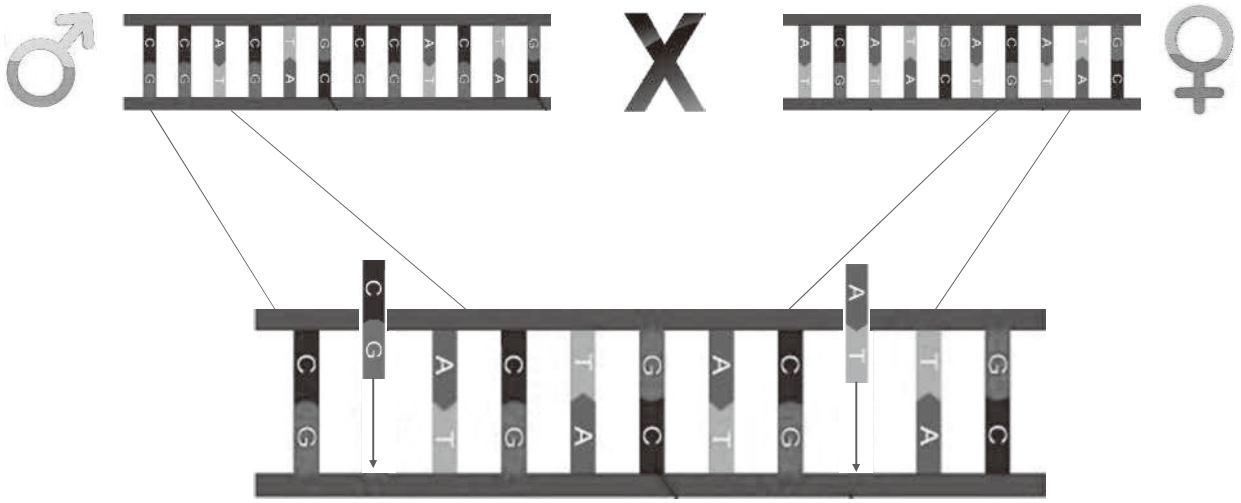
Imputation with genomic software



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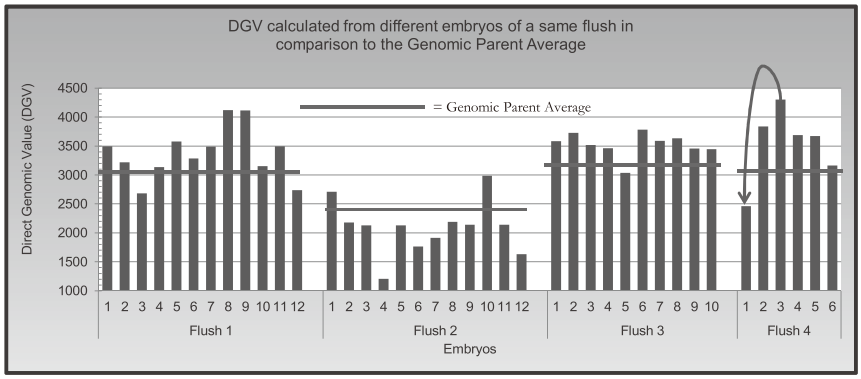
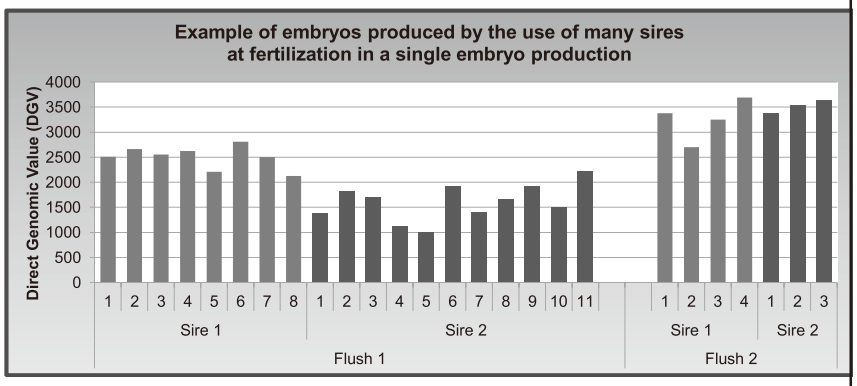
Variation intra embryo flushes

- Some crosses generated very variable Direct Genomic Values (DGV) between different embryos (flushes #1 and #2)
- For others, the DGV obtained were very similar (flush #3).
- In some cases, very large divergences (1842 pts. of DGV) were found between two embryos from a same production (flush #4).



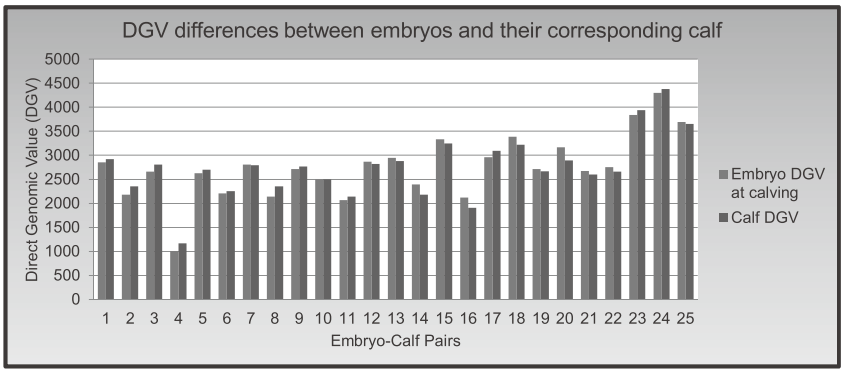
Parentage possible

- Multiple sires can be used for one embryo production.
- Pedigree validation is part of the QC of genomic analysis and then, sire identification is executed.
- It is then possible to do more matings in a shorter period of time while being able to identify the pedigree of each embryo.



DGV differences between embryo and corresponding calf – Study 1

- The 25 first calves born from genotyped embryos were genotyped to measure the accuracy of our amplification method.
- For the DGV, a mean divergence of 106 ± 68 pts. was calculated for the first 25 samples (4.3 ± 3.6% difference).



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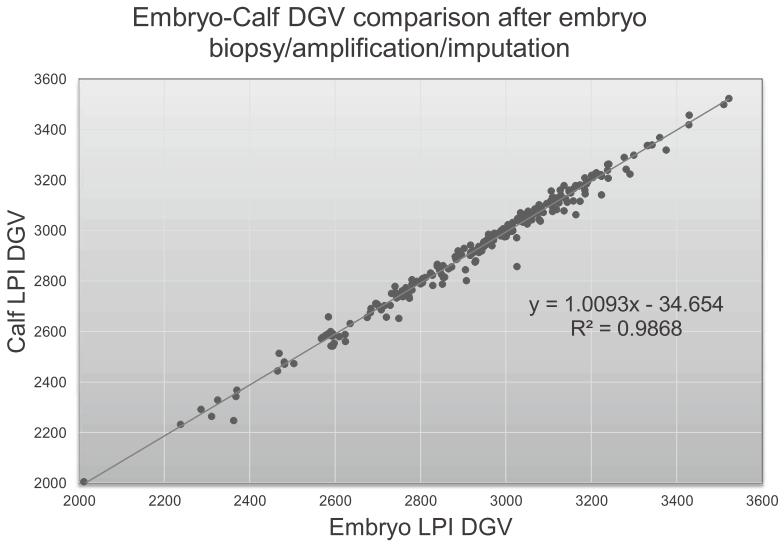
DGV comparison after embryo biopsy-amplification-imputation



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2nd study performed by Boviteq

56 calves had 50k genotypes and were considered in this study. The genotypes from embryos were imputed using a specially adapted version of FImpute (V2). Parents of the embryos had 50k genotypes, therefore family information was the main source of information for imputation.

Figure 7: Embryo DGV (before imputation) vs calf DGV for LPI (From same genomic data)

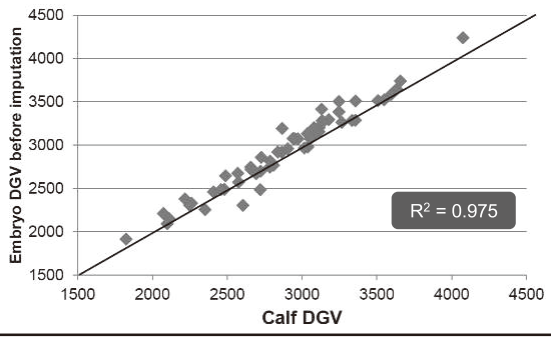
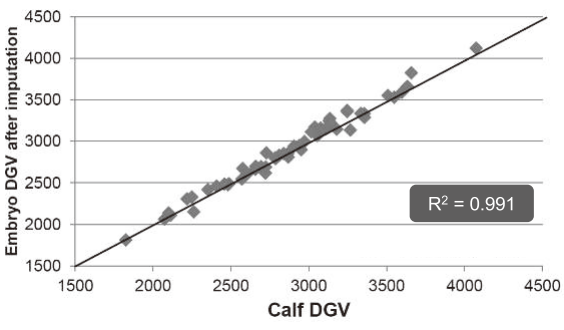


Figure 8: Embryo DGV (after imputation) vs calf DGV for LPI (From same genomic data)



Conclusions from 2nd study

- Most of errors in biopsies are due to allele drop-out (calling a heterozygous locus as homozygous). For most embryos the genotyping error rate was less than 2%.
- The post processing of embryo genotypes by imputation improves genotype quality especially for embryos with a high genotyping error rate. However, having good DNA extraction and amplification methods remains very important.
- Having genomic information for both parents is essential for embryo imputation. The parent information allows correcting a large portion of Mendelian conflicts. Given this, it is important to ensure that both the sire and the dam of embryo biopsies are genotyped with the 50k panel.
- Differences between the DGVs of embryos and calves ranged from -131 to 165 points of LPI, and 46 embryos out of 56 had LPI difference of <100.

IETS – Data Retrieval Committee

Recent statistics on embryo collections from in vivo flushes and from In Vitro Fertilization (IVF)

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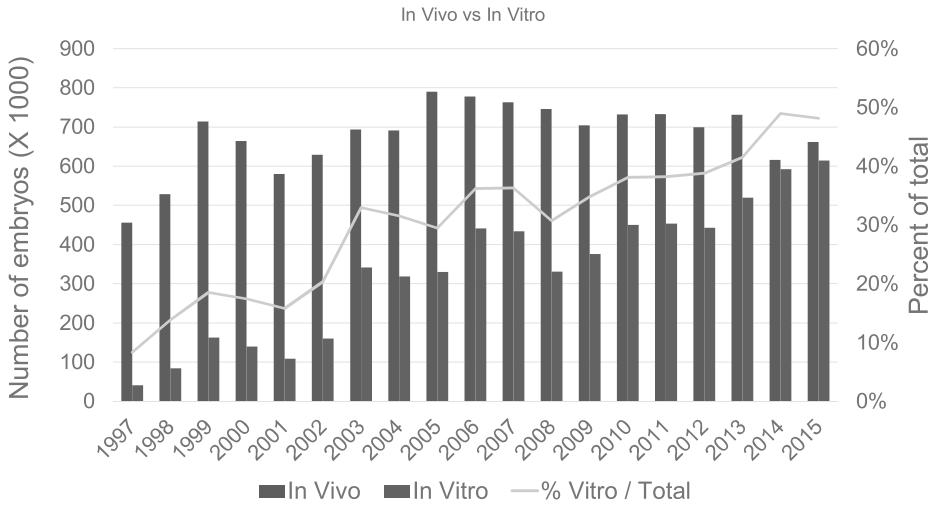
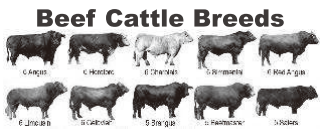
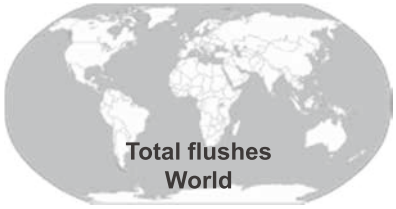
IVF - Evolving Technology





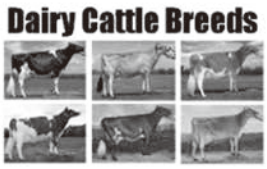
In Vivo Embryos 2015



- 100,739 flushes
  - 6.6 emb/flush

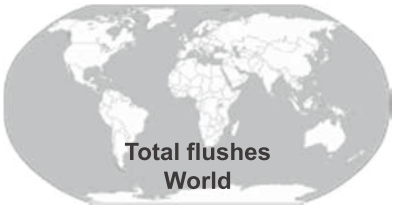
ASIA
- 14,689 flushes
  - 7.2 emb/flush

North America
- 53,536 flushes
  - 6.7 emb/flush



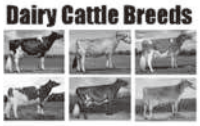
In Vivo Embryos 2015

Japan
- Conv. semen: 2,527 flushes
- Sexed semen: 0



- Conv. semen: 37,942 flushes
- Sexed semen: 4,339 flushes (10%)

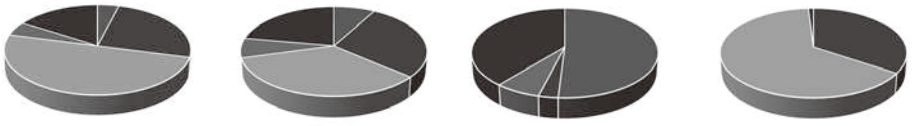
North America
- Conv. semen: 1,756 flushes
  - Canada (41%)
- Sexed semen: 2,828 flushes
  - Canada (19%)



In Vivo Embryos

Embryo Transfer 2015

	Fresh ET	Frozen Domestic ET	Frozen Imported ET	Frozen for Export
World	67,365	74,022	2,094	15,731
Japan	2,531	6,231	1,070	0
Canada	16,855	20,985	58	5,408
USA	33,584	25,537	0	10,109
South America	3,640	5,245	145	0



Note: In pie charts, **green** represents **other countries** not listed above

IVF Embryos 2015
(Dairy + Beef)

World

111,704 OPUs

↓

2,061,205 oocytes → 18.5 oocytes/OPU

↓ 29.7% dev. rate

612,709 embryos → 5.5 embryos/OPU

Asia

3,177 OPUs

↓

59,224 oocytes → 18.6 oocytes/OPU

↓ 16.0 % dev. rate

9,438 embryos → 3.0 embryos/OPU

North America

35,980 OPUs

↓

683,717 oocytes → 19.0 oocytes/OPU

↓ 31.1 % dev. rate

212,046 embryos → 5.9 embryos/OPU

OPU = Ovum Pick-Up

IVF Embryos
(Dairy + Beef)

Asia

OPUs

3,177 OPUs

↓

59,224 oocytes → 18.6 oocytes/OPU

↓ 16.0 % dev. rate

9,438 embryos → 3.0 embryos/OPU

Slaughterhouse ovaries

35,335 aspirations

↓

714,783 oocytes → 20.2 oocytes/OPU

↓ 7.9 % dev. rate

56,740 embryos → 1.6 embryos/OPU

OPU = Ovum Pick-Up

IVF Embryos

	Fresh ET	Frozen ET (Domestic emb)	Frozen ET (Imported Emb)	Frozen Export
World	304,946	97,589	1,638	718
USA	58,579	30,183	0	0
Canada	4,309	1,844	0	172
Japan	800	1,164	0	0
South America	224,066	58,821	0	0

IVF Embryos – % of ET using Fresh Embryos

	2013	2014	2015
World	89.9%	81.3%	74.0%
North America	81.0%	76.7%	67.1%
South America	95.1%	84.0%	79.2%
Asia	57.2%	No Data	50.1%

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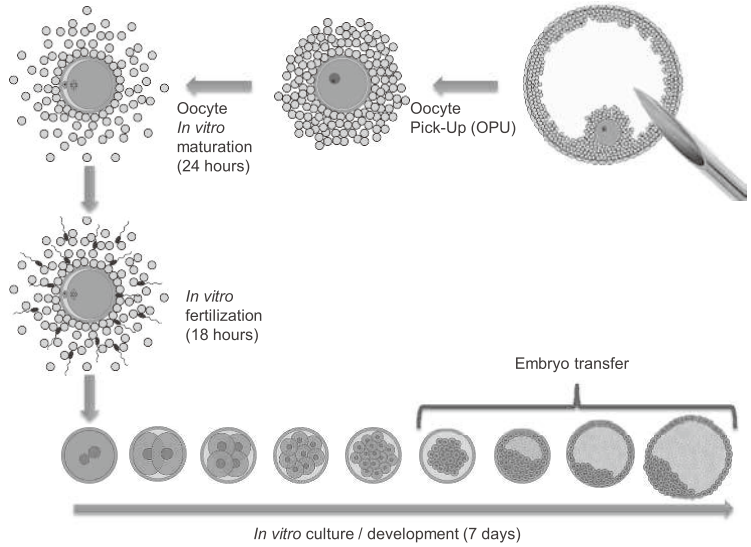
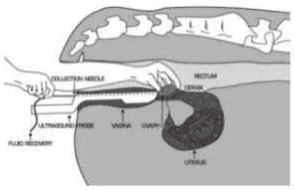
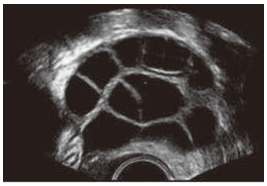
IVF – In Vitro Fertilization







Why is IVF used more?

- **Improvement of in vitro culture systems**
  - Migration from a 1-step system with serum +/- co-culture to sequential defined or semi-defined systems
  - IVF embryos today comparable to in vivo embryos – increased cryotolerance

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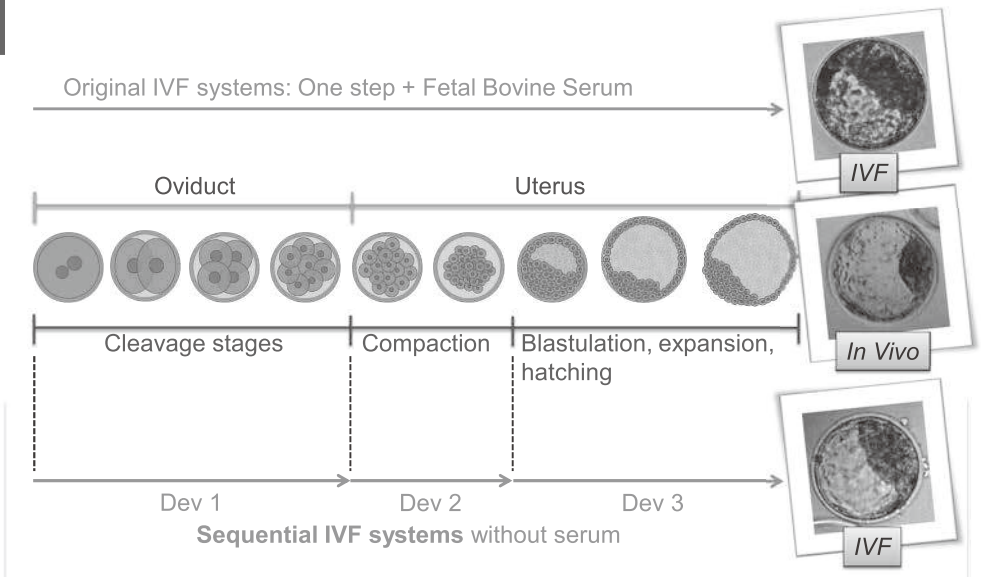
The ideal in vitro culture system



Why is IVF used more?

- Improvement of in vitro culture systems
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  - IVF embryos today comparable to in vivo embryos – increased cryotolerance
- **Introduction sexed semen in IVF**
  - **IVF remains a fixed-cost technology – more expensive**
  - **Producing over 90% female embryos per IVF cycle advantageous**

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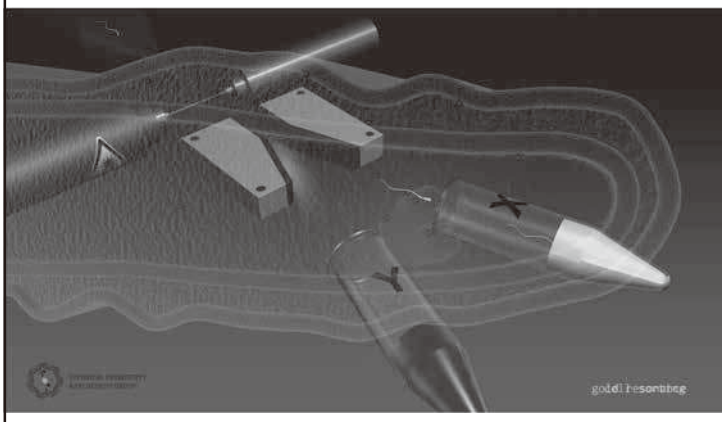
Semen Sexing



- Can use frozen sexed semen or reverse sorted semen

Why is IVF used more?

- Improvement of in vitro culture systems
  - Migration from a 1-step system with serum +/- co-culture to sequential defined or semi-defined systems
  - IVF embryos today comparable to in vivo embryos – increased cryotolerance
- Introduction sexed semen in IVF
  - IVF remains a fixed-cost technology – more expensive
  - Producing over 90% female embryos per IVF cycle advantageous
- **Genomics has changed the bovine industry**
  - **Life-span of an elite bull today much shorter**
  - **Any technology, such as IVF, that can increase embryo production in same period of time is key to take advantage of elite genetics**

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Accelerating genetic gain between generations by using IVF

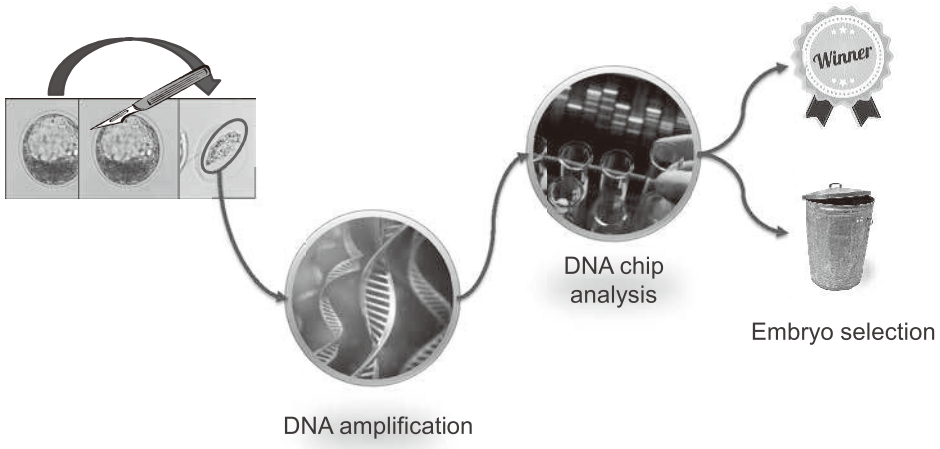
Comparison of embryo production methods
(in vitro vs *in vivo)*

45 to 60 days period

	In vitro	In vivo
Semen	Non-sexed	Non-sexed
OPU or Flush	4	1
Oocyte or Structure	51.6	7.3
Viable embryos	21.2	4.3
Female embryos	10.2	2.1
Gestation (J60)	4.3 (42%)	1.2 (59.4%)

3.6 X more!

Embryo Genomics



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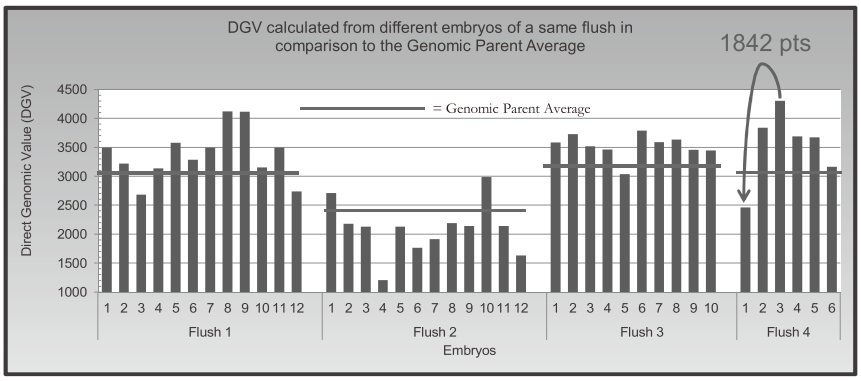
Why genomic evaluation of embryos?



High variation between embryos from a single mating

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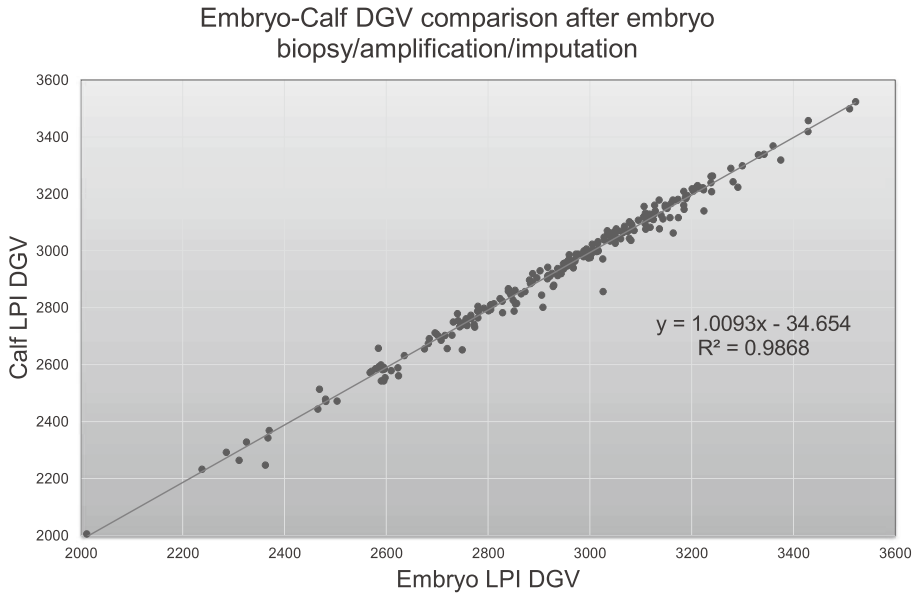
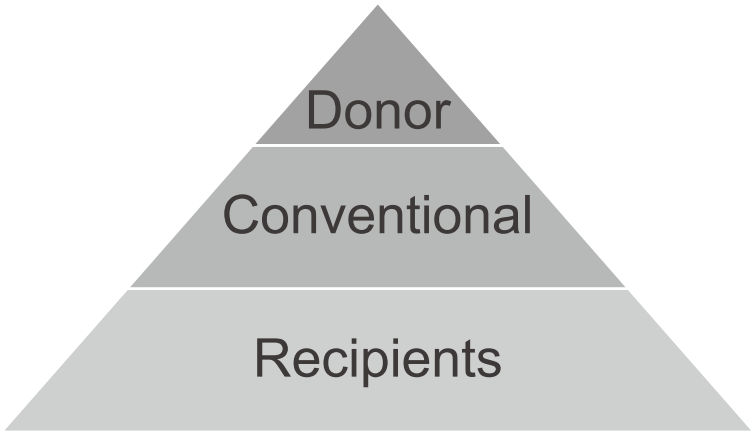
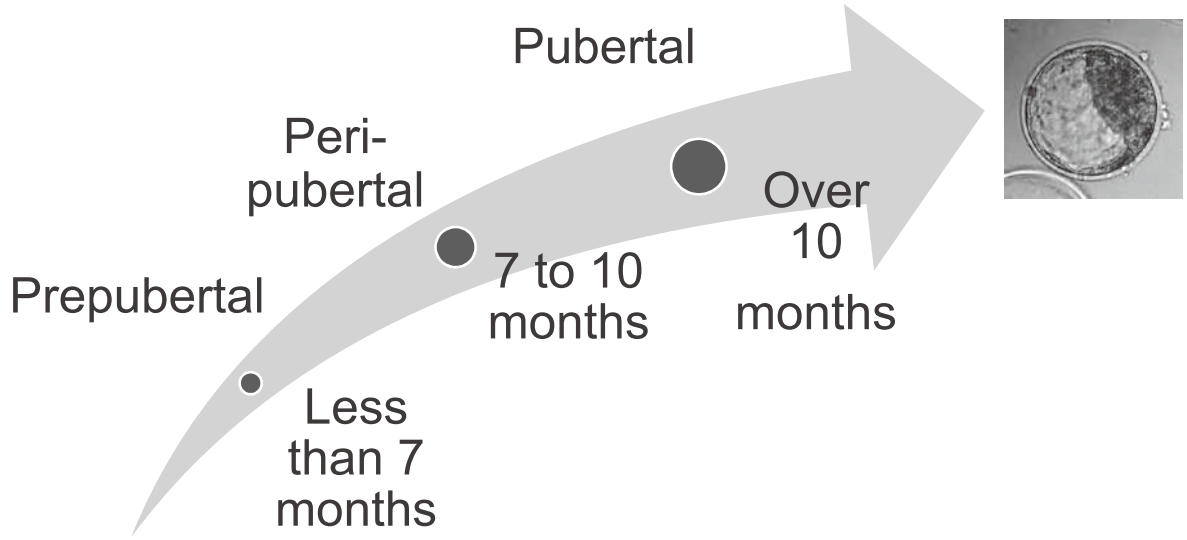


IVF Solutions – Genetic Strategy

Reproductive strategies differ based on genetic ranking

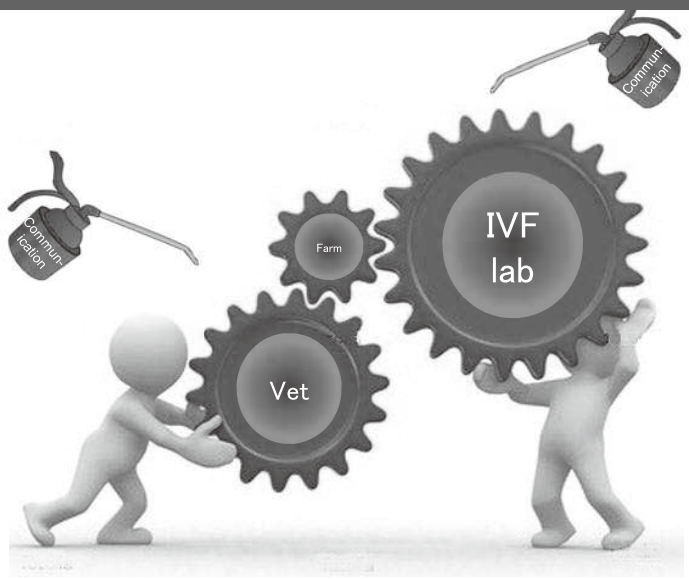


Genetic race – Huge challenge in IVF



A successful IVF program

- **If we want this to work – we must work as partners**



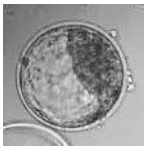
The BIGGEST challenges for commercial IVF

- Manage Expectations !
- All clients are in it for gestations
  - Not simply for many oocytes
  - Not simply for many embryos
  - But highest number of QUALITY EMBRYOS
- This may seem evident, but you would be surprised by the number of clients that are disappointed with results

A successful IVF program

Superovulation

OPU



IVF

Recipients

OOCYTE QUALITY

EMBRYO QUALITY

RECIPIENT QUALITY

And ONLY 1% luck

SEMEX Genetics for Life

Donor parameters that must be considered

- Superovulation or natural cycles
- What is an ideal donor
- Nutrition

A successful IVF program

OOCYTE QUALITY

The ugly truth …

Garbage 'IN'

Garbage 'OUT'

A successful IVF program

OOCYTE QUALITY

Garbage 'IN'

What do we mean?

Quality can be affected by intrinsic factors or extrinsic factors.

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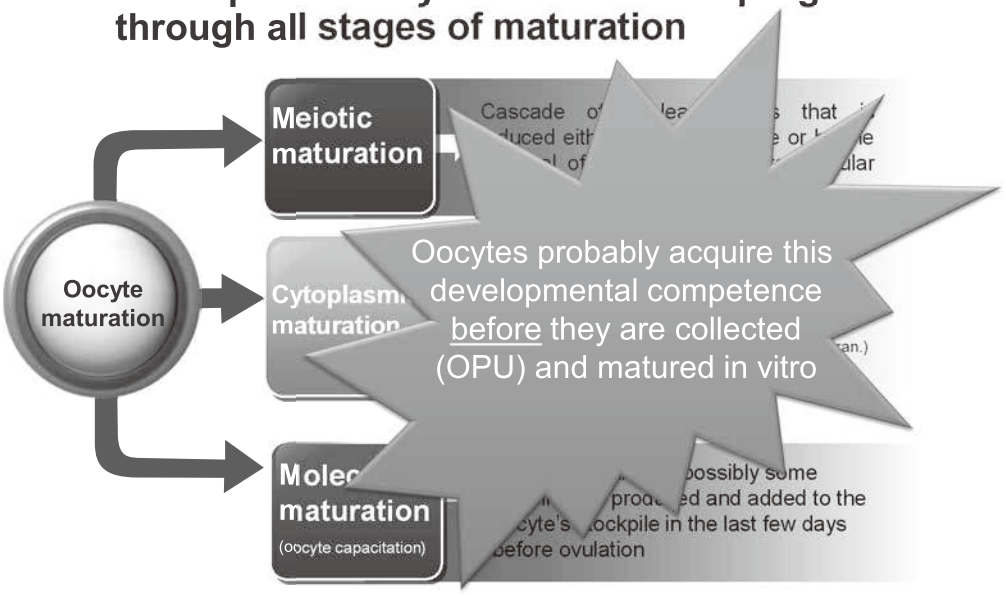
A successful IVF program

OOCYTE QUALITY

Intrinsic factors

Using the best **synchronisation** and **superovulation** protocol for donor animals to produce developmentally competent oocytes for IVF

A competent oocyte will be able to progress through all stages of maturation



Contribution of the oocyte to embryo quality. MA Sirard et al Theriogenology 2006 65 p126-136

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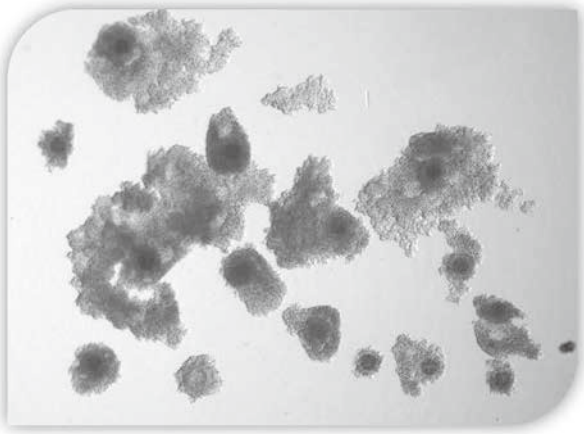
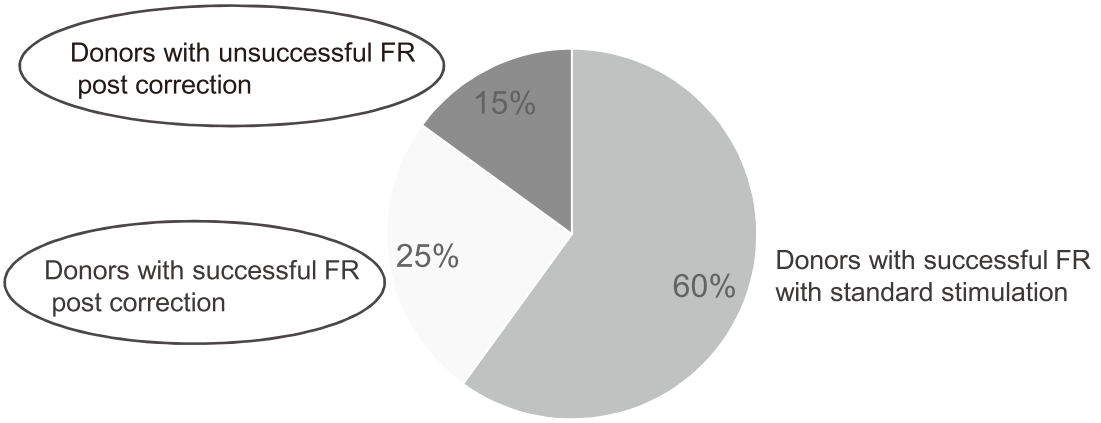


Oocytes that possess an inherent developmental competence will produce viable embryos.

It is impossible under a microscope to determine if an oocyte possesses this competence.

Acquisition of developmental competence is a result of the transcription of key genes that will be triggered by external signals.

How many donors have a successful follicular response following standard stimulation programs ?



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Donor potential

Theriogenology
Volume 65, Issue 1, 7 January 2006, Pages 102–115
IETS 2006 Pre- and Post-Conference Symposia
IETS 2006

Effect of hormonal stimulation on bovine follicular response and oocyte developmental competence in a commercial operation

Jean Durocher, Nathalie Morin, Patrick Blondin

Table 6
Oocyte collection results for the three categories of donor animals (mean ± S.E.M.)

Category of donor animal	No. of animals	No. oocyte collection sessions	Collection sessions/animal	Mean no. oocytes
Low outcome	8	49	6.1	5.7 ± 3.2 a
Medium outcome	11	86	7.8	10.2 ± 4.0 b
High outcome	12	67	5.6	15.7 ± 7.1 c

Different letters (a–c) differ significantly within a column ($P < 0.05$).

Table 7
Embryo development for the three categories of donor animals (mean or percentage ± S.E.M.)

Category of donor animal	Mean no. of viable embryos	Percentage of viable embryos (%)	Mean no. of transferable embryos	Percentage of transferable embryos (%)
Low outcome	3.1 ± 2.3 a	53 ± 25	2.7 ± 2.2 a	47 ± 25
Medium outcome	5.9 ± 3.6 b	60 ± 24	5.2 ± 3.5 b	53 ± 23
High outcome	9.5 ± 5.9 c	61 ± 24	8.4 ± 5.5 c	54 ± 24

Different letters (a–c) significantly within a column ($P < 0.05$).

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Donor Potential – ideal donor

BIOLOGY *of* REPRODUCTION

Antral Follicle Count Reliably Predicts Number of Morphologically Healthy Oocytes and Follicles in Ovaries of Young Adult Cattle[1]

J.L.H. Ireland[3], D. Scheetz[3], F. Jimenez-Krassel[3], A.P.N. Themmen[6], F. Ward[7], P. Lonergan[7], G.W. Smith[4], G.I. Perez[5], A.C.O. Evans[7] and J.J. Ireland[2,3]

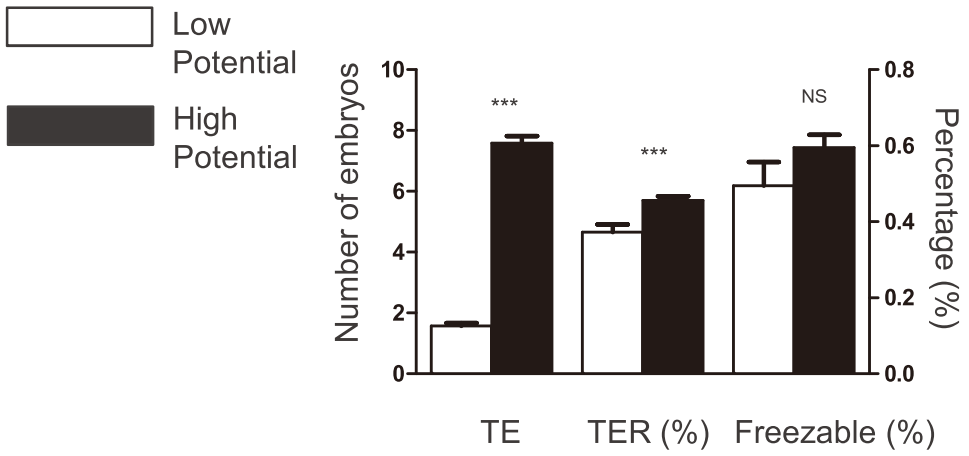
Methods to predict numbers of healthy oocytes in the ovaries of young adults could have important diagnostic relevance in family planning and animal agriculture. We have observed that peak antral follicle count (AFC) determined

Correlation between phenotype, genotype and antral follicle population in beef heifers

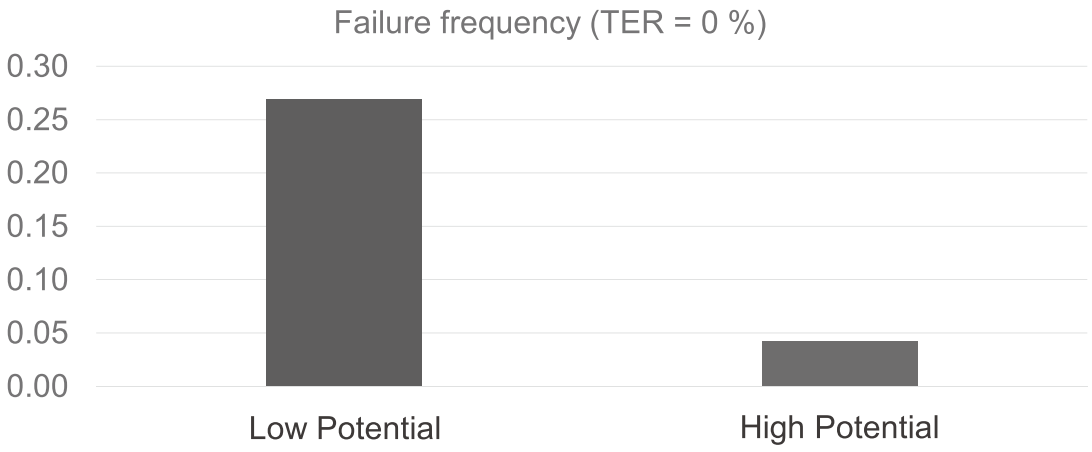
F. Morotti, G.M.G. Santos, C. Koetz Júnior, K.C. Silva-Santos, V.M. Roso, M.M. Seneda

Based on these studies, AFC in heifers from weaning to yearling age is highly variable between individuals and repeatable within the same female. Additionally, there is no correlation between phenotypic or genotypic characteristics and the antral follicle population. However, AFC can be slightly affected by finishing precocity at weaning.

Donor Potential – ideal donor



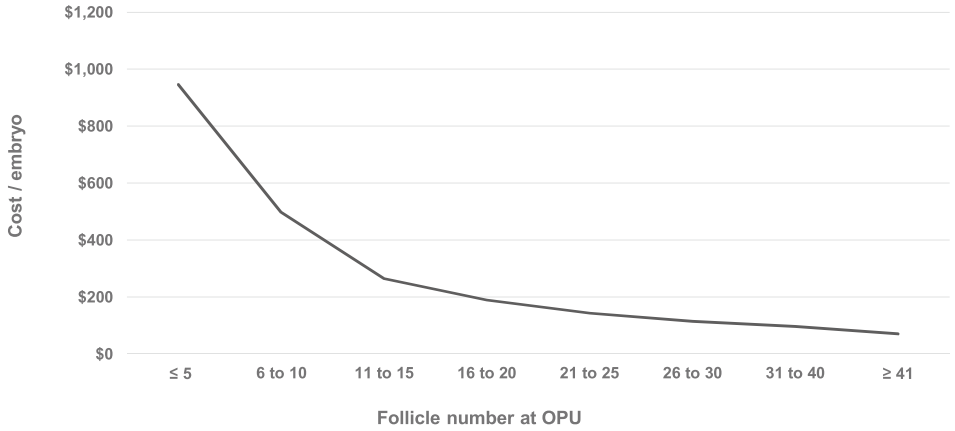
Donor Potential – ideal donor



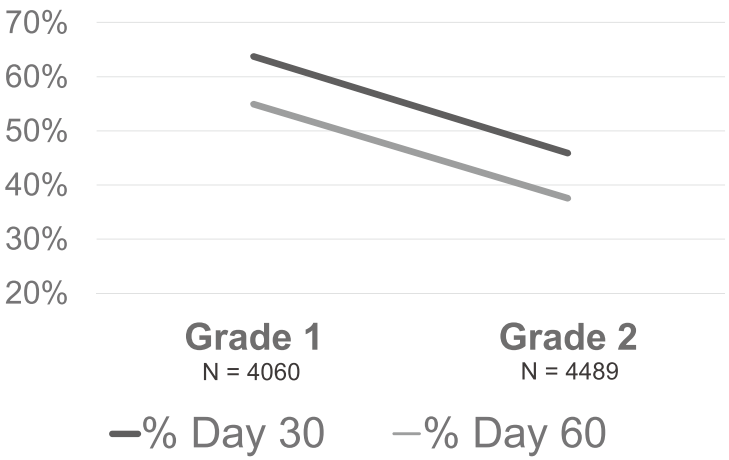
SEMEX Genetics for Life

Donor Potential – cost per embryo

IVF is a fixed-cost model
The potential of a donor will be directly correlated to the cost per embryo



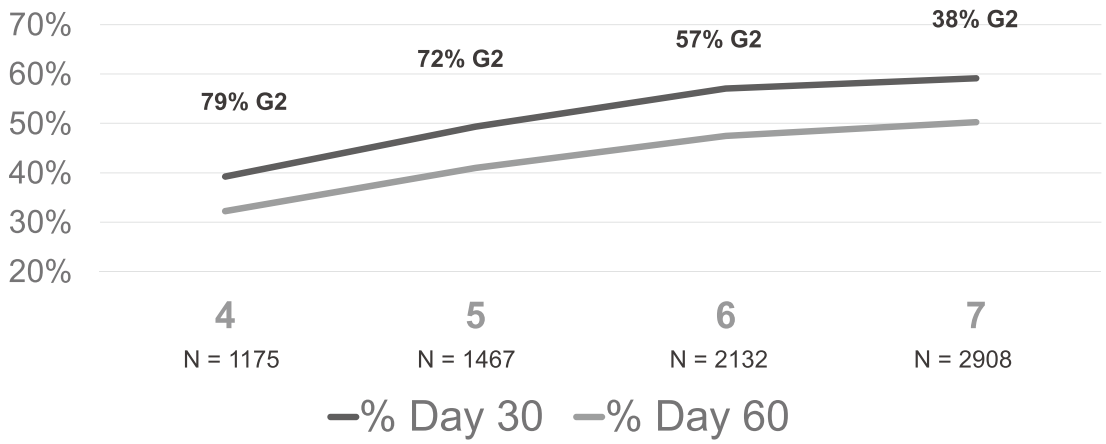
Pregnancy Rates per Embryo Grade Fresh Embryos



Embryo Grade will have a **significant** impact on pregnancy rates
As oocyte quality is correlated to embryo quality, it is imperative donors are superovulated efficiently to increase oocyte quality

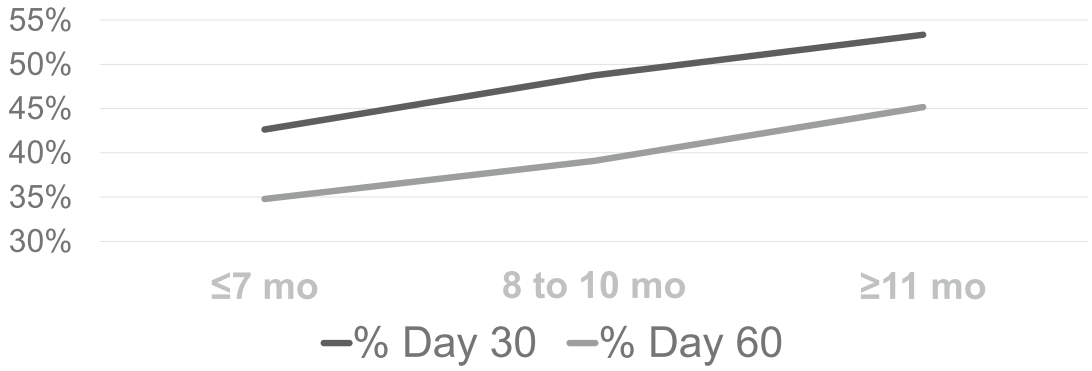
For these IVF sessions, no embryos were frozen so all embryos were transferred fresh

Pregnancy Rates per Embryo Grade
Fresh Embryos



For these IVF sessions, no embryos were frozen so all embryos were transferred fresh

Pregnancy Rates per Donor Age
Fresh Embryos



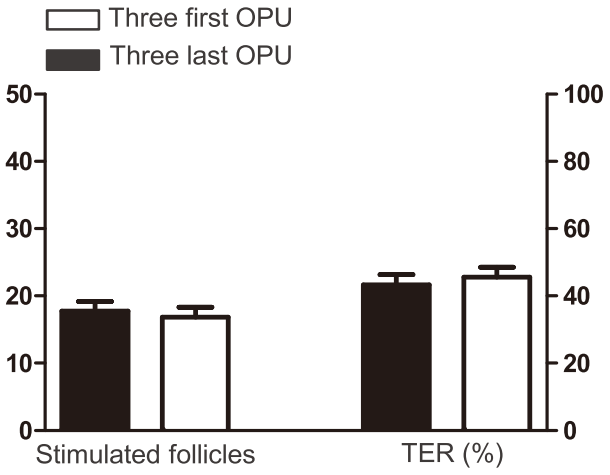
For these IVF sessions, no embryos were frozen so all embryos were transferred fresh

Repeated stimulations

Donors ≥ 10 collections

Every 2 weeks collections

N = 68 donors



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Donor Nutrition

CSIRO PUBLISHING

Reproduction, Fertility and Development, 2017, **29**, 58–65
http://dx.doi.org/10.1071/RD16395

Effects of dry matter and energy intake on quality of oocytes and embryos in ruminants

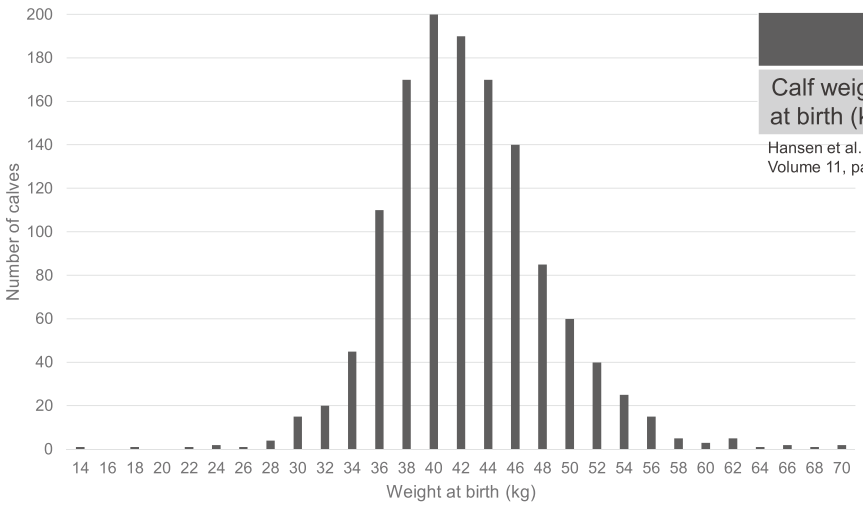
Roberto Sartori[A,C], *Camila Spies*[A] *and Milo C. Wiltbank*[B]

High feed intake, especially associated with moderate to high body condition, before and through superstimulation protocols, natural or induced single-ovulations or before ovum pick-up has detrimental effects on the quality of oocytes or embryos. Feed restriction or high energy supply can be used strategically to obtain either more or better quality oocytes or embryos.

IVF pregnancies

- We have seen some differences in pregnancies following the transfer of IVF embryos produced in our systems
  - 10-20 % of recipients present a delay in mammary development
  - 10 % of recipients calve after 282 days of gestation
    - We will induce recipients that do not demonstrate mammary development up to 14 days prior to due date
  - We have also seen cases of enlarge umbilical cords
    - Probably due to induction cases

IVF calves



	Small Line	Large Line
Calf weight at birth (kg)	39.4	42.0

Hansen et al. Advances in Dairy Technology (1999) Volume 11, page 39

Conclusion

- IVF is a growing technology and will continue to be an important ART worldwide
- If we want to be commercial successful, we must collaborate together as a team
  - Veterinarian + IVF lab + Farmer
- It's all about QUALITY embryos and pregnancies
  - Synchronisation + superovulation
  - Donor potential
  - Nutrition
- Must continue research related to the safe movement of germlasm around the world

Boviteq Team

Dr Christian Vigneault

Dr FX Grand

Valérie Fournier

Thank you

Questions?

Dr Shantille Kruze

Melissa Bowers

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