<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome</td>
<td>2</td>
</tr>
<tr>
<td>OvaGenix Staff</td>
<td>3</td>
</tr>
<tr>
<td>Services Provided</td>
<td>4</td>
</tr>
<tr>
<td>General Terms</td>
<td>5</td>
</tr>
<tr>
<td>Donor, Sire, &amp; Recipient Selection</td>
<td>6 - 7</td>
</tr>
<tr>
<td>Procedures for Donor Stimulation</td>
<td>8 - 9</td>
</tr>
<tr>
<td>Recipient &amp; Donor Heat Detection and Donor Artificial Insemination</td>
<td>10 - 11</td>
</tr>
<tr>
<td>What to Expect the Day of Collections</td>
<td>12 - 14</td>
</tr>
<tr>
<td>Stages &amp; Grades of Embryos</td>
<td>15 - 16</td>
</tr>
<tr>
<td>Storing Frozen Embryos</td>
<td>17</td>
</tr>
<tr>
<td>Labeling of Cane Tabs, Canes, &amp; Goblets</td>
<td>18</td>
</tr>
<tr>
<td>Copy of General Sales Policy and Liability Statement</td>
<td>19</td>
</tr>
<tr>
<td>Contact information</td>
<td>20</td>
</tr>
</tbody>
</table>
Dear New Client,

    We would like to take this opportunity to thank you for choosing OvaGenix as your reproductive and Embryo Transfer provider. We are excited about working with you to achieve your reproductive goals.

    The following is a packet of information on the “How to and What to expect” with your ET program. It contains information on Estrous Synchronization, Follicle Stimulating Hormones (FSH), checking heat, semen handling and breeding, and what to expect the day of collections and transfers. In order to better identify our goals with one another, we ask that you take a few minutes to look at the following information.

    If you have any questions regarding any information, please feel free to give us a call. Thank you once again for giving us the opportunity to meet your ET and other reproductive needs.

    The OvaGenix Staff
OvaGenix Staff

Charles R. Looney, Ph.D.

Don Wideman, Jr MS

Jane Pryor, MS

Chelsie Steinhauser, PhD

Suzanne Menges, MS

Jonathan Chachere, MS

Shelby Grubelnik, MS

Jacob Calloway, BS

Cathryn Looney

Leighton Chachere
Services Provided

◆ Conventional Embryo Transfer

Donor Superovulation and Collection

Donor Single Embryo Collection

Embryo Freezing and Storage

Embryo Transfer

On the Farm Collections

Embryo Shipping

◆ Special Services

In-Vitro Fertilization (IVF)

Ultra Sound Diagnostics

Fetal Sexing

Tissue Banking
General Terms

**Ova** – used to refer to the female gamete (egg) and unfertilized oocytes (UFO)

**Embryo** – used to describe a fertilized egg

**Degenerate** – used to describe an ova that became fertilized, but somewhere between fertilization and removal, the embryo died

**Superovulation** – larger than normal number of ovulations from the treatment with fertility drugs

**In-Vitro** – “in glass”, refers to a process performed in a laboratory and outside the body

**In-Vivo** – occurring in a living organism or under natural circumstances

**Donor** – the female from which embryos are recovered

**Follicle** – a structure on the ovary which contains the immature ovum

**Corpus Luteum (CL)** – a structure on the ovary from which the ovum has been released from the follicle. This structure replaces the follicle structure

**Estrous** – used to refer to the 18 - 23 day cycle of the cow

**Estrus** – used to describe standing heat
**Donor, Sire, & Recipient Selection**

When selecting cattle for an Embryo Transfer (ET) program, whether it is donors, recipients, or sires there are certain criteria you want to establish.

**Donor Selection:**
- 50 days post-partum
- Body condition score of 5 or better
- Nutrition increased 3 weeks prior to program starting and continued until 4 weeks after the program
- Genetic superiority: birth weight, milking ability, and pre and post weaning growth
- Reproductive ability: reproductively mature for heifers – having regular estrous cycles, no reproductive problems – retained placenta, metritis, or cystic ovarian disease.
- Good udder and sound condition

**Recipient Selection:**
- 50 days post-partum
- Body condition score of 5 or better
- Nutrition increased 3 weeks prior to program starting and continued until 4 weeks after the program
- Reproductive ability: reproductively mature for heifers – having regular estrous cycles, no reproductive problems – retained placenta, metritis, or cystic ovarian disease.
- Good udder and sound condition

**Sire Selection:**
- Well-proven bulls
- Produce high quality semen (should be ET certified)
- Use sire summaries to evaluate EPD’s (Expected Progeny Differences – indicate the genetic worth of an animal as a parent when compared to another animal of the same breed)
Cattle should be current on all vaccination and be on a de-worming schedule.

**De-worming and Vaccination Schedule:**

- Cattle should be de-wormed twice a year
- Cattle that have been or are currently in warm southern climates with standing water should be treated for liver flukes.
- Vaccinations – IBR, BVD, PI3, BRSV, Lepto 5, and 7 way clostridia.
- 1 to 3 months prior to calving, administer 8 – way clostridium and scourguard 3KC.

Check with you local veterinarian for a specific plan tailored to your region.
Procedures for Donor Stimulation:

Once a date has been selected to collect your donors, you will receive a copy of a stimulation schedule along with the drugs that you will be using. The schedule you receive is very detailed and explains when to give shots, how much the dosage is for each donor, and when to expect heats on your donors. This schedule will also contain information on synchronizing your recipient cattle. The following is a brief explanation on how to use vaginal inserts, mix the follicle stimulating hormone (FSH) drugs (Folltropin, Ovagen, Sioux BioChem), how to check heat, and artificially inseminate your donors.

Vaginal Insert Application:
A vaginal inserts used in the synchronization of the estrous cycle in cows. It delivers progesterone at a controlled rate into the blood stream of the cow.
1. Wash the external area of the vulva with a diluted Nolvasan solution and rinse with clean water. Vaginitis is a real possibility if this is not done.
2. Rinse the applicator gun between applications.
3. Wash the vaginal insert with a diluted Nolvasan solution.
4. Load the vaginal insert in the applicator gun making sure the string end of the vaginal insert is loaded first.
5. Lubricate the end of the applicator gun with a non-spermicidal lubricant and insert the vaginal insert in the vagina.
6. Position the vaginal insert as far forward in the vagina as possible, push the plunger to expel the vaginal insert. If it is difficult to position the vaginal insert in the vagina, rotate the gun between 10 and 2 o’clock until is slips forward.
7. To remove the vaginal insert, pull on the string. Sometimes the vaginal insert is completely drawn into the vagina and the string will not be visible externally. If this occurs, spread open the vulva and manually search for the string.
Vaginal inserts may be cleaned with water, then a diluted Nolvasan solution, and then rinsed in clean water and can be used for a second time. The vaginal insert needs to be allowed time to air dry, with no exposure to the sun, and then once thoroughly dry, placed in zip-lock bags and saved for a second use.

**Mixing FSH (Folltropin, Ovagen, Sioux BioChem):**

1. Mix prescribed amounts of diluent with FSH powder – be careful as to not to cause bubbles. Let the powder dissolve thoroughly.
2. Be sure to follow stimulation instructions for each donor. Using a 3cc syringe and a sterile 20 x 1 ½” needle for each injection. Pull up the desired amount for that particular donor and administer injections intramuscularly in the lower back of the leg. **Refrigerate any unused portions of FSH.**
3. Injections are given both morning and evening with 8 to 14 hours between injections. For example, an injection could be properly given at 8 am and again at 6 pm or at 7 am and again at 7 pm.
4. Once injections are completed (at the end of the 3 or 4 day schedule) you may freeze any unused portion of the FSH mixture. Be sure to record the date of freezing on the FSH bottle.
Recipient and Donor Heat Detection and Donor Artificial Insemination:

Donor Heat Detection:
On the third or fourth day of shots, the donors are given 2.5cc of Estrumate (or 6cc Lutalyse) at both the morning and evening times. The donors should start showing signs of estrus (heat) in 36 to 48 hours. You need to carefully watch your donors during the critical heat detection period. This is a very vital part of your program. Don’t be alarmed if donors show estrus early or late. Be sure to record on the Stimulation and Breeding sheet the time and date that the donor showed standing heat.

Donor Artificial Insemination:
At standing heat the donor should be inseminated with 1 straw of semen, then 2 straws at the second breeding (12 hours post estrus), and 1 straw 12 hours later. Should you have a donor that does not show estrus, call us for proper instructions. When handling semen, be sure to keep cane in the neck of the tank, below the frost line. Quickly remove needed straws and replace the cane immediately back in the tank. Thaw semen in a 35 degrees Celsius (95 degrees F) water bath for 45 seconds. Be sure to save your straws so that you may properly record the sire name, registration number, cane code and collection code on the paperwork. This is very important. Proper identification of the semen should be checked prior to breeding.

Recipient Heat Detection:
At the same time your donors are showing heat, so should your recipients. Proper detection and record information of the heats is also very vital to your embryo program. Simply record the recipient number, date and time she was in STANDING HEAT. If you have a recipient that showed signs of estrus (see list below), but never had a standing heat, have her available to be checked at the end of the day. Once heat checking is complete, please take your list and carefully check over it. Your
recipients should be easily and clearly identified – **No Duplicate Numbers**.

**Frequently Observed Signs of Estrus:**

1. Nervous and restless  
2. Fence walking  
3. Bawling  
4. Trailing other cows  
5. Head butting  
6. Licking  
7. Mounts other cows  
8. Signs of clear mucus  
9. Ruffled tailhead  
10. External genitalia is red and swollen

The above signs are indications of an animal coming into or going out of estrus. A definition for an acceptable estrus is an animal that is standing to be ridden – **Standing Heat**. Estrus should be observed early in the morning, afternoon, and in the evening for at least 1 hour. If an animal’s tailhead has been painted and the paint is rubbed off this is a good indication of a heat. We do recommend the use of the Heatwatch system, especially for an ET program.

You will find a Recipient Heat form in this package, which can be used to record the cow number, date and time of heat.

*Anatomy of the Female Reproductive Tract*
What to Expect the Day of Collections:

What is Needed:
1. Electricity
2. Running Water
3. Clean, dust free, temperature controlled (72 F) environment for lab purposes
4. Covered chute with a squeeze
5. Heat and a/c for the lab
6. Sturdy table or desk for the lab
7. Bathroom – close by
8. Paperwork containing: Donor – Sire assignments, registration numbers, when the donors were in heat, and a list of recipients and when they were in heat

What to expect:
On the day of collections it is very important that everything run smoothly and quickly to maximize the number and quality of recovered oocytes. If you feel that additional help is needed please do not hesitate to get it. It is very important that on this day there is adequate help available.

The morning of collections, donors need to be up and ready. Donors having early heats or donors that are a priority to be transferred need to be the first to be collected. When a donor comes into the chute, her ovaries are first palpated per rectum to estimate the number of corpus luteum (CL) and follicles. This palpation gives an estimate as to the possible number of ova that will be produced. Once this is completed the donor is given an epidural block of Lidocaine Hydrochloride. The donor cow’s vulva area is then cleaned with water and the tail is pulled forward and tied to the chute with a quick release knot. Now it is time to collect your donor.

When a donor is collected for embryos, a silicon catheter is inserted into the uterine body. Once the catheter is in place a tiny balloon on the end of the catheter is inflated in order to hold the catheter in place. After the balloon
is inflated, the media tubing is then connected and the media begins to enter the uterine body. Once the uterine body is filled with media, the media is then released into a filter that will collect your embryos. Once your donor is finished being collected it is time to bring in the next donor. Just as when administering any drugs, the donor should be observed for any adverse affects.

While the next donor is being prepped for collections, the lab technician takes the filter from the first donor into the “lab” and rinses the filter. Once the filter is rinsed, the grid dish is searched for all embryos. After all the embryos have been recovered, the technician separates out the viable embryos from the degenerate embryos. Once embryos are obtained, they are then assigned stages and grades. Embryos are graded according to an international standard developed by the International Embryo Transfer Society (IETS). This system assigns a number for the embryo stage, then a second number for the embryo grade (see page 14). If embryos are to be frozen, the quicker they are in the freezer the better. We will allow embryos to sit out for no more than 2 ½ hours before they have to be in the freezer. Embryos are frozen in a cryoprotectant, Glycerol or Ethylene Glycol, to prevent damage during the freezing process. Glycerol is commonly known as “3 step thaw” and Ethylene Glycol is commonly known as “direct transfer” or “1 step thaw”. Embryos are loaded in 1/4cc straws with one embryo per straw. Each straw contains a label with information as to the donor, sire, registration numbers, date frozen, and the embryo number (see page 17). Straws are then packaged in canes. A cane tab identifies each cane with a specific number (see page 17). The body of the cane contains the same information that is found on the straw label (see page 17). Straws, goblets, and cane tabs are either in white or yellow. Embryos packaged in white signify the embryos are frozen in Glycerol; if embryos are packaged in yellow this signifies they are frozen in ethylene glycol, direct transfer.

Once all donors have been collected and freezing is taking
place, if necessary, then we will begin to transfer embryos. While collections are taking place, recipient cows should be brought up and sorted according to their heats. Cows showing early heats should be brought in first, followed by the next closest group of heats and so on down to the late heats. Cows that do not show a heat or were unobserved heats should come in last. Late heats will work best for frozen embryos if necessary. After all fresh embryos have been transferred, it will then be decided as to whether or not any embryos will be thawed for leftover recipients.

It is important to remember that embryos are a priority and we will be doing what is in the best interest for your embryos. This is why we ask that everyone be ready to work, cattle are up and sorted when we arrive, and that there be no other cattle work or any interruptions the day of collections.
Stages and Grades of Embryos

When embryos are evaluated they are assigned a stage code, which refers to the developmental stage of the embryo, and a grade code, which refers to the quality of the embryo. These stages and grades are subjective and assigned by our trained embryologist. When there is a monetary value placed on grades of embryos, please don’t ask our embryologists to change their assessment of the embryos. As stated earlier, our embryologist grade embryos according the guidelines set forth by IETS.

Embryo Grades:

Grade 1: Excellent or Good – the embryos is consistent with the stage of development, embryo mass has individual cells (blastomeres) that are uniform in color, size and density. There are no extruded cells off the cell mass. The zona pellucida is smooth with no flat or concave surfaces.

Grade 2: Good to Fair – the embryo has a few irregularities in the cell shape, size, color and density. There are a few extruded cells from the cell mass.

Grade 3: Poor – the embryo has major irregularities. The cell mass tends to be very loose.

*Note: Lower grade embryos do not result in a lower quality calf, but can significantly reduce pregnancy rates if transferred.
Embryo Stages:
The following diagram shows the stages of development:

1. 1-cell (day 1)
2. 2-cell (day 2)
2. 4-cell (day 3)
2. 8-cell (day 4)
2. 16-cell (day 5)
3. Early morula (day 5-6)
4. Morula (day 6)
5. Early blastocyst (day 7)
6. Blastocyst (day 7-8)
7. Expanded blastocyst (day 8-9)
8. Hatched blastocyst (day 9)
9. Expanding hatched blastocyst (day 9-10)
Storing Frozen Embryos:

If you are storing your embryos, preferably keep them in a tank separate from your semen. If this option is unavailable, then it is suggested that you store them in canisters separate from your semen. The canister containing your embryos needs to be clearly labeled. You need to note that embryos should always stay immersed in liquid nitrogen. If we need to store your embryos, please let us know ahead of time.

If you need help in arranging an embryo shipment please contact us for more information. When shipping embryos there are some simple rules that could save heartache and money:

1. Contact the person that you are shipping the embryos to to find out what overnight courier service is in that area.
2. Obtain the exact 911 address of the farm or a description of the location (example - 3 miles west on Hwy 999 and 1 mile east of Hwy 222).
3. Make sure there is the correct phone number of the receiver
4. Ship the embryos over night in a dry shipper that is properly charged. Dry shippers need to be fully charged over night then topped off for 4 hours. After 4 hours, pour off the liquid nitrogen. No liquid nitrogen is allowed in the shipper.
5. Call the receiver of the embryos with the air bill number so the they can call the carrier in case the shipper is delayed.
6. Insurance is important when shipping embryos and should be the responsibility of the shipper.
7. A copy of the Certificate of Freezing should accompany the embryo shipment. The certificate should show which embryos are being shipped along with showing what stages and grades those embryos are and how they were froze and how they should be thawed.

It is extremely important that proper handling and storage be observed in order to maintain the quality of frozen embryos.
Labeling of Cane Tabs, Straw Plugs, Canes, & Goblets

- E1503 XX
- TX0000

- DT1 E1503 XX 123456 Super Donor
- 00JA01 XX 789123 Super Sire

- Super Donor
  - D.T.
  - or
- Super Sire
  - 10% GLY

(The Goblet sits in this area)

- 5 Emb
- # 1 – 5

- E1503XX
- TX0000
- 00JA01
- 7.0d

Super Donor
Super Sire

Donor: Super Donor
Sire: Super Sire

123456
789123

7.0 Day

E1503XX
TX0000
00JA01
5 EMB #1-5
7.0 Day
Copy of General Sales Policy

- **Credit Approval:**
  All new accounts must complete a credit application. If an In Clinic donor program is available, a signed Embryo Transfer Service Agreement must also be returned. (enclosed)

- **Billing:**
  Invoices will be mailed after each flush for all services provided during the current billing cycle

- **Prompt Pay Discounts:**
  All accounts paid within 15 days of the invoice date are eligible for a 2% discount. Large programs are eligible for volume discounts.

- **Finance Charges:**
  Finance charges will be assessed at 1 ½% per month (18% per annum) on all accounts 60 days past due.

- **Account Status:**
  When multiple dates are scheduled, accounts must be paid in full before supplies for next flush / transfer are dispensed.

- **Delinquent Accounts:**
  Accounts greater than two months (60 days) delinquent will be placed on COD status until credit has been re-approved. Finance charges will continue to accrue on COD accounts.

- **Liability Statement:**
  If needed, OvaGenix will store your frozen embryos and/or semen at a cost of 5.00 per can per quarter with a minimum fee of 15.00 per quarter. **OvaGenix assumes NO LIABILITY for this storage.** It is the responsibility of the owner to insure his embryos / semen if he so chooses.

---

**Please sign and return.**

I __________________________________________________________ representing _______________________________ ranch (Print)

I acknowledge and understand the above policies as stated by OvaGenix and will adhere to the conditions of payment and account status.

Signed ___________________________________________ Date: ________________________________________

**FAX to: 979-731-1086**
Contact us at

Main office and shipping address:

4700 Elmo Weedon Rd
Suite 103
College Station, TX 77845

Billing Address:

P.O. Box 3038
Bryan, TX 77805

Contact Numbers:
Office: 979-731-1043
Toll free: 1-866-4-EMBRYO 1-866-436-2796
From outside the US: 01-866-436-2796
Fax: 979-731-1086

Email: info@ovagenix.com
Web Site: www.ovagenix.com