



ET Animals Registration Guide



ET Animals Registration Guide

Contents	page
1. Glossary	3
2. Embryo Transfer	
- Pre flush	5
- ABC form	6
- Completing the Flush Form	7
- Parentage Verified	8
- Calf registration	9
3. IVF Procedures	9
4. Imported Embryos	9
5. Buying and Selling Embryos	9
- DBC form	10
6. DNA testing	
- Hair sample collection protocols	11
7. Pricing	12

1. Glossary

ABC Form	Flush Form. Complete this form to record the details of a flush and identify each embryo recovered from a flush.
AI	Artificial Insemination
DBC Form	An application form to be completed upon sale or export of an embryo, whether implanted or frozen.
DNA Testing	A test provided to record the molecular DNA relevant for use in determining the parents of the animal.
Donor Dam	The biological dam from which embryos' have been flushed
ET	Embryo Transfer
Flush	The procedure of recovering one or more embryos after superovulating the female, then fertilising the resulting eggs.
HA	Holstein Australia
IVF	In-Vitro Fertilisation
Parentage Verified	The parents of an animal are verified by matching DNA of the animal against DNA of the parents. This means the animal and both its sire and dam is required to be DNA Tested. If a sire is an AI sire, it most likely has already been DNA tested.
Recipient Dam	The female cow in which the embryo has been implanted.



Embryo Transfer and IVF Procedures for Farmers

Holstein Australia (HA) provides a service for farmers allowing registration of calves born from an Embryo Transfer or In-Vitro Fertilisation program. Embryo Transfer programs have become popular since the first ET animal was registered in the 1970s.

In order to accurately record the calf details, a set of procedures is provided by HA. By following these procedures, a farmer can take comfort in knowing there would be minimal errors when carrying out these programs.

HA requires ALL calves born from these programs to be parentage verified through DNA testing.

HA has recently simplified the steps required to register an ET calf, as well as reduced the cost to members.

2. EMBRYO TRANSFER

STEP 1. Pre-Flush Preparation

Prior to flushing the Donor Dam, the Donor Dam should be DNA Tested. The results of this test are then used to verify the parentage of the resulting calf. By DNA testing the Donor Dam at this step you are ensuring her DNA information is available when you are ready to register the calf without delay. (Occasionally the donor dam is not available for DNA testing when the calf is ready to be registered. For example, the dam may die before its ET calf is born. If this does happen, contact HA and speak to an ET Officer)

STEP 2. Flush the Cow

HA recommends you fill out a [*Flush Form*](#) as soon as the flush procedure has taken place. Your local veterinarian may use their own “Flush Form”. The same details need to be completed on their form. The “Flush Form” is also called the “ABC Form”; these are the same form.

The “Flush Form” is available for download from the Holstein Australia website; complete the form and make 2 copies. The original is required to be sent to HA Office.

The “Flush Form” identifies the donor dam, sire/s used, along with details of every embryo recovered.

Embryos, once recovered, are either implanted immediately into a recipient, or frozen for later use. When frozen, they are stored in identified straw/s. Frozen embryos can be thawed and implanted at a later date. The details of each of these steps must also be recorded on a “Flush Form”. This means a farmer may have to complete a “Flush Form” on several occasions.

See next page for an example of a blank Flush Form



The ABC Form is the standardised Format of the International Embryo Transfer Society.

ABC
FLUSH FORM

A. CERTIFICATE OF EMBRYO RECOVERY

PRESCRIBED Lodging Fee per flush to accompany Certificate A.

Donor Cow Name _____ Herdbook No. _____ DNA Case No. _____

Donor Cow Owner _____ Address _____

_____ HFAA Member No. _____ Tel. No. _____ Onset _____ AM
 _____ Oestrus Date _____ PM
DD/MM/YY

Service Sire (1) _____ Herdbook No. _____ Breeding Date _____
DD/MM/YY

ID Code _____ Freeze Date or Batch No. _____ Sexed Semen X or Y _____ Recovery Date _____
DD/MM/YY

Service Sire (2) _____ Herdbook No. _____ Total Recovered _____

ID Code _____ Freeze Date or Batch No. _____ Sexed Semen X or Y _____ No. Cleaved/Degen. _____

Signature _____ Date _____ No. Unfertilised _____
 _____ ET Code _____ No. Transferred _____ (See B)
 _____ Are the embryos IVF? Y/N _____
 _____ Lodging Fee \$ _____

B. CERTIFICATE OF EMBRYO TRANSFER

Record ALL fresh transfers (downwards) and freezing details (upwards) on date of embryo recovery. Lodge BREED COPY (original) as soon as possible, but within 6 months of flushing in the case of fresh embryos. Delete non-pregnancies before lodgement.

EMBRYO IDENTIFICATION: RECIPIENT

One embryo was transferred to each of the following recipients:

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15.	Recip. Tatt or Ear Tag No.	Recipient Registration No.	Breed	Days Since Oestrus	Stage Code	Qual. Code	Embryo Manipulated N, D, F, M or U	Thawed		Pregnancy Confirmed	Comments
								Straw No.	Transfer Date		

Frozen Embryo Cane No.	Frozen Straw Id No.	No. Embs. /Straw	No. X Washed	Stage Code	Quality Code	Embryo Manipulated N, D, F, M or U	Zona Intact	Trypsin Treated	Comments

EMBRYO IDENTIFICATION: FROZEN EMBRYO (complete upwards as part of Certificate of Freezing)

Type of container
 Straw _____ Ampule _____ Other _____ Each container labelled to show firm code, breed, reg.no. of donor, freeze. Str. or Amp.no.

Signature _____ Date _____ Phone(____) _____
 _____ Practitioner that transferred/froze the embryos _____ ET Code _____

*Use comments column for any special notations and/or to identify the location of the opposite half of a divided embryo. Pregnancy can be noted.

C. CERTIFICATE OF FREEZING

Time from recovery to onset of freezing _____ (hrs.) Cryoprotectant and concentration, final molality and cooling procedure _____

How Frozen: Seed Temp. _____ Cooling Rate _____ Plunge Temp. _____ Other _____

Recommended method of thawing and dilution _____

N.B. Conditions for lodging Embryo Certificates appear on page2. Please complete this form and forward the original to Holstein Australia, PO Box 489, Hawthorn BC, 3122, within 6 months, together with applicable fees. Take two copies, one for the PRACTITIONER and one for the OWNER to keep. Complete DBC form in the event of a change of ownership in a transferred or frozen embryo. DBC forms are available on request from Holstein Australia and on this website.

Completing the Flush Form

- First, after original flush, fill out the “Flush Form”, recording ALL embryos recovered and whether each embryo is implanted or frozen. *For implanted embryos a second “Flush Form” is not required.*
- Embryos that have been frozen and stored require a new “Flush Form” to be completed and forwarded to the HA Office each time an embryo is thawed and implanted into a recipient dam.
A separate form is required for each donor dam but several embryos from the same flush can be placed on the one form.
When completing the second form please make sure you provide the original recovery date as well as the date of transfer into recipient.

The following summarises the information required in order to complete the “Flush Form”:

- Donor Dam’s full identity
 - Including Full Name & Herdbook number
Note: Just writing “Daisy” as an Identification is not enough
- Sire’s Identity
 - Use Herdbook Number and Full Name
No Nicknames please e.g. “EMORY” is NOT a NASIS code
 - If there are two sires used both sires must be identified.
- Breeding (AI) and Recovery (Flush) dates.
- 1. Total number of Embryos’ recovered. *(Note: complete for flush only)*
2. Total number of Embryos’ implanted. *(Note: complete for flush only)*
3. Total number of Embryo’s frozen. *(Note: complete for flush only)*
- Record the identity of the embryo

If frozen, the straw in which embryo is stored has a label with an identification code. It is this code which is to be recorded on the “Flush Form” to identify the frozen embryo. Some straws may have more than one embryo in it and this also needs to be recorded. Please note, it is a requirement of HA that no more than **one embryo** is implanted into a recipient.

For fresh, or thawed and implanted embryos, the Recipient Cow Identification needs to be provided. (This identification can be an Ear tag Id, Tattoo, Herd Test Number, Nickname, Herdbook number if registered, etc. This is sufficient to identify the recipient cow.)

The completed form should be forwarded to HA. HA recommends you send in this form as soon as possible after the flush has taken place.

If the “Flush Form” is **sent in early**, the details will be available when the calf is born and subsequently registered. This makes the registration process easier by reducing the number of possible errors.

HA recommends you provide full name and herdbook number when providing the donor dam and sire’s identification, along with the original recovery date.

There is a charge when submitting the original “Flush Form”. Subsequent forms submitted regarding thaw and implantation of embryos will not incur a charge.

It is required that you supply details of ALL embryos recovered during the flush on the first “Flush Form”. If you supply extra information regarding a flush (e.g. more embryos than originally specified) after the “Flush Form” has been submitted, extra charges will apply.



STEP 3. Parentage Verified

After the calf is born you are able to register your calf. It is easier if a farmer registers the calf and requests a DNA Testing Kit at the same time. There are two exceptions to this. The first is when two sires are used during a flush and the second for IVF calves. Please see below for more detailed instructions for IVF and two sire flushes as in both cases, the calf cannot be registered until it has been parentage verified.

HA requires **ALL ET and IVF calves MUST be Parentage Verified.**

If the Donor Dam has already been DNA Tested, this should be a simple procedure of DNA Testing the calf. A parentage request will be made from HA.

If the Donor Dam has not been DNA Tested, you will receive a DNA Testing kit for each of the calf and its dam. (HA recommends you DNA test the dam prior to flushing her and recovering embryos)

Q. What happens if 2 Sires were used during the Flush?

Proceed with step 1 and 2, including both sire identifications on the “Flush Form”. In step 3, ALL calves from the flush MUST be parentage verified PRIOR to registering the calf. You must contact HA office and request DNA testing kits for

each calf to be parentage verified. Once their parentage is known, the farmer can proceed to register the calf.

STEP 4. Register the Calf

When registering the calf you will be required to provide the following details in addition to the normal registration information:

- Recovery Date
- Recipient Cow Identification

If your calf has not been parentage verified at the time of registration (i.e. you registered and requested a DNA test kit at the same time), your registration certificate will be held until the test results are received and the parents are correct. (The calf registration is PENDING until verification occurs)

3. IVF PROCEDURES

Follow the ET procedures Step 1 to 4, except as follows:

At procedure 3

ALL IVF calves MUST be DNA verified PRIOR to registering the calves.

4. IMPORTED EMBRYOS

Please discuss these with the registrar if you are considering importing embryos.

NOTE: ALL registrations, in this case, are sent in to HA on **paper**.

Telephone HA on 03 9835 7600 to speak to an ET Officer.

5. BUYING AND SELLING EMBRYOS

(Frozen and/or recipient cows in calf)

The seller (Vendor) is required to submit the HA's "DBC form" for the transfer of ownership of an embryo or recipient in calf. *See over the for a blank DBC form.* Information required on this form:

- Buyer's details
- Sale Date
- Donor Dam's Id
- Sire details
- Recovery date of Flush (*Important*)
- Number of embryo's sold
- Recipient dam details or Frozen Straw Ids

Send this form to HA, PO Box 489, Hawthorn East, Victoria 3122

It is important to send in this form as soon as possible after the sale of an embryo in order to have these details available for the new owner to register the impending calf.



D. APPLICATION FOR EMBRYO EXPORT/CHANGE OF OWNERSHIP

Application for domestic/International Movement. To be submitted to breed organisation in exporting country, together with Lodging Fee.

Name of Buyer _____

Address _____

State _____ Country _____

Sale Date _____ Export Date _____ Certification of DNA Genotyping/Blood Typing needed by Buyer: YES/NO

Donor Cow _____ Herdbook No. _____ DNA Case No. _____
cc

Owner _____ Breeding Date _____

Address _____ Recovery Date _____
DD/MM/YY

Service Sire (1) _____ Herdbook No. _____
 Sexed Semen X or Y _____ cc

Service Sire (2) _____ Herdbook No. _____
 Sexed Semen X or Y _____ cc

Name of Seller _____ Signature _____ No. Sold _____
Owner of Donor Dam on Date of Recovery

Address _____ Lodging Fee \$ _____

B. RECIPIENT IDENTIFICATION – SALE OF FRESH/THAWED EMBRYOS

Information copied from Breeder's original ABC document (Flush Form) previously lodged with breed organisation.

Date of Embryo Transfer _____ Surgical _____ Non-Surgical _____ Freeze Date _____ Str. Or Amp
DD/MM/YY on Str. Or Amp. Number/s _____

Signature _____ Firm _____
Practitioner freezing the embryo

ET Code _____ Phone () _____

Tattoo or Ear Tag No.s	Herdbook Registration No.	Breed	Days since Oestrus	Stage Code	Quality Code	Embryo Manipulated N, D, F, M or U	Embryo Fresh/Frozen	Comments
1.	_____	_____	_____	_____	_____	_____	_____	_____
2.	_____	_____	_____	_____	_____	_____	_____	_____
3.	_____	_____	_____	_____	_____	_____	_____	_____
4.	_____	_____	_____	_____	_____	_____	_____	_____

C. EMBRYO IDENTIFICATION – SALE OF FROZEN EMBRYOS

Information copied from Breeder's original ABC document (Flush Form) previously lodged with breed organisation.

Type of container Straw _____ Ampule _____ Other _____ Each container labelled to show firm code, breed, reg.no. of donor, freeze. Str. or Amp.no.

Cane No.	Straw or Amp. No.	No. Embs./ Str. Or Amp.	No. X Washed	Stage Code	Quality Code	Embryo Manipulated N, D, F, M or U	Zona Intact	Trypsin Treated	Comments
_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____	_____	_____

Time from recovery to onset of freezing _____ (hrs.) Cryoprotectant and concentration, final molality and cooling procedure _____

How Frozen: Seed Temp. _____ Cooling Rate _____ Plunge Temp. _____ Other _____

Recommended method of thawing and dilution _____

Signature _____ Date _____ Phone() _____
Practitioner that transferred/froze the embryos ET Code _____

N.B. Conditions for lodging Embryo Certificates appear on page 2. Please forward the original completed form, together with fees, to Holstein Australia, PO Box 489, Hawthorn BC, 3122, within 30 days of sale or change of ownership. Photocopy twice, for SELLER'S COPY and BUYER'S COPY.

6. DNA TESTING

How to get a DNA Test

1. Ring HA to request a DNA testing kit. (Ph: 03 9835 7600) This will be sent to you.

The DNA testing kit comprises the following:

- # A sample card in a plastic sealable bag
- # A label identifying the animal you are going to be testing.
- # A letter of request to DNA Test your animal

One DNA testing kit is provided for each animal requested. (i.e. If you are testing 9 animals, 9 kits will be sent out)

2. Follow the instructions on the back of the card. Attach hair samples (including hair root) and label. Place the card (with hair sample and matching label attached) into the clear plastic bag.
3. RETURN the sample/s to
ET Registrations, HA, PO Box 489, HAWTHORN BC, Victoria 3122

(Note: HA requires that all samples are sent through our Office in order to receive the discount from our service provider.)

HA will forward the sample/s to the laboratory. When results are received and finalised, charges will be applied to your member account and you will receive a copy of the results from HA.

Protocol for Collection of Hair Samples for DNA Analysis

DNA is contained in the root or follicle of a hair, not in the shaft itself. Therefore it is essential that the hair follicle is attached to each plucked hair being submitted for DNA analysis.

SHORT, FINE BODY HAIRS ARE NOT SUITABLE FOR DNA ANALYSIS. ONLY THICK COARSE, THICK TAIL HAIRS WILL BE ACCEPTED.

Collect long thick hairs from the switch or brush of the tail.

1. It is essential that hairs submitted for DNA analysis are thoroughly **dry**.
2. Collect a minimum of **20** hairs from the **tail** of the animal to be tested. **Pluck** the hairs from the switch or brush of the tail, **DO NOT CUT THE HAIRS**. (You could wrap the hair around a pencil and pull.)
3. Ensure that each extracted hair has a **follicle (bulbous hair root) attached**.
4. Place all of the hairs from one animal **onto the card** supplied with the DNA kit and affix with the supplied label, as instructed on the **back of the**

card. Place the card back into the **snap-lock plastic bag** (provided when you request your DNA test kit). **Do not place the hairs directly into envelopes.**

5. Place the labelled samples that are in plastic bags into an envelope. Post the sample(s) to HA.

Q. What do I do if my cow does not have a tail?

If your cow does not have a tail you can collect from inside the ear, or perhaps even under the belly – just as long as you do not collect short, fine body hair.

7. PRICING

Price Structure for DNA Testing *(prices current at time of printing)*

Fees will be charged to the member account after the testing has been completed.

Labelled DNA Test samples are to be forwarded to HA for processing.

ALL Prices are GST Inclusive.

Lodgement Fee, ABC (Flush) Form	\$22.00
Lodgement Fee, DBC Form	\$22.00
DNA Type from Hair sample	\$36.30
Parentage determination/verification	\$5.50
Request of URGENT DNA profiling	\$11.00

If a full DNA type is unable to resolve parentage then additional tests are available at a cost of \$7.70 per test per animal.



Holstein

A U S T R A L I A

www.holstein.com.au

24-36 Camberwell Road
HAWTHORN EAST VIC 3122

Phone 03 9835 7600
Fax 03 9835 7699
Email enquiry@holstein.com.au