

Section 6 – Guidelines for Recording Artificial Insemination and Embryo Transfer and Reporting Fertility

Section 6 – Al and ET Version October, 2017

Network. Guidelines. Certification.

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Change Summary

Date of Change	Nature of Change
August 17	Combined old Section 6 and old Section 8 into new Section 6.
August 17	Reformatted according to new template.
August 17	Reordered with AI, followed by ET and then fertility reporting.
August 17	Edited heading to better reflect contents. Save as V17.00.
August 17	Accept all changes and save as V17.01.



August 17	Add annexes. Now V17.02. Edit headers for clarity. Remove breed code table – now replaced by link to Interbull website where master copy is held.
August 17	Stopped Track change sand accepted all previous changes.
August 17	Moved the file to the new template (v2017_08_29).
August 17	Fixed some wrong bulleted list.
August 17	Edit headers correct language errors.
August 16	Update version in title to August 2017.
Oct. 2017	Hyperlinks have been corrected
Feb. 2018	Bubalus species in italics and minor corrections on numbering of some bullets. First table in Annex 1 had some empty cells that have been taken from the original and fixed



1 Bovine Semen Straw Marking

1.1 Objective

The guidelines define the **minimum information** to be printed on a bovine semen straw.

If additional information is to be printed, general recommendations are also given in order to help the users.

1.2 Field of application

The guidelines apply to bovine semen straws used for international trade, for either fresh or deep-frozen semen.

1.3 Definitions

To code the identification of semen in bars and print it on semen straws for an use on field during the insemination act by AI technicians or farmers in do it yourself, following recommendations have to be implemented:

- a. "2a" means two digits with an alphanumeric format.
- b. "3n" means three digits with a numeric format.
- c. "Bovine" means domestic animals of the genera *Bos, Bubalus* and *Bison* (include in particular the bovine species *Bos taurus, Zebu Bos indicus*, Indian buffalo *Bubulus bubalis*, American bison *Bison bison* and European bison *Bison bonasus*).
- d. "Bull" means a bovine male as defined above.
- e. "Ejaculate" means the semen released by one ejaculation.
- f. "Collection" means the entire successive ejaculates from the same donor in the same day.
- g. "Collection sequence for a given location and a given day" means the rank of the ejaculate within the bull collection (/n) or for all the bulls (/nn). It is also called charge number.
- h. "Semen collection centre" means an approved and supervised establishment in which semen is collected and processed for use in artificial insemination.
- i. Semen processing centre" means an establishment in which semen is processed for use in artificial insemination.
- j. "ISO country code" refers to the 2a list of codes ISO 3166.
- k. "International identification" means a unique registration number provided by the country for all the bovine animals and preceded with the ISO country code.
- 1. "Bull code" means any code used to identify the bull for the management.
- m. "Uniform bull code" means the unique identification used by NAAB comprising the so-called "stud code" (3n), the breed (2a) and a number (5n) unique within the "stud" and the breed.
- n. "Bar code" means a system for coding alphanumeric numbers in bars deciphered by reader.



- o. "Batch of semen" means a group of semen straws produced from ejaculate(s) of a specific bull, on a specific day, in a Semen Collection Centre (SCC), with the same specific treatment (ie extenders, sexing, specific dilution...).
- p. "Batch identification" means a unique number to identify a batch of semen within a SCC either a serial number or combination of bull ID, collection date, ejaculate number. The Batch identification format is left to the action of SCC.
- q. "Stud or marketing code" means a unique code assign by the NAAB (National Association of Animal Breeders) to identify SCC or AI marketing organisation. A fee is paid at first registration and then each year in case of commercial activity in the US.
- r. "Barcode number" means a stud or marketing code + batch identification.

1.4 Straw identification

1.4.1 Summary of the straw identification as a minimum requirement

- a. Semen collection or processing centre code
- b. Breed (2a).
- c. Identification of the bull-
- d. Collection code (yyddd).

1.4.2 Printing

The printer should be an ink jet printer to ensure the legibility of the information.

1.4.3 Order

Guidelines do not address the order of the information.

1.5 Information related with the semen collection or processing centre

The collection or processing centre from which semen is issued should be identified with a code. According to legal basis or to industry agreement this code should be either the collection code or the processing code. Within a country, centre codes printed on straws is either processing or collection and this information is available on reference lists.

If the semen is intended to be used within the European Union (EU), according to the Directive 88/407, the code should be the official EU code assigned for the approved "semen collection centre".

Outside of the EU another code to identify the processing centre can be used, for example the "stud code" assigned by the NAAB.

1.6 Information related to the bull

1.6.1 Breed

The recommended format is 2a.

The ICAR list of the most relevant breeds for the international trade in semen is maintained by the Interbull Centre and available <u>here</u> on the Interbull website. The breed code can be presented alone or as an integrated part of the uniform bull code.



1.6.2 Identification of the bull

It can be either the Interbull international identification (IID) code or a world-wide unique bull code.

The international identification comprises the ISO country code (2a) and a registration number of the bull within the country (max 12n), for instance FR1234567890. This international identification is used for any purpose including traceability. It may or may not be the HB number.

If a bull code is used, it must be cross-referenced on the transport documents with the international identification of the bull.

This bull code can be:

- a. The "uniform bull code" from NAAB (example 132HO12345).
- b. A unique national bull number preceded by the ISO country code (example FR12345) .

1.7 Information related with the semen

1.7.1 Collection code

It is recommended to print the collection date with the Julian format "YYDDD" where YY is the two last digits of the year (99, 01) and DDD is the day number (from 001 to 366).

The collection sequence is considered as an additional information, but if it is printed, it should be adjusted to the date separated with a slash "YYDDD/1".

1.7.2 Format for additional information

- a. **Name**. Either the short name (commercial name) or the full name can be used.
- b. **Collection** sequence. It should be adjusted to the date separated with a slash "YYDDD/1".
- c. **Compulsory information**. Within the EU, semen produced has to be labelled with its IBR status. The format for this has to be defined by the European Commission and will be part of this recommendation as soon as it is available.

1.8 Barcode identification of straw

To code the identification of semen in bars and print it on semen straws for an use on field during the insemination act by AI technicians or farmers in do it yourself, following recommendations have to be implemented.

1.8.1 General rules

- a. Barcode doesn't substitute for official visible identification of semen straws that is printed according to ICAR Guidelines, in 1.4 above, page 6.
- b. Barcode with the system 128C (see a. in 1.9.6 on page 10 below) is highly recommended.
- c. The barcode must be as short as possible because it's the principal factor to obtain a high percentage of success when reading it. According to the state of art in 2008, for an easy reading on field, a maximal number of 13 digits is suggested.
- d. Barcode is recommended to contain only numeric characters (see b. in 1.9.6 on page 10 below).



- e. Some characteristics of the straws (ie colour...) affects the readability of the barcode. So it's recommended to test straws before use with barcode.
- f. The barcode number (n. in 1.3 on page 5 above) refers to an unique ID for any batch of semen (see p. in 1.3 on page 5 above).
- g. The format of this number
 - Refers for it's first 3 digits to an unique reference number of SCC allocated by ICAR as described in 1.7.1 on page 7 above and defined in h. in 1.3 on page 5 above.
 - Refers for the other digits to an unique batch identification as defined in p. in 1.3 on page 5 above.
- h. The list of ID of SCC utilising a barcode system is maintained by ICAR. This list is unique in the world and consistent with the list of SCC ID (Stud and marketing codes) allocated by the NAAB.

1.8.2 Allocation of ID of SCC and publishing barcode format

- a. Any SCC utilising a barcode system for international usage has to inform ICAR. ICAR will allocate a unique ID to the SCC.
- b. SCC provides ICAR with the ID stud or marketing code allocated by the NAAB if it already has one.
- c. An allocated ID will be valid for 20 years after the day when the SCC stops its activity to guaranty its uniqueness in bar coding. It doesn't change if the SCC modifies its system of barcode.
- d. At the same time, the SCC informs ICAR of the format of the barcode number. ICAR publishes this format on the web site and renders it accessible to any user (§4).
- e. ICAR and NAAB manage together the system of allocation of ID SCC and fix various problems arising in using an unique ID for the 2 organisations:

1.8.3 Management of barcodes within SCC and for the movements of semen

- a. Any SCC running a barcode system maintains a data base where any barcode number on straw is cross referenced with the official data printed on straws. Optional information may also be attached to the data base.
- b. Any client receiving semen may get from its supplier (distributor, AI Company...) the necessary data to cross reference barcode number with the official straw identification in accordance with these ICAR guidelines and eventually optional requested information.
- c. After reading it is highly recommended that the barcode number is stored in the user data base as a raw data.

1.9 Explanatory notes

Comments on the recommendation

1.9.1 History of the discussions

Several attempts have been made to define an international recommendation for straw identification from which are the following:

a. ICAR proposal of September 1995



- b. IFAB proposal of June 1998
- c. QualiVet proposal of November 1998

All these approaches were to define precisely the entire sequence to be printed on the straw and tried to combine the requests of the different countries. As a result, the previous statements resulted in rather long identification and eventually failed to reach a full agreement from the different countries.

The actual recommendation tries more to set up the principle of the identification rather than to reach a full agreement on the sequence printed on the straws.

Basic ideas were:

- a. The straw should not be considered like a database by itself.
- b. The minimum information for official recording purposes is 'centre/bull/date' and for field recording by the technician 'bull/date'.
- c. For ease of use by the technician on farm and accuracy, the number of data items should be kept as few as possible and in large print.

1.9.2 Semen collection centre

The "semen collection centre" is a specific facility for the collection of bull semen and should not be confused or replaced by the 'owner identification'.

It is the approved and supervised "semen collection centre" which should be under an obligation to ensure that the semen has been obtained from animals whose health status is such as to ensure that the risk of spread of animal disease is eliminated, and has been collected, processed, stored and transported in accordance with hygienic rules and rules which preserve its health status.

1.9.3 Collection code

Printing a date instead of a code is advisable for transparency to the customer.

Most people prefer having a "real" date like "11 March 99" than a Julian date YYDDD comprising year + day in the year.

The main reason why the Julian format was chosen was the ambiguity of the information 02/05/03 that could be naturally interpreted in different countries as DDMMYY YYMMDD or MMDDYY. Another reason is the compactness of the Julian format (5n) and the ease of reading with the sequence number (99032/1).

The collection sequence is considered as additional information because lots of centres mix the ejaculates of the same collection and thus do not want to systematically print "/1" for nothing.

1.9.4 Name

Some people support a short name that is easy to read for the technician and others prefers a full name to avoid confusion between bulls. No agreement could be reached for the format.

1.9.5 Identification

The international identification is up to now the only identification universally accepted world-wide. It was logical to recommend that this be the minimum printed on the straw. But since this identification is long, it is not practical to read it in liquid nitrogen neither to record it on farm for the insemination. Every country is thus using either the bull name or a bull



code. An agreement was reached to not impose the international identification for those countries used to managing unique bull codes, but it can also be used when required.

1.9.6 Barcode identification of straw

- a. We choose to recommend the type of barcode for several reasons: readers are not able to read all the types of barcodes and the 128 C type is compact (about 17 mm long for a 10 digit numeric barcode and 23 to 25 mm long for a 13 digit numeric code). However as technologies advances this recommendation may change.
- b. In a barcode 128C, alphanumeric characters take 3 to 4 times more space than numeric characters. Considering the elements to write on a straw, numeric characters are presently more compatible with the available space.

1.10 Procedure to handle breed codes on semen straws

1.10.1 Article 1 - Purpose

The general purpose of the list of breed codes is **to facilitate traceability of semen** that is traded across country borders. The code should thus be used to identify the breed of the bull on semen straws that are used in another country than the country of origin (sampling)."

1.10.2 Article 2 - Exceptions

The breed codes printed on straws do not apply:

- a. To identification of breeds in the international genetic evaluations for bulls offered by Interbull.
- b. To procedures of registration of the progeny: the use of the breed code printed on the straw doesn't make provision for the breed of the calf born out of the insemination using the semen unit nor it's registration in the Herd-Book of the breed of the sire.

1.10.3 Article 3 – New Breed Codes

Breeds can be added to the code list, provided semen from bulls of the breed is exported in a **significant number** and to a significant number of countries.

In 2004 "significant number" means that more than 10 000 doses have been exported in more than 3 countries. These figures may change according to the experience in processing such demands; then new rules will be published.

1.10.4 Article 4 – Evidence Required

Any party requesting that a breed is added to the list of codes should provide **unequivocal evidence** that:

- a. The breed does not belong to a breed already on the list.
- b. The breed is recognized as a separate breed, e.g. recognized by a breed society.
- c. There is significant international exchange of genetic material from the breed, e.g. by showing country of origin, number of doses (semen straws) produced in country of origin, number of doses (semen straws) exported, according to article 3.



1.10.5 Article 5 – Other Party Support

Any party requesting that a (local) breed is added to an already **existing group of breeds** should provide unequivocal evidence that the request is warranted. The request should be seconded by at least one other party representing a breed already included in the group.

1.10.6 Article 6 – Unique and Relevant

New breed codes should be unique and should be assigned based primarily on the name and/or abbreviation of the breed used in the country of origin. The second character in the breed code should be exchanged if the most logical 2-character code is already in use.

1.10.7 Article 7 – Interbull Centre Role

The list of breed codes is maintained by the Interbull Centre. **Requests for updating** or adding breed codes should be submitted to the Interbull Centre by e-mail, fax or letter. Requests, and results of requests, should be officially announced on the Interbull web-site accommodating the breed code list.

1.10.8 Article 8 – ICAR Website

This procedure is published and up-dated on the ICAR web site as part of Section 6 of the ICAR Guidelines.

1.10.9 Article 9 - Appeals

If a requesting party disagrees with the result of the procedure handled as describe before it may submit the case to ICAR board, that will make the final decision.

2 Bovine Embryo Production and Transfer

2.1 Object of the recommendation

The purpose of this recommendation is to improve quality of data in Embryo Production and Transfer on cattle in respect of assessment parentage of calves born out of this technology. It takes into account existing rules or guidelines already laid down to guaranty high level of exchanges at international level and is considered as an extension of those rules. It recommends the minimum items that should be recorded for using embryo data and the minimum of controls that data must undergo for being declared as valid.

It is a complementary addition to international rules governing embryo trade such as:

- a. Veterinarian requirements issued by EU or other national/international bodies.
- b. Zootechnical requirements issued by EU or other national/international bodies.
- c. Technological guidelines adopted by the IETS.

2.2 Field of application of the recommendation

The recommendation applies usage of embryo data to establish parentage of calves born out of embryos prior to registration in the herd-book. It also provides elements to insure embryos traceability.

It applies on females from which embryos are recovered, their sires and to the recipient female whatever the technology used to produce embryos subject to future transfer, such as classical production technique, IVF, splitting, embryonic cloning.



It applies for embryos produced within country or imported from the other countries

It doesn't apply to recording of data used for technology purposes such as:

- a. Assessment of embryo's quality.
- b. Processing of embryos for freezing, or other technique (splitting, sex assessment) Then IETS guidelines including forms have to be used for international exchanges of embryos.

DNA references (or blood types as exceptions) have to be provided for the genetic parents of embryos. DNA references are the ISAG list of markers.

2.3 Definitions

- a. AI: insemination to produce embryos from a donor female, heifer or cow.
- b. **Donor female:** female chosen as genetic mother of future calves born out of embryos.
- c. **Double** AI: two AI carried within a short lap of time, e. g 48 hours, on the same female with or not the same bull often to produce embryos. This information is recorded to avoid rejection when verification of dates.
- d. **Embryo transfer:** Implantation of embryos produced in vivo or in vitro into a recipient female
- e. **IETS**: International Embryo Transfer Society: professional forum for the exchange of information among practitioners, scientists, educators regulatory officials. IETS is providing a handbook of forms and certificates for the benefit of practitioners. The updated IETS forms are available on www.iets.org
- f. **IVF**: in vitro fertilisation. Technique used to fertilize oocytes recovered from donor females with bull semen outside the genital tractus of donor female on the lab. Oocytes may be recovered either by ovum pick up either at the abattoir.
- g. **Operator**: Person qualified to recover embryos from donor females and or to perform transfers of embryos. Also person qualified to carry out OPU of IVF. An operator is a member or acts under the responsibility of a team.
- h. **OPU**: Ovum pick up. Technique allowing recovering oocytes out of an ovary from a living donor female. Then oocytes are fertilized on the lab by bull semen.
- i. **Recipient female**: Female treated to be subject to embryo transfer. After calving she will be called as "non-genetic mother".
- j. **Recovering embryos**: Technique used to recover embryos by flushing a donor female inseminated with semen from one or two bulls.
- k. **Registration**: In the sense of this recommendation "registration" of an animal, recipient , donor cow or bull, refers to an animal with an unique identification notified to a data base. The same term applies for herds.
- 1. **Reproductive cloning**: technique used to multiply nuclei of embryos produced in vivo or in vitro.
- m. **Teams**: Officially approved bodies to collect embryos or oocytes and/or to transfer embryos. References of approved teams are published by national or international



authorities (codes, address, responsible person), with the scope of approval: nationally- internationally.

2.4 Recording of relevant data

Data that have to be recorded in order to insure the parentage of calves born out of embryos and the traceability of embryos refer to:

- a. Recovering embryos, including IVF procedures (see 2.4.1 below).
- b. Embryo transfer (see 2.4.2 below).

Moreover it will be mentioned minimum items that should be mentioned on straws containing frozen embryos (see section 2.4.2 below)

The provisions to follow movements of embryos is given in Annex 3. Embryos storage and movements on page 20 below.

2.4.1 Recording data at different steps of embryo production

2.4.1.1 Summary of items to be recorded when embryos are recovered

When embryos are recovered, some items have to be registered compulsory, by hand (paper form) or by electronic devices (as example laptop computers, PDA.). Those data will be used to constitute the basic databases to trace back embryos history according to the various situations.

When embryos are imported, relevant data have the same status as recovering data.

Requested data are, either on farm recovering or importation:

In any situation:

- a. Recovering's references of the approved team and/or operator carrying out operations.
- b. Date of collection (or date of importation).
- c. Date of freezing (if different).
- d. ID Herd of the donor at time of embryo recovery.
- e. ID Donor female.
- f. Nature of recovering: embryos or Oocytes.

Only for embryos recovering:

- a. Possible ID sire(s if double AI have been carried out with 2 different bulls).
- b. Age of embryos.

Only for Oocytes recovering:

- a. Nature of recovering: abattoir or OPU.
- 2.4.1.2 Summary of items to be recorded at fertilisation process or reproductive cloning (when relevant)

These items address any embryo (or on straw to identify embryos):

- a. Lab of fertilisation.
- b. Date of fertilisation.



- c. Possible ID sire(s).
- d. Operator of cloning.
- e. Date of cloning.
- 2.4.1.3 Extra information
 - a. Indication of genotyping by biopsy.
 - b. Technical data such as codes requested by the IETS forms set.
- 2.4.1.4 Data for parentage validation
 - a. Embryo reference number (which may contain recovering reference number).
 - b. Identification of the approved team and / or operator.
 - c. Date of freezing.
 - d. Embryo's sire(s): ID + breed code.
 - e. Embryo's dam: ID + breed code.
 - f. ID Herd of the donor.
 - g. Age of embryo (s).

If embryos are imported relevant data may be obtain from documents accompanying embryos:

- a. Herd Book pedigrees.
- b. IETS appropriate forms.

Recommendation does not address the order of items. The description of order has to be mentioned when data are exchanged.

Note

DNA has to be collected from parents, markers (or blood types) have to be provided along with the other data.

2.4.2 Identification of embryos on straws

On each straw containing embryos, an unique reference number has to be printed or handwritten in order to set cross-reference with following items on papers or accessible electronic files, that render possible to follow physical movements of embryos:

- a. Identification of the approved team/operator that has recovered embryo(s).
- b. Date of freezing.
- c. Embryo's sire(s): ID + breed code.
- d. Embryo's dam: ID + breed code.
- e. Number of embryos per straw.

In addition to the unique reference number, more information may be laid down on straws according to the needs of the clients or the breeding organisations. This recommendation does not address the order of items.



The structure of the unique reference number isn't requested by this recommendation. It includes usually the team / operator code, the reference of recovering intra year and the rank within recovering operation.

In situation of embryos produced for export purposes it is strongly recommended that teams implement the IETS identification system.

2.4.3 Summary of items to be recorded when embryos are transferred

When embryos are transferred into recipients, some items have to be registered compulsory, by hand (paper form) or by electronic devices (such as laptop computers, PDA.). Those data will be used to constitute the basic database to assess parentage of the calves born out of embryos allowing distinction between genetic mother and biological mother.

Items addressing any embryo are:

- a. Operator.
- b. ID Herd of recipient.
- c. ID Recipient female.
- d. Date of transfer.
- e. Embryo's identification.

Recommendation does not address the order of items. The description of order has to be mentioned when data are exchanged.

2.4.4 Details on recorded items

2.4.4.1 Recovering reference number

Reference of the officially approved team that has recovered embryos and the intra team year number.

2.4.4.2 Embryo's reference number

Refers to recovering reference number to describe produced embryos: number of embryo produced intra team by an approved team.

2.4.4.3 Operators

Technicians or vets working under the responsibility of approved teams for recording or transfer embryos. Recording of individual operators is not compulsory.

2.4.4.4 Dates

The date of each operation has to be recorded. Dates of recovering embryos and freezing are usually the same.

2.4.4.5 Identification of herds and females

Herds and females have to be identified within the national system of registration dedicated to genetic data processing. The identification number of females including country code has to be recorded for each donor or recipient female.

2.4.4.6 AI bull

Donor females have to be inseminated by semen of AI bulls, known through the reference of their semen. The bull's identification is that defined by the "ICAR guidelines for straw



identification for bovine semen" as the international identification code or a world-wide unique bull code (refer to 1.6.2 on page 7 above).

2.4.4.7 Double AI

The existence of a double AI has to be mentioned, either by recording of a code, either automatically.

2.4.4.8 Breed codes

Those of the ICAR list (available <u>here</u>) for bulls and donors have to be used for international trade. In case of production of embryos, from breeds that are not on the list, teams are free to use any other code, provided that they not already on the list.

2.4.4.9 Herd

A herd may be either a farm or a station.

2.4.4.10 Age of embryos.

Embryos produced in vivo or in vitro, are transferred at stage of blastocytes, usually 7 days.

2.5 Transmission of embryo data for parentage assessment

- a. Data have to be transmitted on a regular frequency to the database where there will be matched with birth data.
- b. Embryo identification data and AI data have to be available in the database.
- c. Transfer records have to be available in the database prior to birth data.
- d. Birth data have to be transmitted by the person in charge of the recipient at calving (sex of the calf is declared at birth and not by molecular analyse).

2.6 Parentage assessment

After calving of a female known as a recipient by indication of embryo transfer instead of AI (or natural service) as reproduction event, the system of parentage assessment has to establish the genetic parents of the calf . Two methods are possible according to the data processing organisation implemented within countries:

- a. Either parentage assessment requires that both parents and the calf are **compulsory analysed for Micro satellite or SNP markers or blood types** consistency on the basis of recorded data described in 2.4 on page 13 above.
- b. Either parentage assessment requires that relevant data undergo successfully tests described in Annex 4. Validation of data on page 21 below and transmitted to the database according to 2.5 above, prior to the transfer data being matched with birth data.

If dates of implantation and of birth are consistent with the gestation length of calf's breed, taking into account the age of the embryo, the genetic parents may be attributed the born calf.

It is recommended that parentage for the more valuable animals of the population **should be confirmed using DNA analysis**.



Note

Parentage of AI bulls, born out of AI or ET, has to be compulsory checked for checked for **Blood typing**, **Micro satellite**, **or SNP parentage analysis in most countries**.

2.7 Quality controls

The efficiency of any information system depends on the quality of data proving that the expected result fits with the goal. For embryos, it deals with the accuracy of the records and with the proof that the progeny from embryo transfer was born from foreseen parents.

It is recommended that the organisation in charge with data processing aimed to assess parentage from calves born out of embryonic techniques carries out controls and implement relevant indicators for failures on each test suggested above, in terms of completeness, integrity, coherence and likelihood of recorded date.

Note

Those quality controls are independent of those requested for the renewal of approval of ET teams.

3 Fertility Reporting for AI organisations

3.1 Scope

Non-Return Rates (NRR) as a management tool for AI industry to characterise bull fertility and technician performance or to compare different semen treatments.

The reproductive performances of any herd or flock is out of scope.

3.2 Aims

- a. To facilitate the understanding of the "Non-return rates" usually provided by AI organisations, recommending a precise description of the method used for the calculation of NRR.
- b. To suggest guidelines for calculations of NRR, in order to facilitate the harmonisation of the calculations between countries or AI organisations.

3.3 Definitions

First insemination =	First insemination to breed an heifer or to breed a cow after the end of each pregnancy.
Non-Return Rate (NRR) =	Percentage of females that are inseminated for the first time during a given period of time (such as a month) and have not been recorded as having returned for another service within a specified number of days (e.g. 24, 56, 90).

3.4 Rules for calculation

3.4.1 Services to consider

Only first inseminations should be considered for the calculation of NRR (agreement for bull fertility in 5th ICAR meeting at TRENTE, 1964).



3.4.2 Females to consider

In a given herd, all females inseminated should be used for NRR calculation (without selection on reproductive parameters).

Female breed(s) should be indicated.

3.4.3 Day of insemination

The day of insemination is Day o.

3.4.4 Interval of returns

Calculation of a NRR (e.g. 56 day NRR) commonly excludes early returns according to the objective of the NRR (e.g. returns within 3 days after the insemination are excluded since it is considered usually as a problem due to females and not to the males).

Thus, both limits of the considered interval should be indicated (e.g. 3-56 day NRR).

3.4.5 Limits on the interval of returns

As a rule, the limits given should be inclusive.

For instance, for a 3-24 day NRR, if an insemination has occurred on Monday (Do), an early return recorded on Tuesday (D1) or Wednesday (D2) is excluded, and a return recorded from Thursday (D3) to D24 is considered.

3.4.6 Exclusion of short returns

The females with short returns, excluded as stated above, could be considered either like non-returned females ("pregnant") or like non-inseminated females. The former will lead to a slight overestimation of NRR, the latter represents a better option but might be more complicated to implement.

As a rule, short returns should be considered like non-inseminated females, i.e. should be eliminated from the file for the given year of calculation. Otherwise the chosen option should be indicated.

3.4.7 Number of first Al

The number of first AI should be indicated for any NRR, since it is related to the precision of the estimation. For example, a NRR of 50% based on 100, 400 or 1600 AI will have a standard deviation of 5, 2.5 or 1.25 units of percentage.

3.4.8 Correction of NRR

Numerous factors have been shown to be able to influence the NRR according to the breeding situation. Some of the factors commonly used are: parity of the female (cow/heifer), technician, day of the week, herd, area of AI, year or season or month, semen price, Do It Yourself or not, herds on milk recording or not, milk production for cows, female breed if several.

As a minimum for correction, NRR should be adjusted for parity (cow/heifer).

In any case, it should be indicated if the NRR have been adjusted or not, and in case which factors have been used for correction.

3.5 The NRR related to the date of each insemination

As stated above, one should indicate:



- a. The given period of time in which females have been inseminated.
- b. The number of females.
- c. The limits of the interval during which the returns have been observed after the date of each insemination (3-24, 18-24...).
- d. Female breed(s).
- e. If females with short returns were considered either pregnant or non-inseminated.
- f. If nrr have been adjusted or not (and if yes, the source of variation taken into account).

The suggested expression of the NRR is as follow:

'Given period' (n=): 'beginning of interval'-'end of interval' day NRR

e.g. for January 2000 (n=1,531): 18-24 day NRR = 68.4%

3.6 The 60 to 90 day NRR

60 to 90 day NRR has been a standard for AI organisations to work out breed receipts on a monthly rather than a daily basis. In that way the NRR of all the females bred in January is calculated at the end of March. The females bred on January first will have about 90 days in which to return. Those bred during the last days of January, however, would have had only about 60 days.

Pay careful attention that the common phrases "18-24 day NRR" and "60 to 90 day NRR" get things confused. "18-24 day" addresses to the two limits of the interval, whereas "60 to 90 day" only addresses to the end of the interval which has the particularity to vary according to the month's day of the insemination.

The same information than for the previous NRR should be indicated.

The suggested expression of the NRR is as follow:

```
'Given period' (n=): 'beginning of interval'-'range of the end of interval' day NRR
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e.g. For the year 1999 (n=15,332): 3-60 to 90 day NRR = 58.9%



4 Annex 1. Incidence of the chosen option for the exclusion of short returns

Let be :

"N" the total number of female inseminated for the first time in a given period

"n1" to "n4" the number of returns within different intervals after the date of insemination

"n5" the number of non-returned females at Day 60

Such as N=n1+n2+n3+n4+n5

If all returns are considered, 60 day NRR =

If short returns are excluded, the table below illustrates the two optional calculations.

Short returns females are considered as	Non-returned females (pregnant)	Non-inseminated females
3-60 day NRR =	$n_1 + n_5$	n
	N	$N - n_1$
18-24 day NRR =	$n_1 + n_2 + n_4 + n_5$	$n_4 + n_5$
	N	$N - (n_1 + n_2)$

5 Annex 2. Consideration of cattle reproductive physiology

Beginning of the interval:

- a. zero will consider the total of the returns.
- b. 3 days will eliminate short returns due to errors in oestrus detection.
- c. 18 days will eliminate the returns which are considered to be related to female failures rather than bull or technician failures.

End of the interval :

- a. 24 days will give early report although not considering late embryonic mortality.
- b. **90** days will give a more precise reflection of bull fertility but is a late indicator to identify unforeseen problems.
- c. 56 days appears as a compromise commonly chosen by AI organisations.

6 Annex 3. Embryos storage and movements

After being collected frozen embryos may be stored in storages centres. They may be moved from one storage centre to other centres before being transferred.

To follow embryos movements following items have to be provided:

- a. Embryos have to move among approved storages
 - Code of approval and address

For any in and out movement, records may be kept



- a. Unique embryo identification reference cross-referenced with data listed in paragraph 2.4.3 on page 15 above.
- b. Date of arrival and previous location (collect on farm or code of the approved storage centre).
- c. Date of exit and destination.

Documentation accompanying embryos according to national regulation has follow the movements (data files may substitute written documents).Following items are recommended:

- a. Documents on embryos identification.
- b. IETS forms if relevant or any technical forms with data having the same purpose.
- c. Pedigrees.
- d. ISAG marker set (or blood types).
- e. Health certificates.

7 Annex 4. Validation of data

After recording data on embryo produced (or imported) or data on transferring, these data have to undergo series of test prior to be used in the genetic system. Those tests may be carried out at various levels according to the organisation and the equipment. From a general point of view, embryos related data undergo the same process as the other reproduction data such as AI. Recommendation doesn't address the way of maintaining and up dating data bases of relevant organisations.

7.1 Completeness and integrity

Each item recorded must be checked against the data model to prove the intrinsic validity of data. All necessary data have to be available prior processing.

7.2 Embryo coherence

Those items have to be checked against existing files to prove their coherence with existing information:

- a. The code of the approved team is known in the base.
- b. The code of the operator recorded is declared by the relevant team.
- c. The herd is registered.
- d. The donor is registered (or the genetic mother).
- e. The AI bull(s) are registered.

Moreover regarding the donor:

- a. The identification corresponds to an animal registered as a female
- b. If two AI are carried out on the same female on the same day an alarm message has to be edited

7.3 Transfer coherence

Items on embryo transfer have to be checked against existing files to prove their coherence with existing information:



- a. The code of the approved team is known in the base.
- b. The code of the operator recorded is declared by the relevant team.
- c. The herd is registered.
- d. the recipient is registered.

Moreover regarding the recipient:

- a. The identification corresponds to an animal registered as a female.
- b. The female is old enough to be bred.
- c. The female is alive.

7.4 Likelihood tests

In order to secure the information likelihood tests have to be carried out:

- 7.4.1 Embryos production
 - a. The donor was registered in the herd the day where embryos were recovered or Oocytes were collected.
 - b. The bull was recognised as an AI bull when the semen was used.
 - c. AI was carried out prior to embryos were recovered in vivo (exception IVF).
 - d. The herd identified is an active one (cattle are recorded within this particular herd).

7.4.2 Embryo transfer

- a. The recipient was registered in the herd the day where embryos were transferred.
- b. The herd identified is an active one (cattle are recorded within this particular herd).
- c. Identification of transferred embryo(s) is in the data base.

8 Annex 5. Survey results

The ICAR board has set up the ICAR Working Group on AI& other relevant technologies (Artificial Insemination and Relevant Technologies WG) in 1998 to satisfy the demand of its members. Duty of the group according to its term of reference, is to set up recommendations in order to improve world wide records used for genetic evaluations and the efficiency of the breeding schemes.

Concerning embryos and associated technologies the need it to cover systematically key aspects, that have not been tackled before. Thus it's important to take into consideration data recording and process to recognise them as valid for genetic purposes, because:

- a. Embryo technology aims at producing animals from top cows of the populations.
- b. It is used for the management of nuclei.
- c. It is an outstanding tool to exchange genetic material.

Embryo technologies mainly means embryo recovering from donors (in vivo or in vitro), embryo freezing and their storage, embryo transfer. New associated technologies are becoming available such as embryo genotyping (to assess the sex, to reveal gene defects or to implement MAS on embryos) and cloning. Harmonisation of codes related to special features of embryos (sex, nuclear transfer for cloning etc.) is underway.



It is necessary to take into account constraints due to national or international legislation and the existing international systems of recording and exchange data on embryos.

- a. The European Union has published two decisions to lay "down the specimen pedigree certificates for the semen and embryos of pure-bred breeding animals of the bovine species... 88/124 /EEC" and to lay down the pedigree and zootechnical certificates by importing breeding animals, semen, ova, embryos, into the EU Decision 96 /510 /CE.
- b. Remark: this EU decision will be up- dated in 2004 (few changes compare to the previous one)
- c. The IETS has produced a set of forms, continuously updated since 1985, dealing with various technical matters related to embryos recovering, processing, freezing, quality control of transfer, exports etc in order to help the work of practitioner and to standardise the coding of the various technical items.

It seems that the strict implementation of regulation and guidelines in using official forms vary among countries according to the national organisations and to the requests of the clients. Nevertheless data requested for various needs are supplied. This point has to be clarified.

The survey presented below is the summary of the work done between 2003 and 2004 by the group. Members of the group are experts of the AI industry of seven countries important in the world AI and/or technically advanced in processing and utilising of AI data.

Organization	Country
NAAB	USA
Swedish Un. or Agric. Science	Sweden
UNCEIA	France
ADR	Germany
Universita di Milano	Italy
CR Delta	The Netherlands
S.V.K.B.	Switzerland
	Organization NAAB Swedish Un. or Agric. Science UNCEIA ADR Universita di Milano CR Delta S.V.K.B.

¹Substitute, Erikson J.A. for USA; R.Powel & K Weigel were substitutes, Irma Robertson gave a strong input in responding to questionnaires & giving comments on behalf of the IETS.

To achieve its goal the group will use the following method:

- a. A questionnaire was build up by the chairman and discussed by the members during a meeting to clarify the questions according to the needs. Validation occurred thanks to e-mail exchanges.
- b. Each member answers any question with or without the help of specialists of this issue in his home country. The individual answers will be gathered and send back to the members as soon as possible.
- c. It will be then put on discussion during the following meeting: explanations and clarifications will probably be necessary.
- d. After validation of answers a summary on the chapter will be done by the chairman and propose to the group for validation.

This material, produced by the answers from 7 countries, has been the source for an ICAR recommendation.



Following topics have to be covered:

Topics General principles	Details Needs for recording
Recording of data	General organisation and information recorded
	Various requirement among countries
Processing and validation	• Data flow
	• Tests
	Quality controls
Integration and use of data	Parentage assessing
in the genetic data systems	Pedigree printing

8.1 General principles

From embryo production to birth of calves from those embryos following steps have to be achieved:

- a. Embryos are recovered from a donor cow, inseminated by a sire or produced from an in-vitro fertilization
- b. Embryos may be produced within countries or imported from an other country
- c. Before they will be transferred frozen embryos may be stored and movements have to be traced
- d. Embryos are transferred into recipient cows
- e. Parents of born calves must be the "genetic parents": donor cow + sire
- f. Embryo teams are operating in various steps of the process, several teams may be involved in the chain process
- g. Embryo teams may be officially approved

Following issues are relevant to deal with data related to embryo technology:

- a. Teams have to render available all data relevant to the proper handling of the embryo to achieve a successful pregnancy to the practitioner in order to have a reasonable chance to get a calf after embryo transfer.
- b. All zootechnical data necessary to establish parentage of calves born out of the recovered embryos have to be available. Users of the technology must get them through ET teams or other bodies.
- c. The transfers have to be processed as the other fecundating events to establish the parentage of calves.
- d. A record of service or insemination, recovery of embryo freezing and/or transfer of embryo with all such events documented and recorded using standardized or approved identification and data recording procedures in order to assure correct parentage of resulting offspring.
- e. Embryos should be traced from their production in the farm or produced in lab to the cow uterus.

It can be added that embryos are complete genetic entities that can result in breeding animals. Many are very costly and identification must be attached to their movement accompanied by documentation because the embryo is a complete genetic entity.



Documents on health status of the donors have to be available for importing or exporting the animals. Those important data are not considered by this questionnaire because the issue is carried out by national authorities.

8.2 Recording of data

In most countries, forms to recover from donor cows(5 countries out of 7 that answered) or to transfer embryos into recipients (7countries/7 countries) are harmonised, so as these used for embryo identification (5/7).

When embryos are traded pedigrees, embryos characteristics (freezing, quality) molecular information or blood typing, are always available and follow them. The same occurs when embryos are imported. (7/7)

Teams are officially approved, in general by the ministry of Agriculture. An official list is available, published by national or international bodies. Teams have to apply for the renewal of their approval and rules have been set up in this respect, using quality control procedures

Data recorded at each step of the process are:

- a. At recovering
 - Recovering reference number (5/7).
 - Date of recovering (7/7).
 - Number of donor's herd (6/7).
 - Possible sires (7/7).
 - Natural service & AI are both possible (4/5).
 - Ovum Pick Up / IVF data may be recorded (4/4).
- b. Technical characteristics (IETS guidelines)
 - Age of embryos at flushing (5/7).
 - Integrity of zona pellucida (7/7).
 - Trypsine washing (7/7).
 - Development stage & quality (7/7).
 - Sex (5/7).
- c. Reference numbers
 - Recovering: team (7/7)/ intra team(5) / year number(3)
 - Embryos Intra team number (2/7), year of recovering (7/7), herd(6/7), operator(7/7)
- d. Transfer
 - Embryo identification (7/7).
 - Recipient (7/7).
 - Date (7/7).
 - Herd (6/7).
 - Team (7/7).

In most situations, ET teams use software, on the farm, to record and transfer ET data. That software are not harmonised within countries.

There are few efforts to harmonise straws identification for embryos

DNA is systematically collected on donors (and sires) by persons of the approved team or vets embryos and teams have access to files were data are recorded.

Embryos stocks are usually not managed by ET teams.

Very few organisations have implemented ISO procedures for embryos collection and transfer.

8.3 Processing and validation data

Processing and validation data used for assessment of parentage of calves born out of embryo transfers follow rules that vary according to countries.

a. In most countries, data recorded on embryos (recovering or import), are registered in the data base used for parentage assessment prior to embryo transfers. In the other countries data are in the herd book data base only at transfer. In the first situation recovering reference and embryos reference are transmitted to the data base. In the second situation there is no harmonisation within country and data transmission varies according to the individual organisations.

Same references are transmitted when embryos are imported. Pedigrees used are those issued by the Herd-Book organisations. In any situation data and reference are available at transfer.

- b. To assess parentage, transfer and embryo data have to be matched in the data base. In most situations transfer data are processed as an AI, semen references being substituted by embryos references. Transfer and embryos reference are introduced before the calf is born in the data base where parentage are established. Eventually parentages are checked with DNA markers of the calf and its parents. In some countries data are recorded and processed at calf birth. Then checks are carried out compulsory using DNA references to verify parentage.
- c. Data recorded on embryos and transfer checked before parentage assessment for integrity and consistency are those describe in part B. Tests for consistence, coherence and likelihood are parallel to those carried out in AI processing: teams, donors, sires recipients are registered in the data base, donor and recipients were in the recorded herd when operations were carried out, recorded dates have to be in line with the biological evens.
- d. Criteria of validation used at birth are those used in AI processing. Age of embryo in rarely taken into consideration. If two sires are possible, decision is made by DNA checks.

8.4 Integration and use of data in the genetic data systems

Herd book organisations (or the Ministry of Agriculture) are setting up rules to describe the process of parentage assessment. Those rules don't vary among Herd Books.

Embryo and transfer data are not used in the genetic system for other purposes other than pedigree classical usage in the genetic systems.

In very few countries embryos are genotyped for desirable traits (QTL), and then genotype stays with the owner of the embryos. If they are genotyped for single traits-colour-gene



defects embryos owner under the behalf of ET organisation (or directly) may deliver this information etc to the Herd Book.

