Understanding and preventing embryonic loss in dairy cows

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Getting them bred!

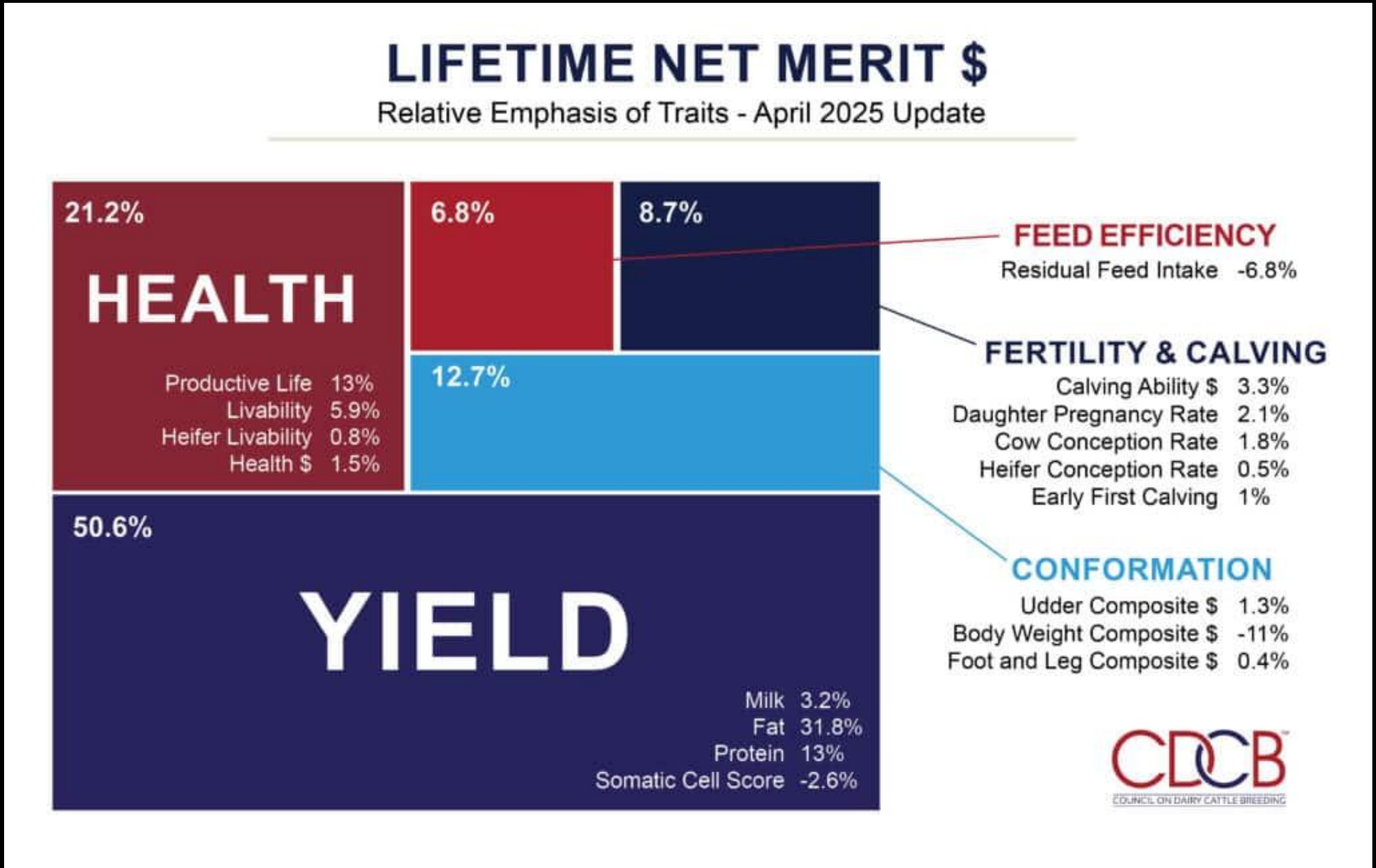
Start with a clean, comfortable, healthy cow in good body condition.

(Management!)

"Good cows" make a difference!

Superior genetics!

Modern genetic selection indices are designed to increase the health, fertility, longevity, efficiency, and productivity of dairy cows.



Do everything possible to reduce the incidence of postpartum disease, particularly metritis and endometritis, after calving

A clean environment is important and should reduce the incidence of metritis.

Photo: Drost Project

Three steps

Three steps to getting cows pregnant:

<u>Step 1:</u> Healthy uterus

<u>Step 2:</u> Healthy ovary

<u>Step 3:</u> Put semen in the cow

Three steps to getting cows pregnant:

Steps 1 and 2 are heavily dependent on management as well as the endocrine and metabolic state that supports milk production.

Everything depends on PEOPLE including Step 3 (unless the bull is doing it)!

Dairy Cow Fertility

Anestrus

Abnormal circulating hormone concentrations

P4/E2

Ovary → Oviduct/Uterus → Calf

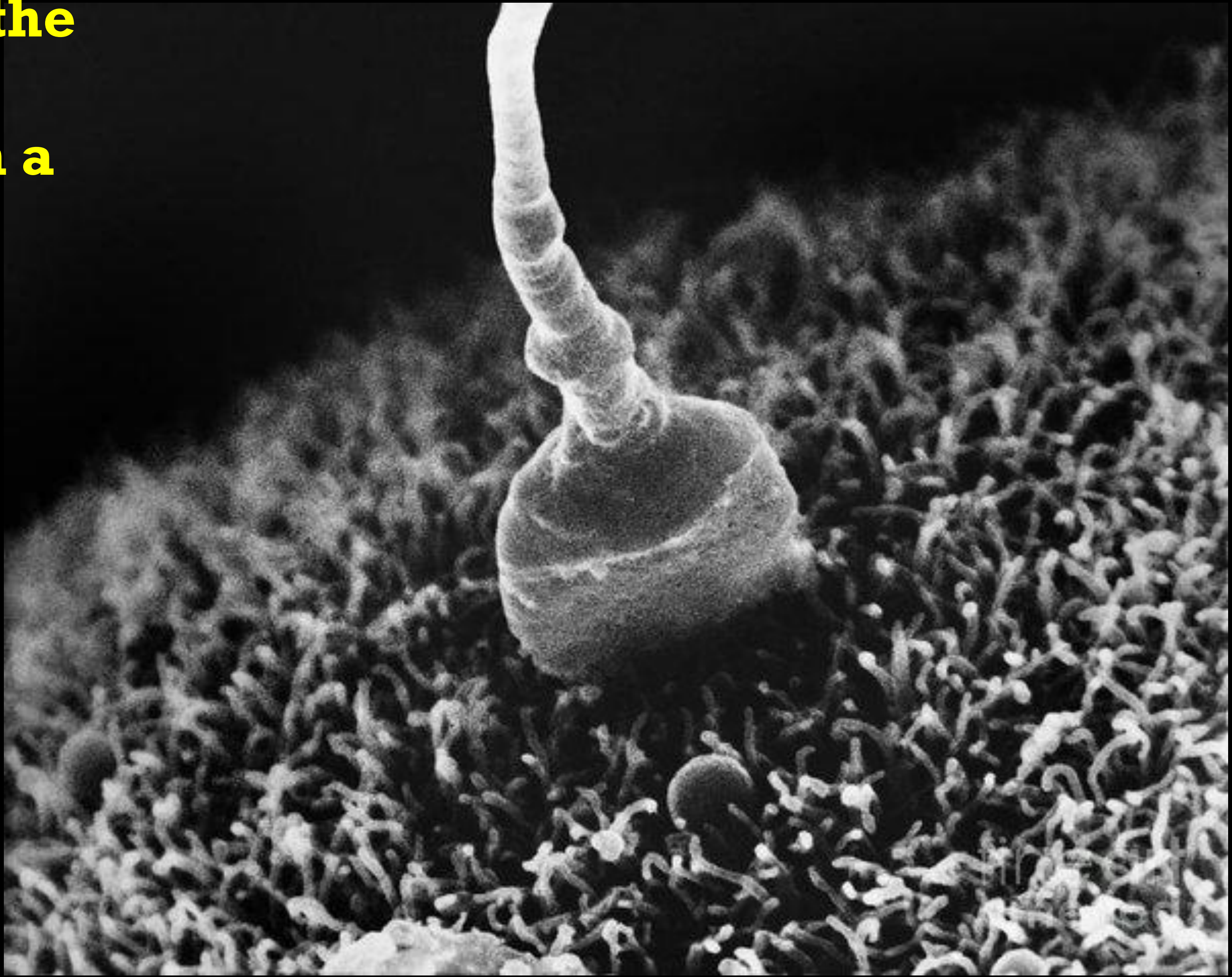
Poor quality gametes and preimplantation embryos

Poor uterine health

Embryonic loss

Fertilization

Fertilization is the union of sperm and egg to form a zygote.

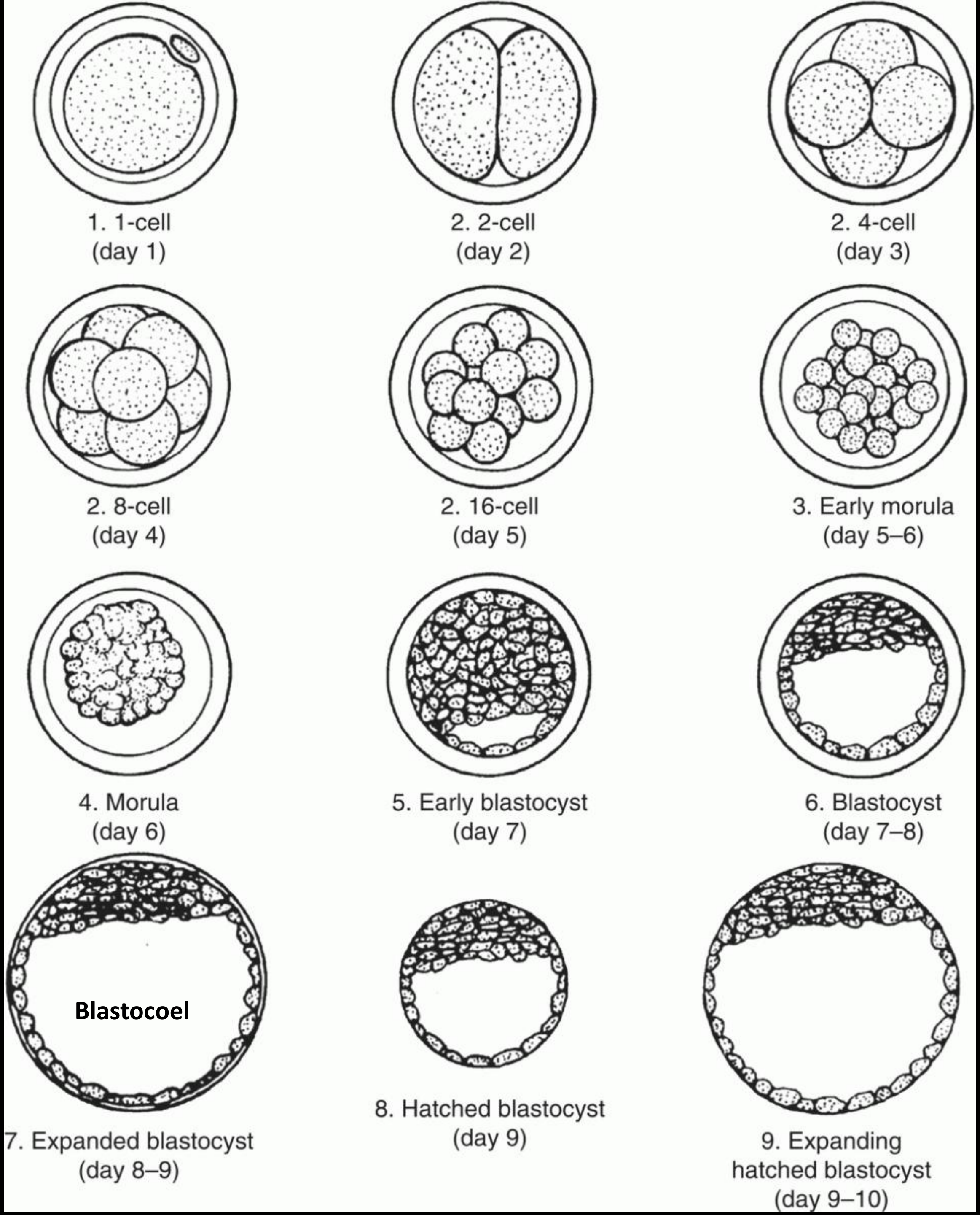


Photograph by David M. Phillips

Early embryonic development

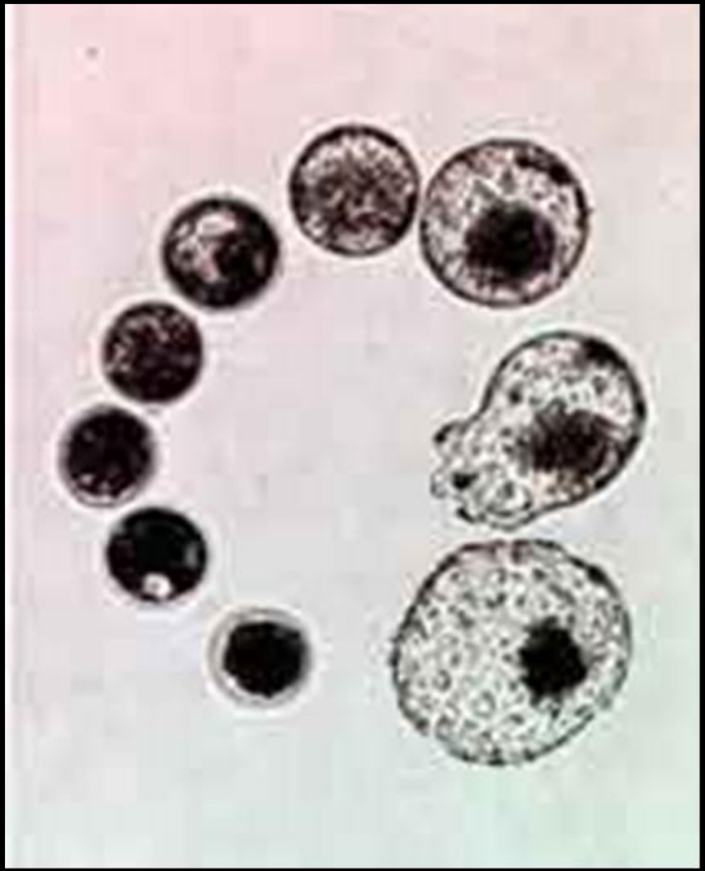
Stages of early embryo development

- Cleavage
- Genome activation
- Compaction
- Cavitation
- Hatching



Early embryonic development
Hatching and elongation

Embryo development in the bovine



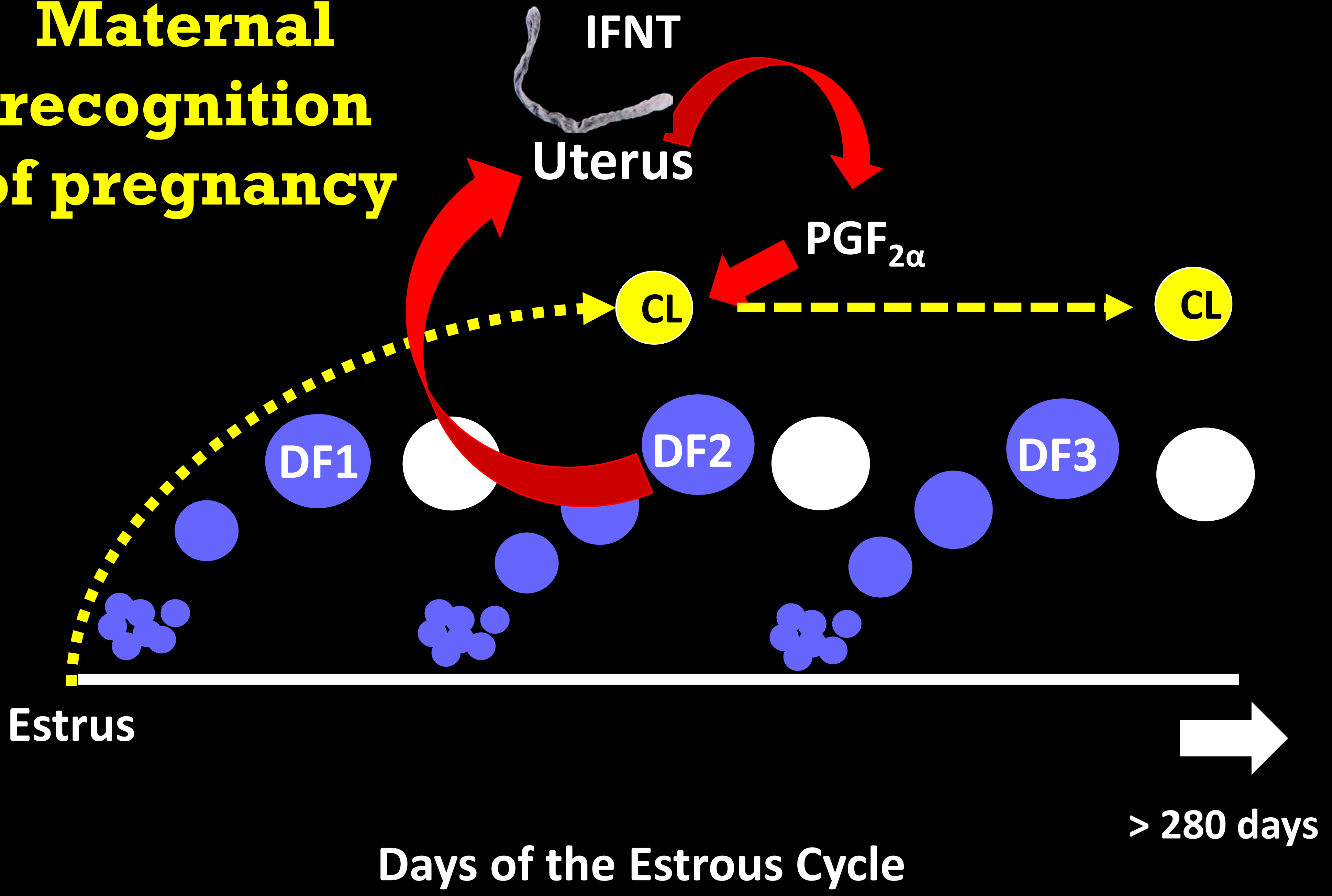
Morula – blastocyst – hatched blastocyst

Spherical

Tubular

Photos courtesy of
The Drost Project (University of Florida)

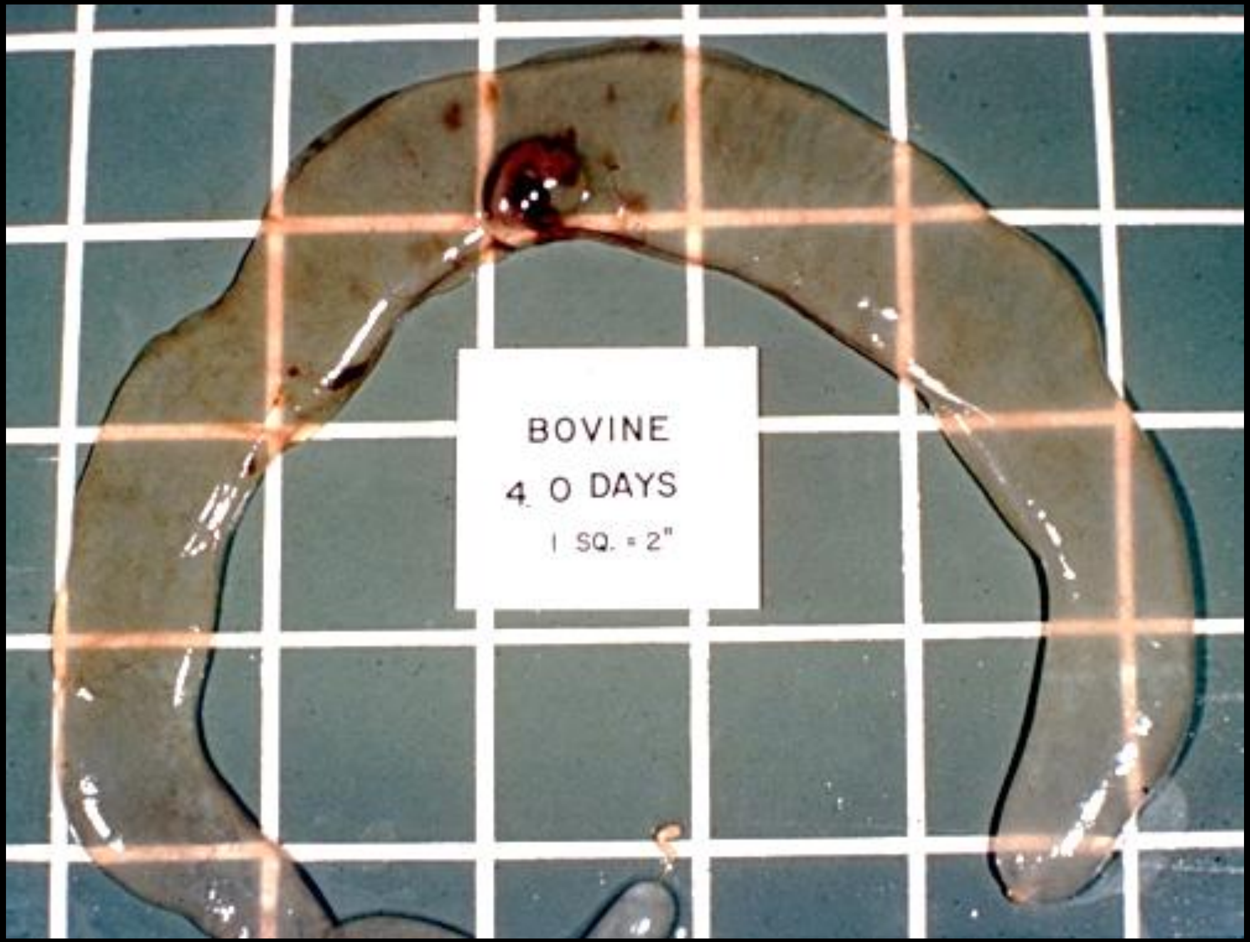
Maternal recognition of pregnancy

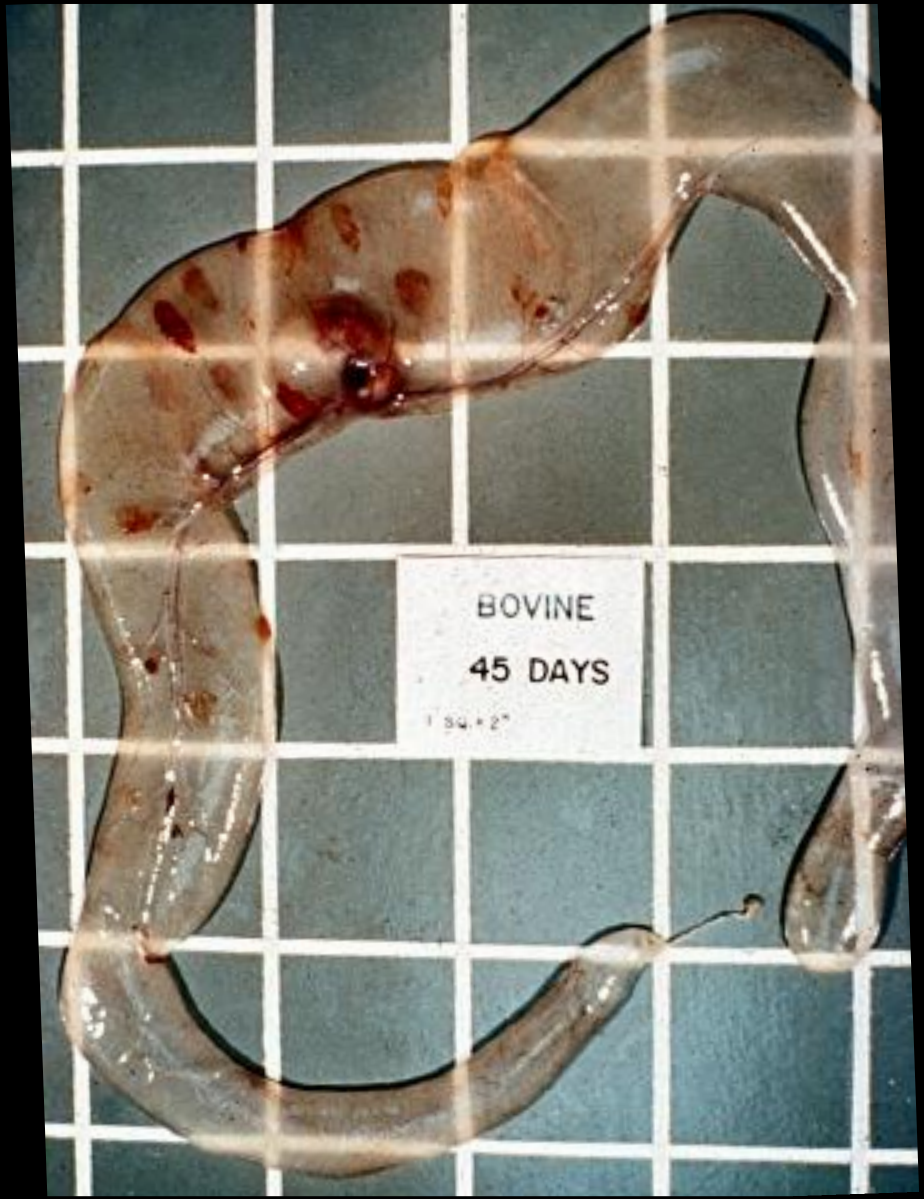


28 to 30 d pregnancy

Photos from The Drost Project; Contributed by Peter Chenoweth

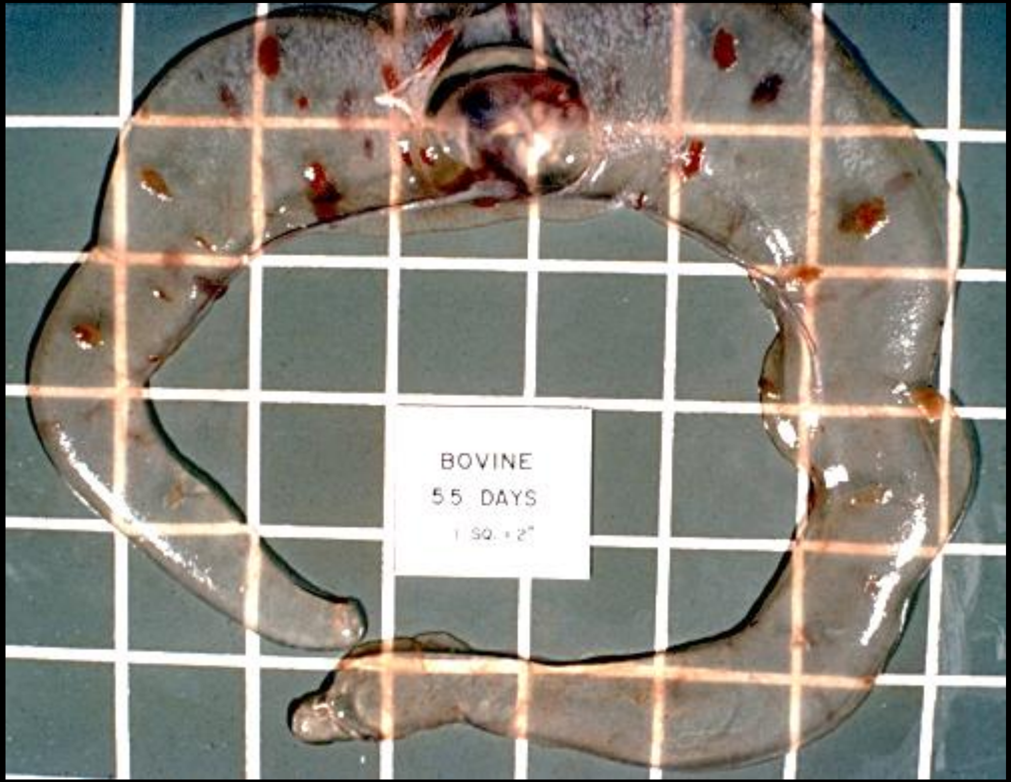
40 to 45 d pregnancy

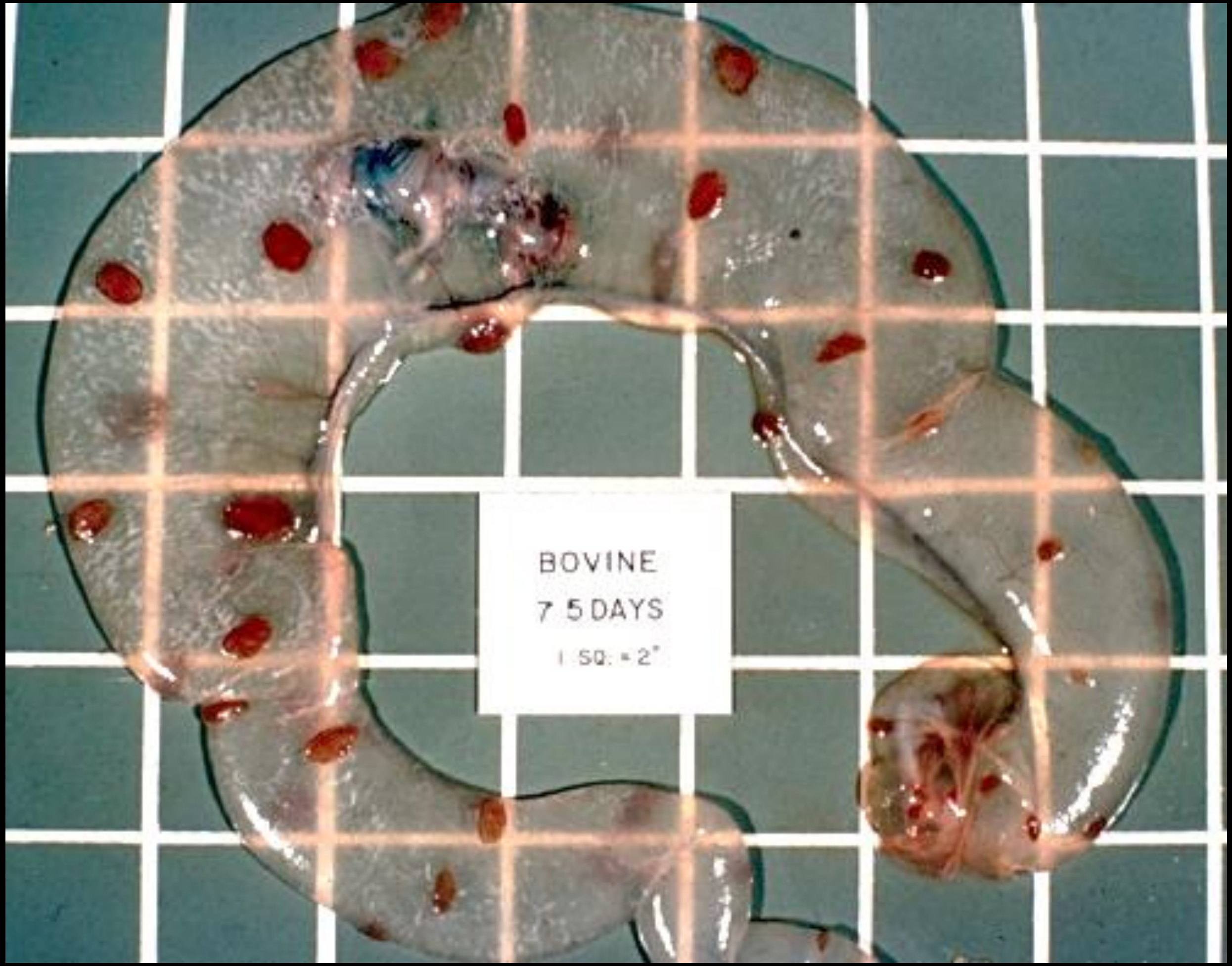




Photos from The Drost Project; Contributed by Peter Chenoweth

55 to 75 d pregnancy

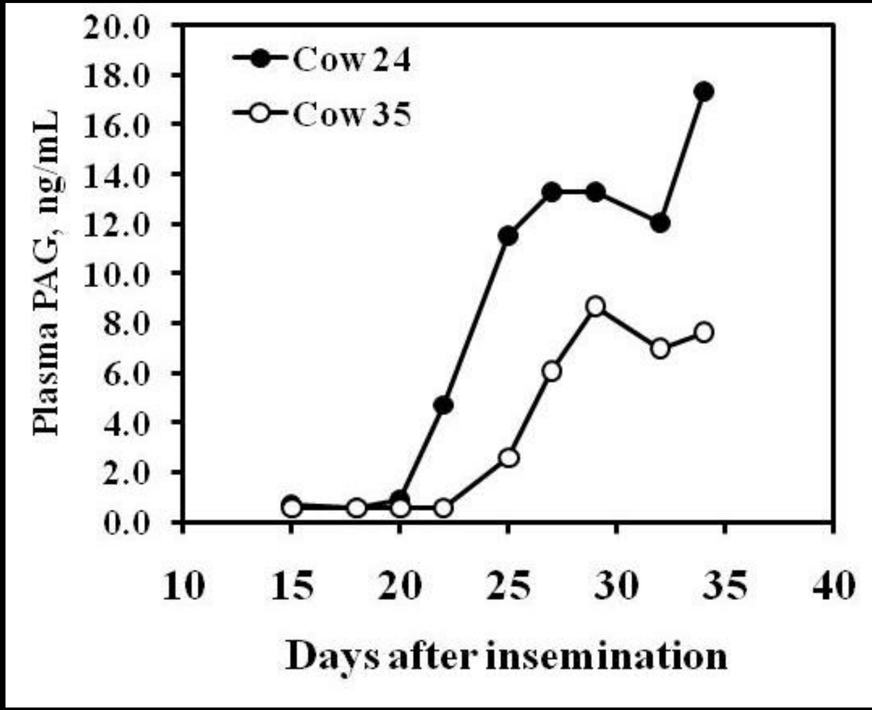


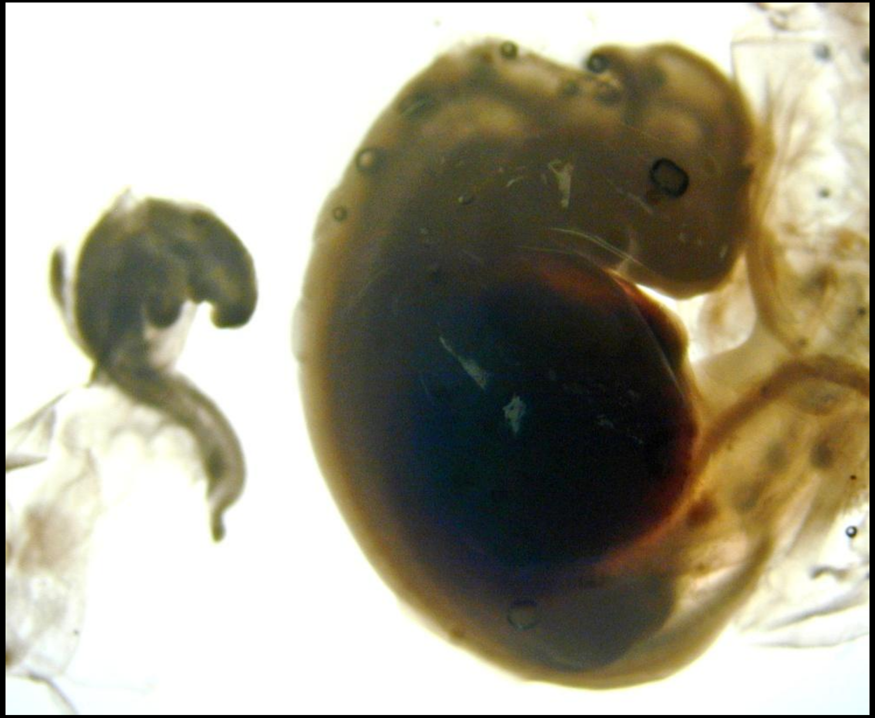


Photos from The Drost Project; Contributed by Peter Chenoweth

35 d pregnancy

Day 35 bovine fetuses





Lactating; Pregnant at 2nd AI

Not lactating; Pregnant at 1st AI

Green et al. (2012) Reproduction 143:699-711

Embryonic loss – when?

Wiltbank et al. (2016) defined four periods of loss:

- First week of pregnancy – fertilization to blastocyst
- Days 8 to 27 – Elongation and maternal recognition of pregnancy
  - 8 to 18
  - 18 to 27
- Placentation (days 28 to 60)
- Embryo/placental growth (d 60 to 90)

Embryonic loss – the numbers

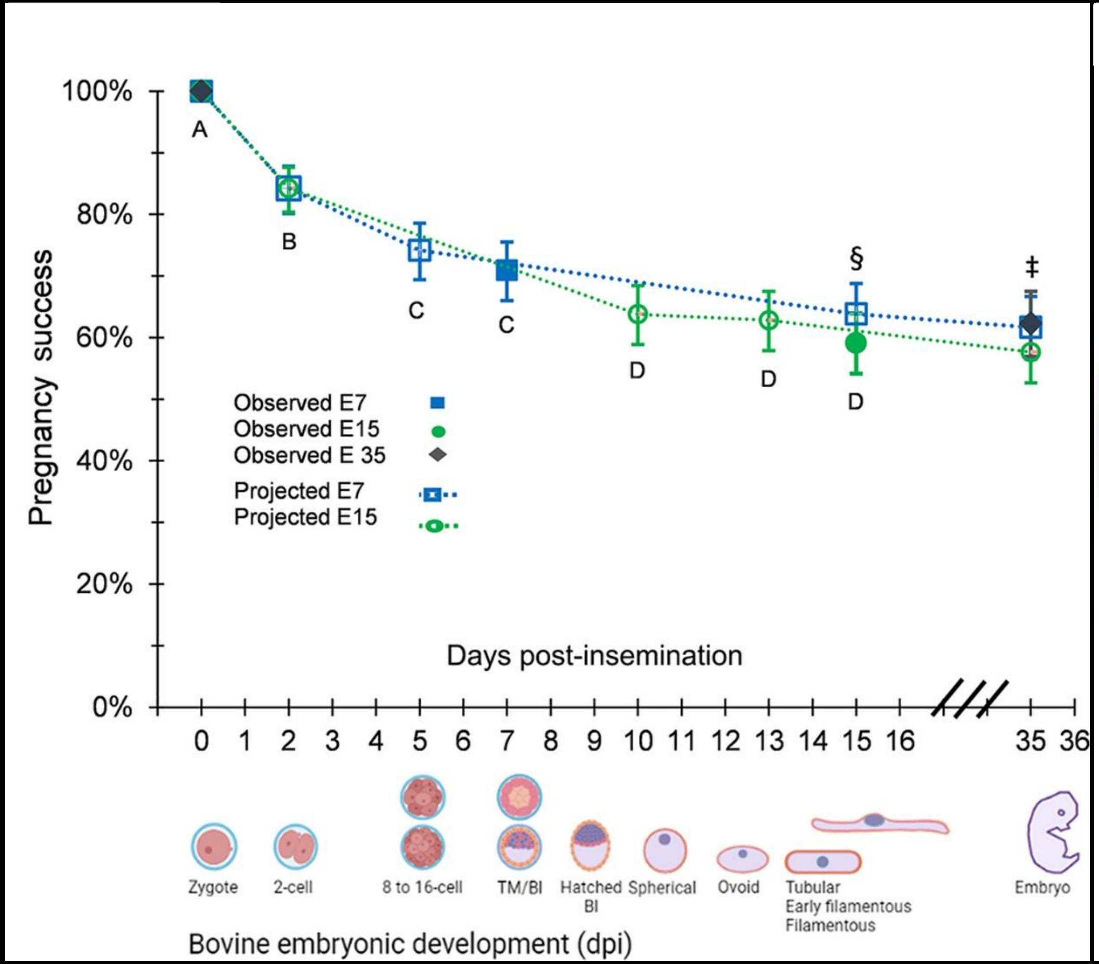
Conclusion from Wiltbank et al. (2016)

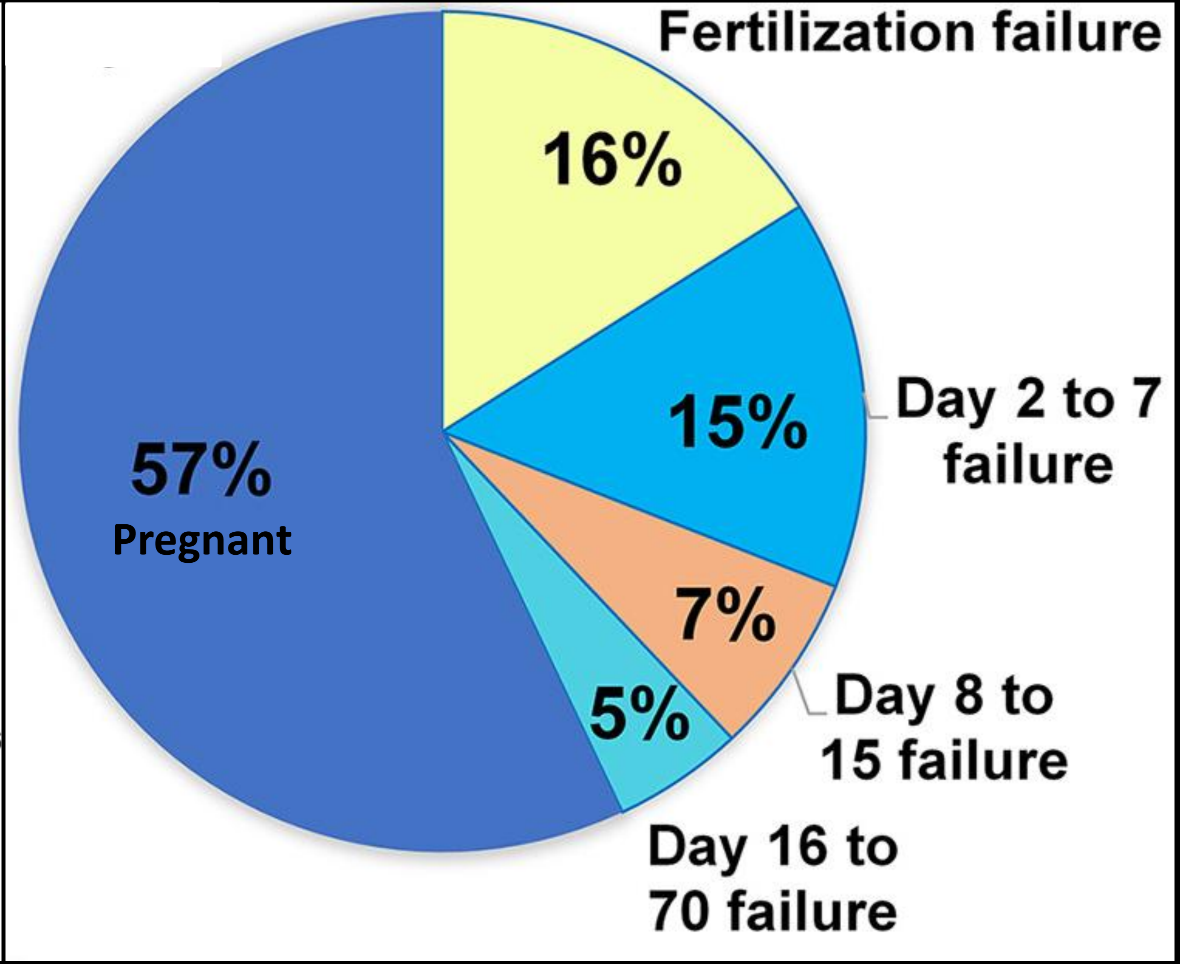
- First week of pregnancy – fertilization to blastocyst (20 to 50% loss)
- Days 8 to 27 – Elongation and maternal recognition of pregnancy (~30% loss)
- Placentation (days 28 to 60) (12% loss)
- Embryo/placental growth (d 60 to 90) (~2% loss)

Wiltbank et al. (2016) Theriogenology 86:239-253

Embryonic loss (recent studies)

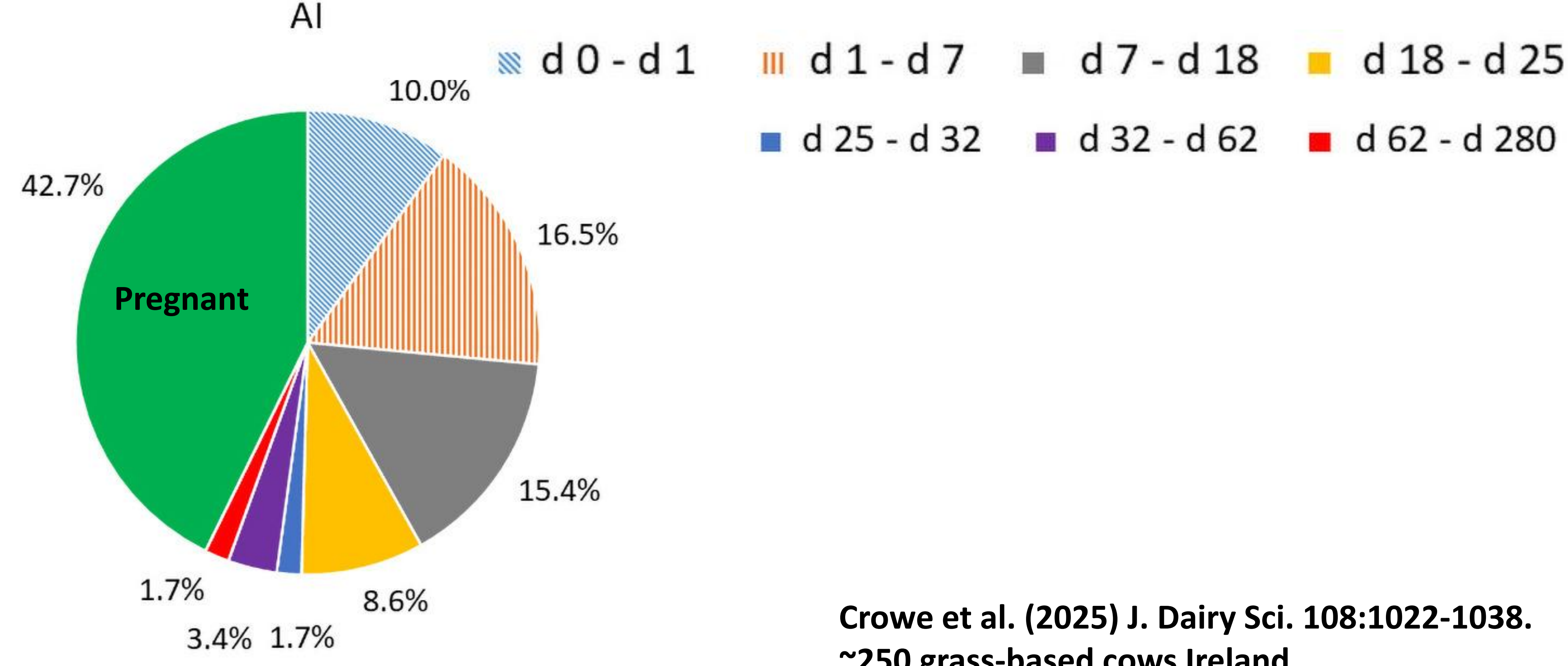
Embryonic loss – recent studies





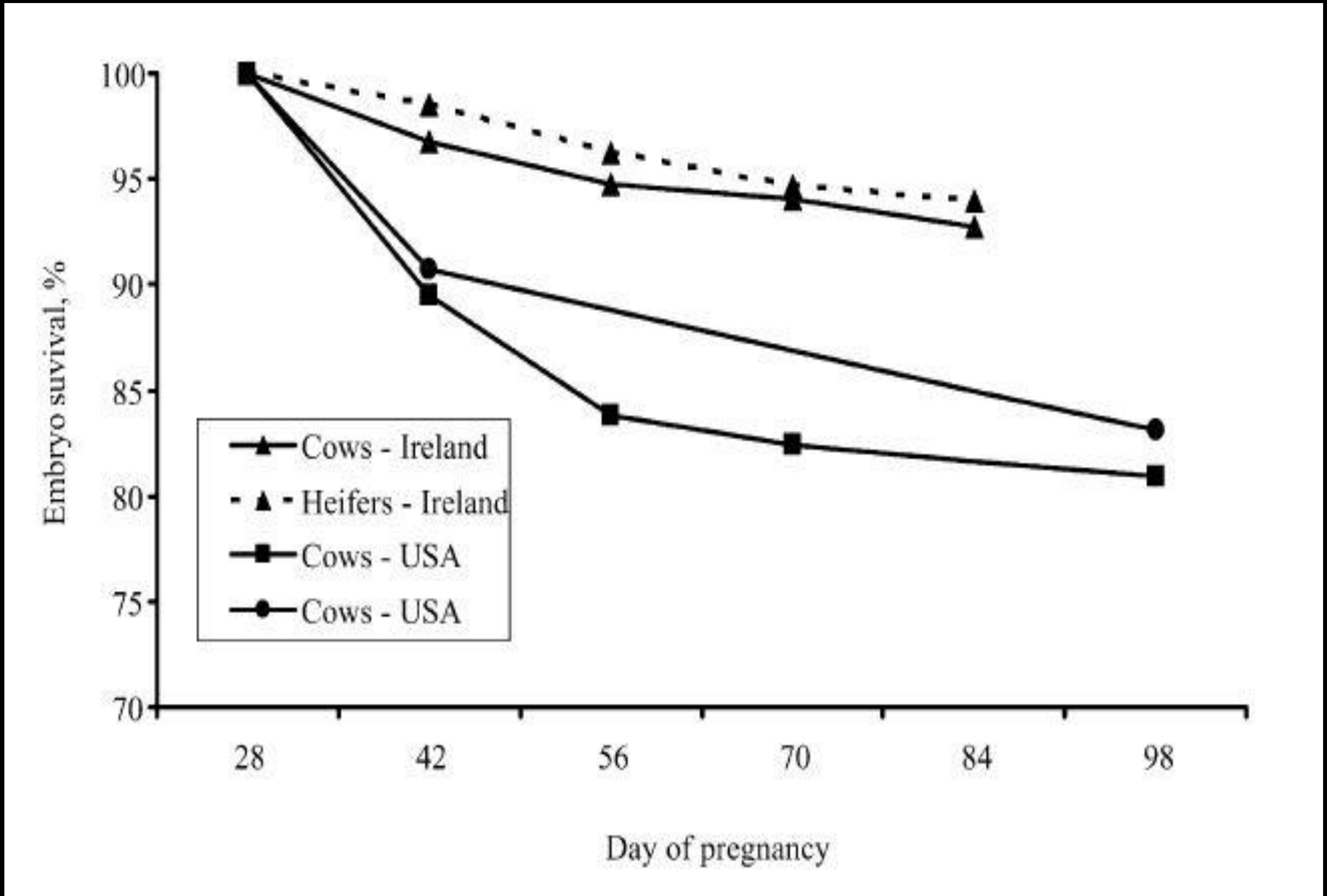
Berg et al. (2022) J. Dairy Sci. 105:9253–9270.
~1500 grass-based cows in New Zealand

Embryonic loss (recent studies)



Crowe et al. (2025) J. Dairy Sci. 108:1022-1038.
~250 grass-based cows Ireland

Embryonic loss (Ireland versus USA)



Santos et al. (2004) Anim. Repro. Sci. 82-83:513-535.

Embryonic loss – Why?

The "why" of embryonic loss depends on "when." There is a progressive decline in the number of viable embryos over time. This progressive decline ends after the placenta is fully formed. The reasons for embryonic loss differ across different times.

Embryonic loss – When?

Wiltbank et al. (2016) defined four periods of loss:

- **First week of pregnancy – fertilization to blastocyst**
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    - 18 to 27
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- Embryo/placental growth (d 60 to 90)

The sire – fertilization loss - No

- Assuming an appropriate timing of insemination with sires of proven fertility, there isn't a lot of evidence that the sire has a major effect on fertilization rates.
- Lethal haplotypes exist in AI sires and can explain < 1% of early embryonic mortality (Charlier et al. 2016).

Charlier et al. (2016) Genome Res. 26:1333-1341.

Sire conception rate

Sire Conception Rate

May represent the sum of multiple effects on embryonic development

NAME	NAAB_CODE	AI_STATUS	NM$_PTA	NM$_REL	PERCENTILE	SCR_PTA	SCR_REL	BREEDINGS
PEAK ZYMURGY-ET	097HO42850	G	1081	75	95	5.6	79	961
OCD CONNOR-ET	200HO12667	G	993	75	84	5.3	79	977
DUCKETT DIESEL-ET	007HO16954	G	725	74	49	4.9	77	867
SIEMERS PARX-ET	200HO12909	G	876	77	69	3.9	76	808
PROGENESIS ELLIOT	200HO12567	I	839	76	63	3.8	60	382
BERRYRIDGE SSI H FRYSKY-ET	250HO16839	G	1054	75	92	3.6	63	445
WINSTAR ALTAPLAYBONUS-ET	011HO15981	G	1084	76	95	3.3	74	745
PEAK ALTAZALEZ-ET	011HO15710	A	922	81	75	3.3	77	879
FLY-HIGHER MOONLAND-ET	777HO12806	G	1148	75	98	3.3	79	977
SIEMERS POST-ET	777HO12823	G	1122	77	97	3.3	62	432
S-S-I SIEMERS GS REAPER-ET	007HO16570	G	1081	76	95	3.2	84	1402
BOMAZ NIKOLAO-P-ET	001HO15649	I	989	85	84	3.1	92	3042
PEAK ALTABRONTIDE-ET	011HO15872	G	997	76	85	3.1	81	1098
VOGUE ZIRCON-P	200HO12521	G	602	76	36	3.1	72	661

CDCB December 2024

Oocyte

The Britt Hypothesis (1991)

Britt, JH. "Effect of short-and long-term changes in energy balance on reproduction." *Proceedings of the Mid-South Ruminant Nutrition Conference*. 1995.

Theoretical Explanation for Latent Effect of Nutrition on Progesterone and Fertility

In an effort to understand how losses in body condition (negative EB) could affect fertility several weeks later, a theoretical model was developed (Britt, 1991; Britt, 1992) based on the rates of follicular growth estimated by Lussier and colleagues (1987). The theory proposed that preantral follicles exposed to adverse environmental conditions would have altered gene expression leading to impaired or altered development. Impaired developing follicles would then result in formation of dysfunctional mature follicles, which would produce poorer oocytes and result in the formation of weakened CL. Adverse environmental conditions that might exacerbate the metabolic perturbations accompanying severe negative EB include heat stress or toxic conditions associated with postpartum disease. The temporal scenario associated with exposure of developing follicles to metabolic perturbations is illustrated in figure 4.

Oocyte

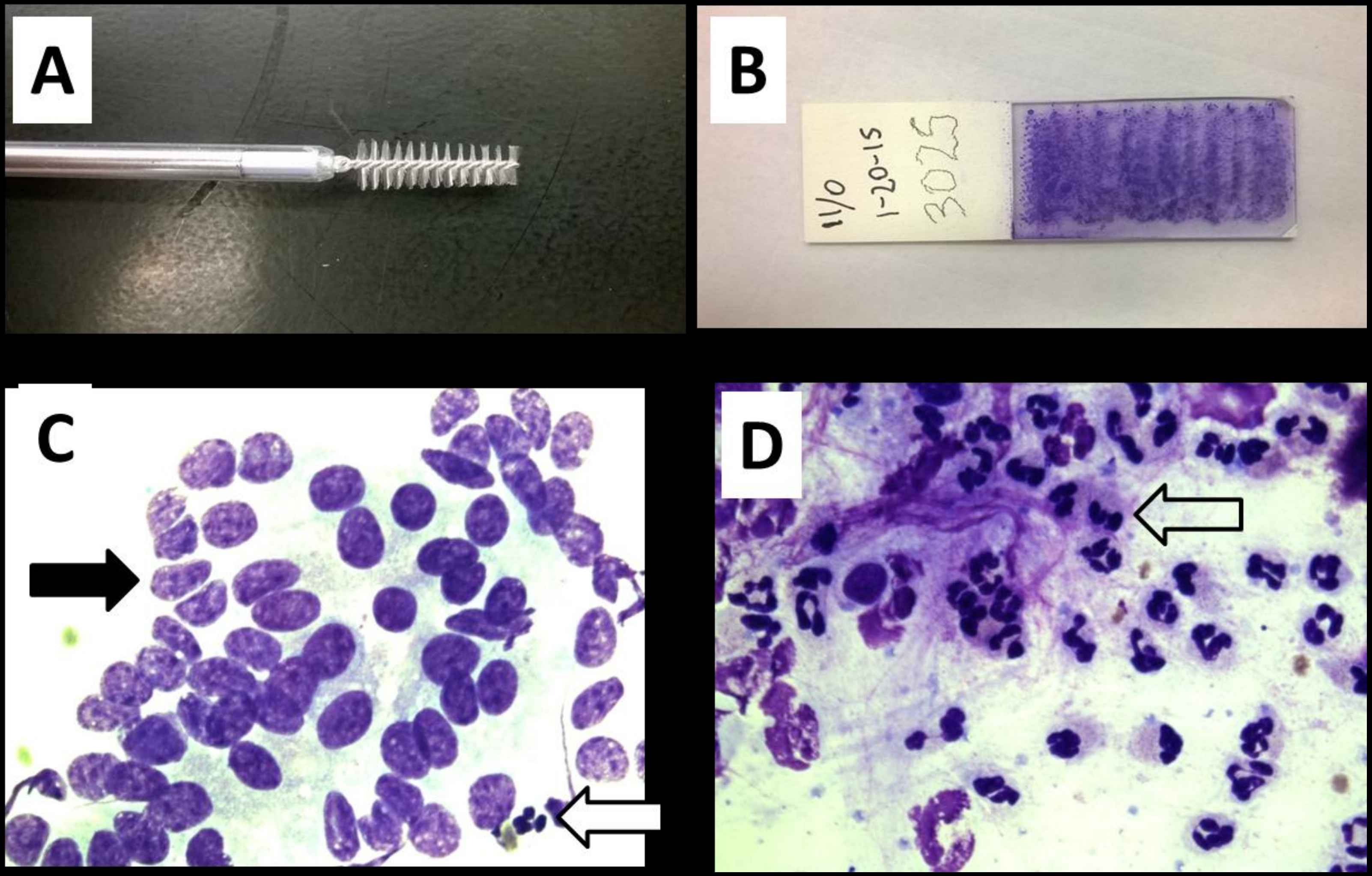
The Britt Hypothesis (1991)

- Toxic to the follicle/oocyte:
  - Elevated BHB and NEFA (Aardema et al. 2019).
  - Inflammatory cytokines and other molecules arising from uterine or mammary infection (Sheldon et al. 2019).
  - Elevated body temperature (Al-Katanani et al. 2002).

Aardema et al. (2019) Anim. Reprod. Sci. 207:131-137.
Sheldon et al. (2019) Ann. Rev. Anim. Biosci. 7:361-384.
Al-Katanani et al. (2002) J. Dairy Sci. 85:390-396.

Uterine cytology

Cytobrush exam for diagnosis of subclinical endometritis



Uterine disease → embryo

Effect of uterine disease on embryo development

(Lactating Holstein Cows – Wisconsin; 70 DIM)

Table 3
Effect of uterine PMNs on ova/embryo recovery, fertilization, and number of transferable/freezable embryos.

Endpoint	PMN <1% (n = 40)	PMN 1%–5% (n = 13)	PMN >5% (n = 12)
CL number	17.7 ± 1.4	15.8 ± 2.3	17.2 ± 1.7
Total ova/embryos recovered	7.8 ± 1.1	9.2 ± 2.4	4.7 ± 1.1
% Recovery	41.5 ± 4.3[a,b]	55.5 ± 8.5[a]	28.4 ± 6.3[b]
Fertilized structures	5.9 ± 7.7[a] →	7.4 ± 1.9[a] →	2.3 ± 0.7[b]
% Fertilized structures	82.3 ± 3.4	81.8 ± 8.8	62.1 ± 11.4
Transferable embryos	4.6 ± 0.7[a] →	5.9 ± 1.7[a] →	1.8 ± 0.6[b]
% Transferable/total	62.3 ± 5.5	61.3 ± 11.6	52.0 ± 12.5
% Transferable/fertilized	74.9 ± 5.4	71.3 ± 11.2	72.5 ± 12.7
Freezable embryos	4.4 ± 0.7[a] →	5.3 ± 1.6[a] →	1.8 ± 0.6[b]
% Freezable/total	59.4 ± 5.4	56.3 ± 12.2	48.9 ± 12.5
% Freezable/fertilized	71.4 ± 5.3	65.4 ± 12.0	69.1 ± 13.3

[a,b] Means within a row with different superscripts differ ($P < 0.05$).

Carvalho et al. (2013) Theriogenology 80:1074-1081.

Uterine disease → Coneption rate

J. Dairy Sci. 100:588–597
https://doi.org/10.3168/jds.2016-11529
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Cytological endometritis at artificial insemination in dairy cows: Prevalence and effect on pregnancy outcome

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†CRV Holding BV, Van Thorenburghlaan 14, Oosterzele 9860, Belgium

<u>Conception rate (AI at ~60 DIM)</u>

Cytology positive at AI (> 1% PMN): 32.7%

Cytology negative at AI (< 1% PMN): 47%

Pascottini (2017) J. Dairy Sci. 100:588-597.

Journal of Animal Science, 2020, Vol. 98, No. 11, 1–20

doi:10.1093/jas/skaa288
Advance Access publication November 3, 2020
Received: 20 July 2020 and **Accepted:** 27 August 2020
Reproduction

REPRODUCTION

The incompletely fulfilled promise of embryo transfer in cattle—why aren't pregnancy rates greater and what can we do about it?

Peter J. Hansen

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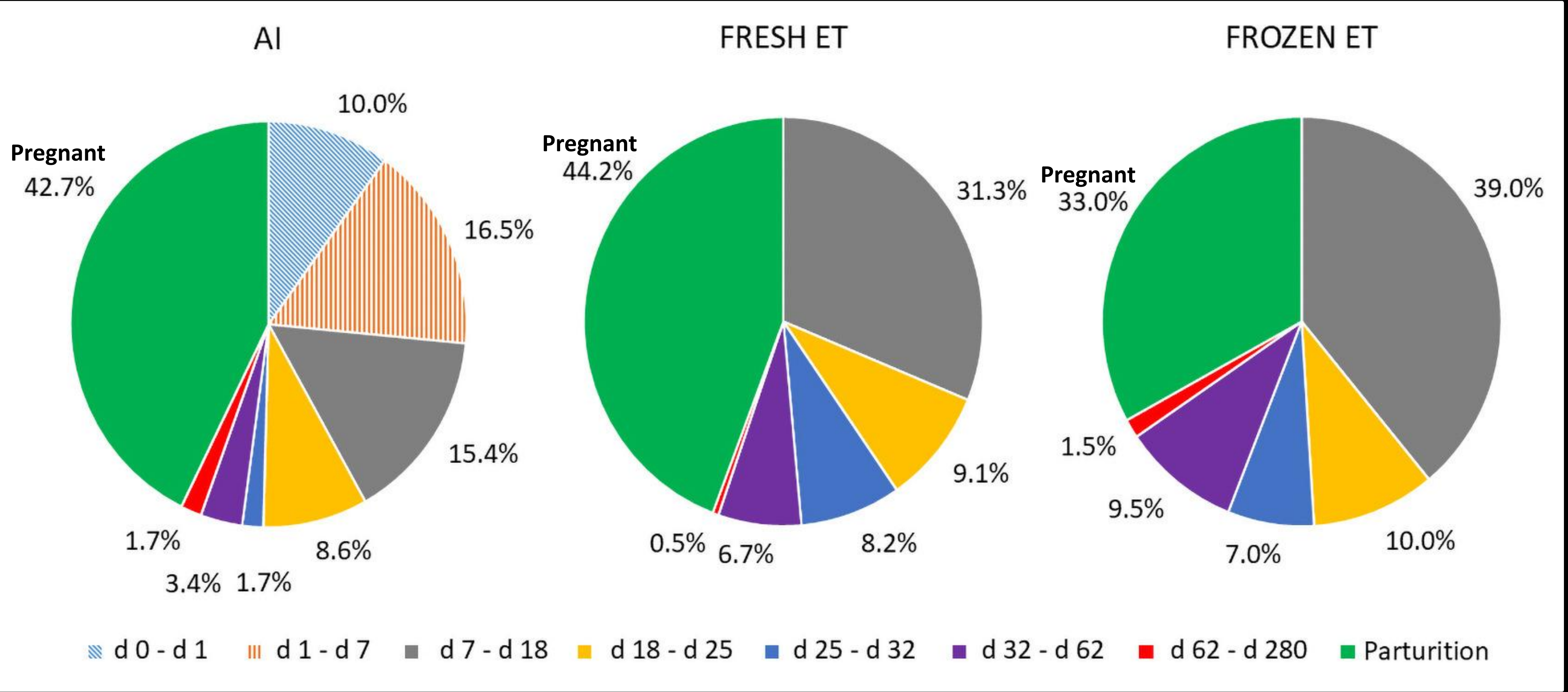
Embryonic loss (recent studies)

Embryonic loss – recent studies

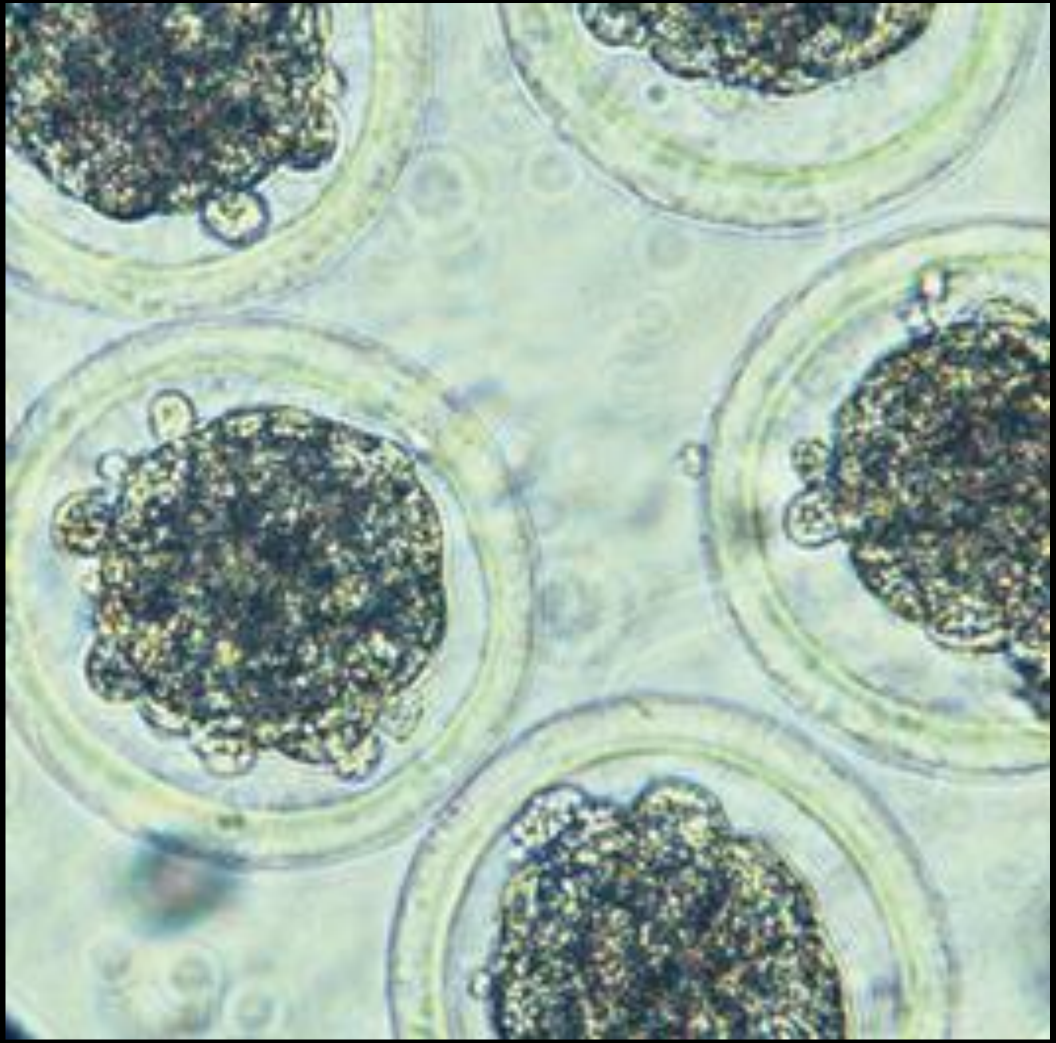


Crowe et al. (2025) J. Dairy Sci. 108:1022-1038.
~1100 grass-based cows Ireland

Embryonic loss – the embryo

Morphological assessment of fertility

Is the microscope good enough?



Embryonic loss – when?

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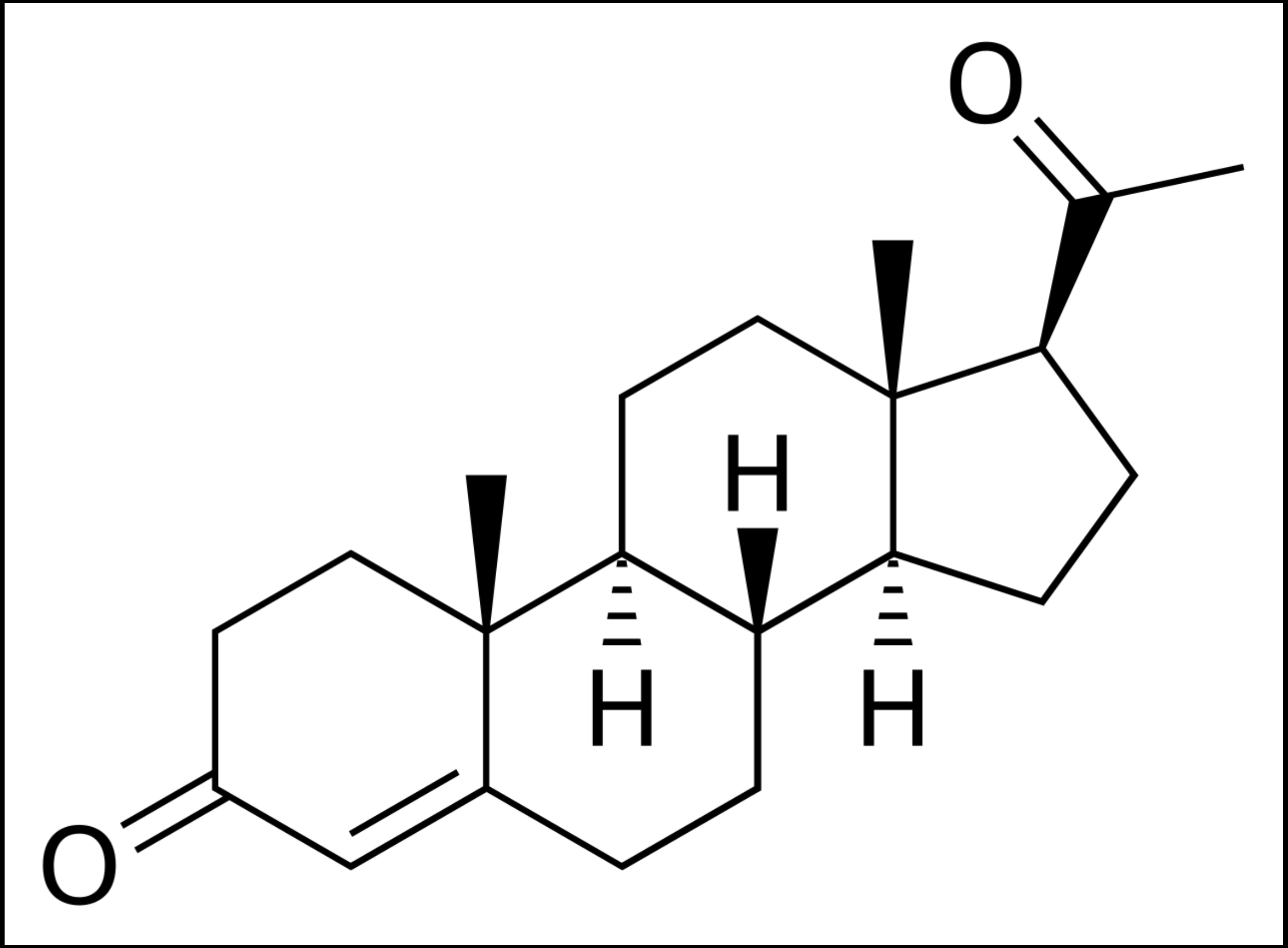
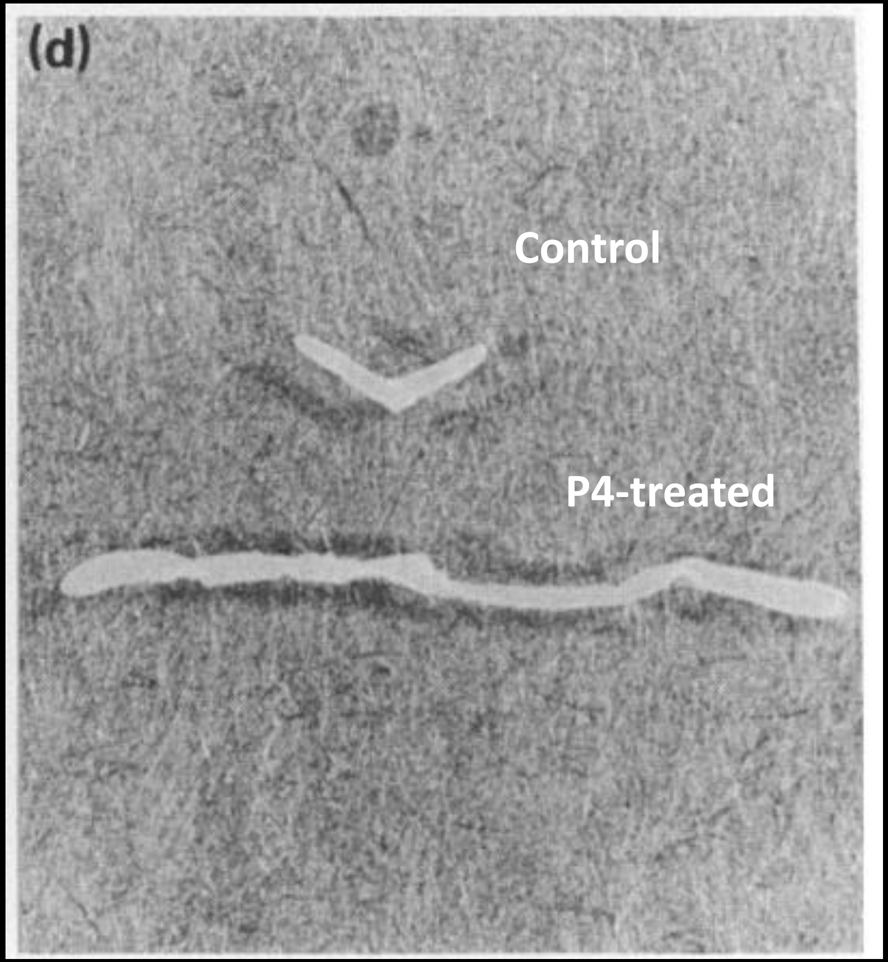
Progesterone!



Progesterone supplementation (first week after AI)



Conceptuses

Garrett et al. (1988)
J. Rerod. Fertil. 84:437-466

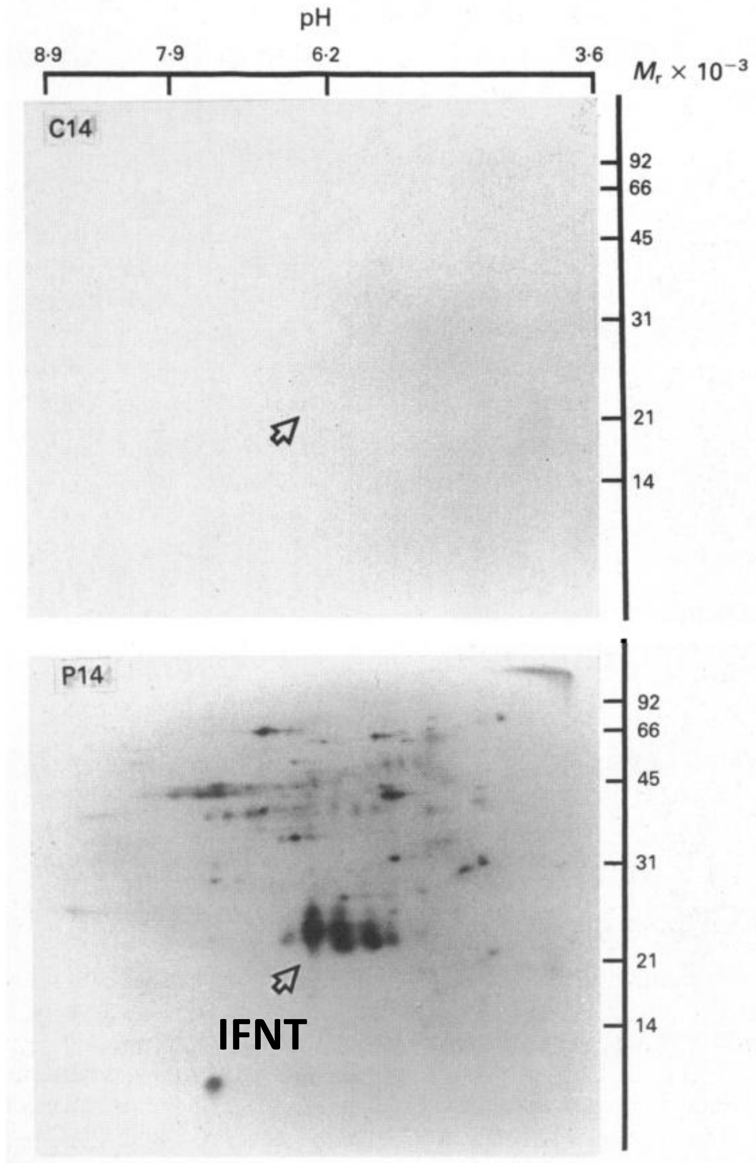


Fig. 3. Fluorographs representative of 2D-PAGE gels of acidic polypeptides in dialysed bovine conceptus culture media from Day 14 control (C14) and progesterone-treated (P14) cows. Arrow denotes position of the bTP-1 complex. Note absence of bTP-1 in culture medium of the control conceptus.

Progesterone to improve fertility – yes or no?

Theriogenology 85 (2016) 1390–1398

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

ELSEVIER

Efficacy of progesterone supplementation during early pregnancy in cows: A meta-analysis

CrossMark

Leyan Yan [a], Robert Robinson [b], Zhendan Shi [a,*], George Mann [c,**]

[a] *Institute of Animal Science, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, China*
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[c] *Division of Animal Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, UK*

Progesterone after AI – yes or no?

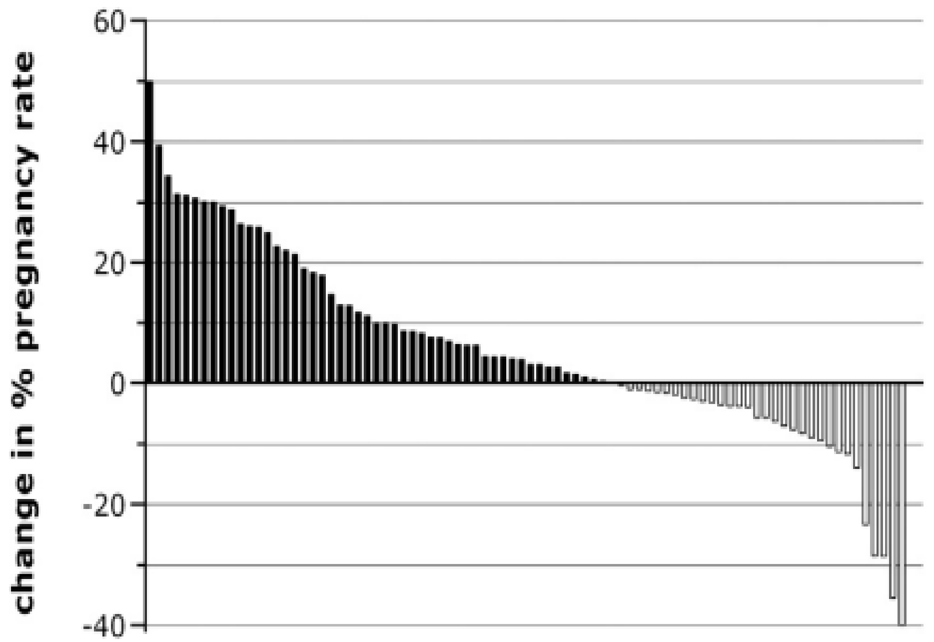

Fig. 1. Summary diagram illustrating the overall variation in the change of pregnancy rate associated with progesterone treatment in 84 experimental studies included in current meta-analysis. The vertical lines show the changes in pregnancy rate after postinsemination progesterone supplementation in individual study. A total of 51 treatments increased, 32 treatments decreased pregnancy rate, and one treatment has no effect resulting in an overall mean increase in pregnancy rate of 1.2% points.

4.1. Conclusions

The results clearly demonstrate that the administration of progesterone treatment too early (<Day 3) or too late (>Day 7) or to cows with relatively good reproductive performance delivers no benefit in terms of pregnancy rate. Furthermore, treatment of cows after cycle or ovulation synchronization is also ineffective. However, the results have clearly demonstrated a consistent increase in the chance of pregnancy in animals of relatively poor fertility supplemented with any form of progesterone by any route of administration during the period of the postovulatory progesterone rise (Day 3–7) after mating at natural estrus.

(Studies were either vaginal or injected P4)

GnRH or hCG – yes or no?

GnRH or hCG after AI – yes or no?

Associa... ...in-
releasir... ...during
the lute... ...alysis

M. Besbac...
[1]Laboratory of...
[2]High Nationa...
[3]Department ...
[4]IHAP, Univer...
[5]Université Cl...

CONCLUSIONS

The present meta-analysis quantifies the change in P/AI in cases of treatment with GnRH and hCG compared with no treatment. The meta-analysis suggests that in certain circumstances GnRH and hCG may produce significant benefits. It did not show any difference between GnRH and hCG. Treatment with GnRH and hCG improved P/AI in primiparous cows with low fertility, whereas treatment of cows with good fertility did not gain any benefit. A buserelin treatment above 10 μg, with an hCG dose above 2,500 IU and a GnRH treatment after d 10 post-AI, were associated with higher P/AI compared with lower doses and earlier treatment.

Embryonic loss – when?

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Embryonic loss

Wiltbank et al. (2016) defined several risk factors for embryonic loss (d 28 to 60):

- Failure to cycle by the start of the breeding season
- Parity (first parity > multiparous)
- Farm
- Change in BCS (greater BCS loss →more loss)
- Uterine disease
- Non-uterine disease (i.e., mastitis)

Wiltbank et al. (2016) Theriogenology 86:239-253

Embryonic loss

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- **Uterine disease**
- Non-uterine disease (i.e., mastitis)

Wiltbank et al. (2016) Theriogenology 86:239-253

Embryonic loss and uterine disease

What are the mechanisms connecting uterine disease with embryonic loss?

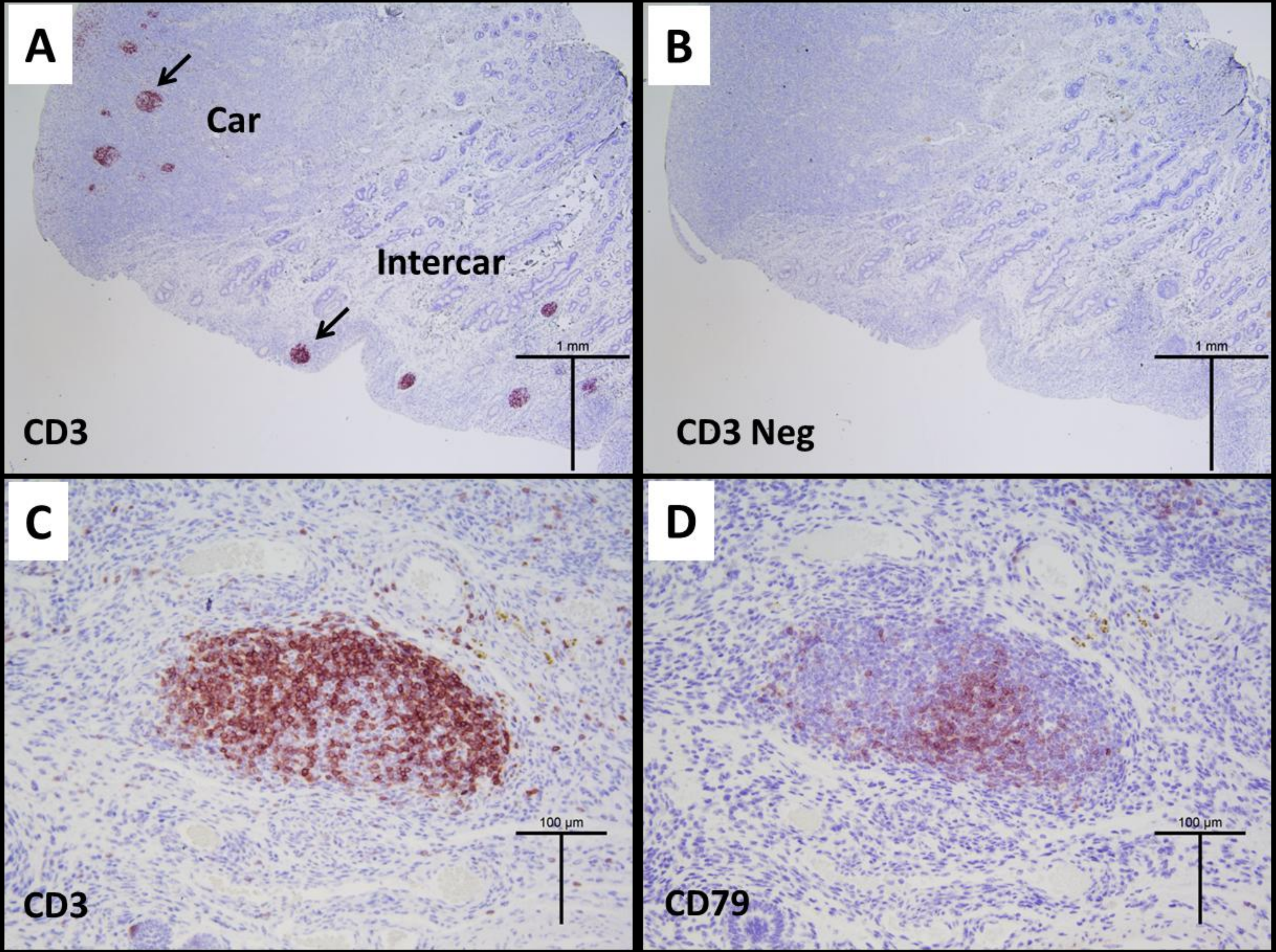
- Residual infection (microbiome; probably not)
- Morphological memory (yes)
- Residual inflammation (yes, probably fades over time)
- Cellular memory (yes)

Lymphocytic foci – postpartum cow

Residual inflammation

Lymphocytic foci in bovine endometrium

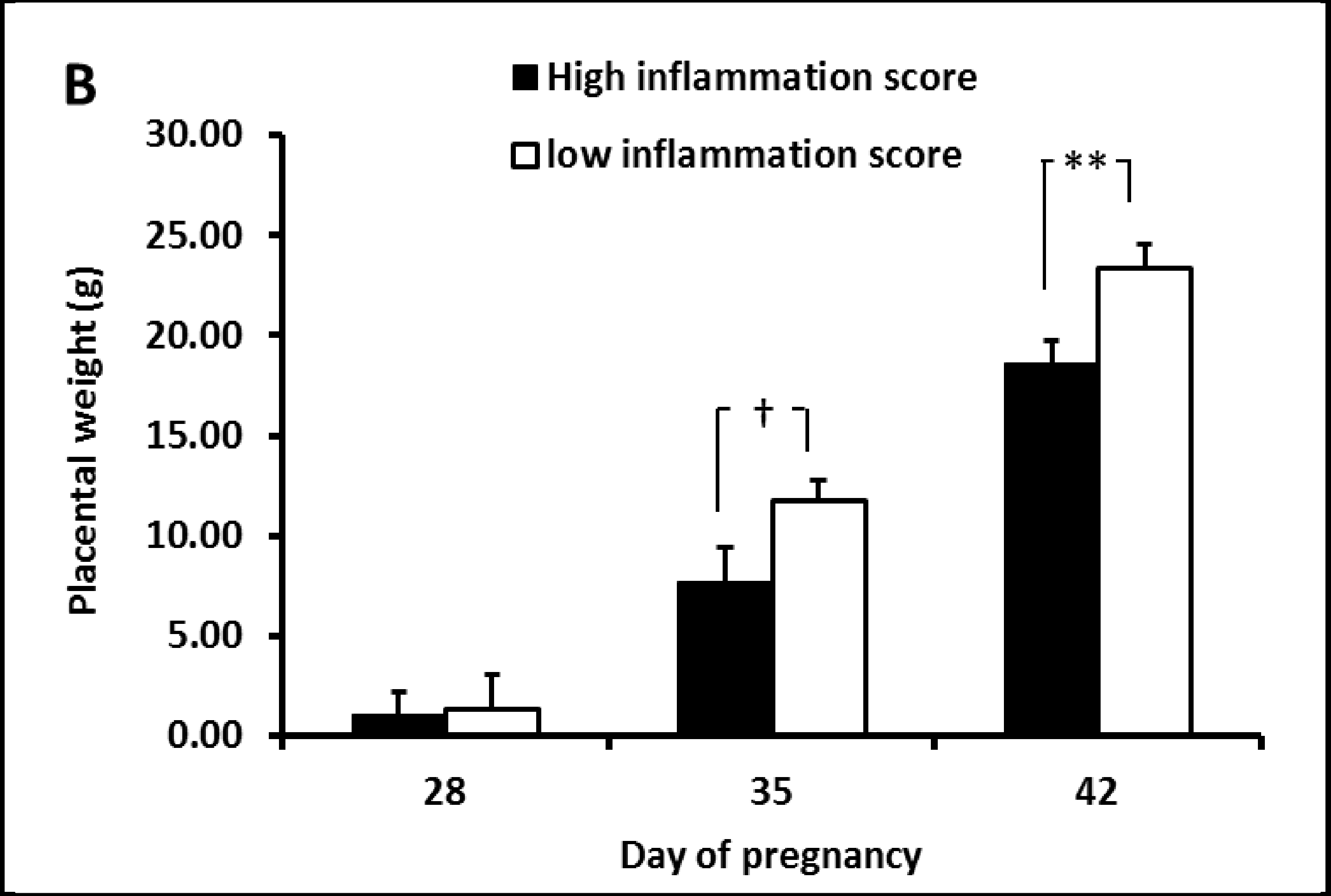
CD3: T cell
CD79: B cell



Lucy, Evans and Poock (2016)
Theriogenology 86:1711-9

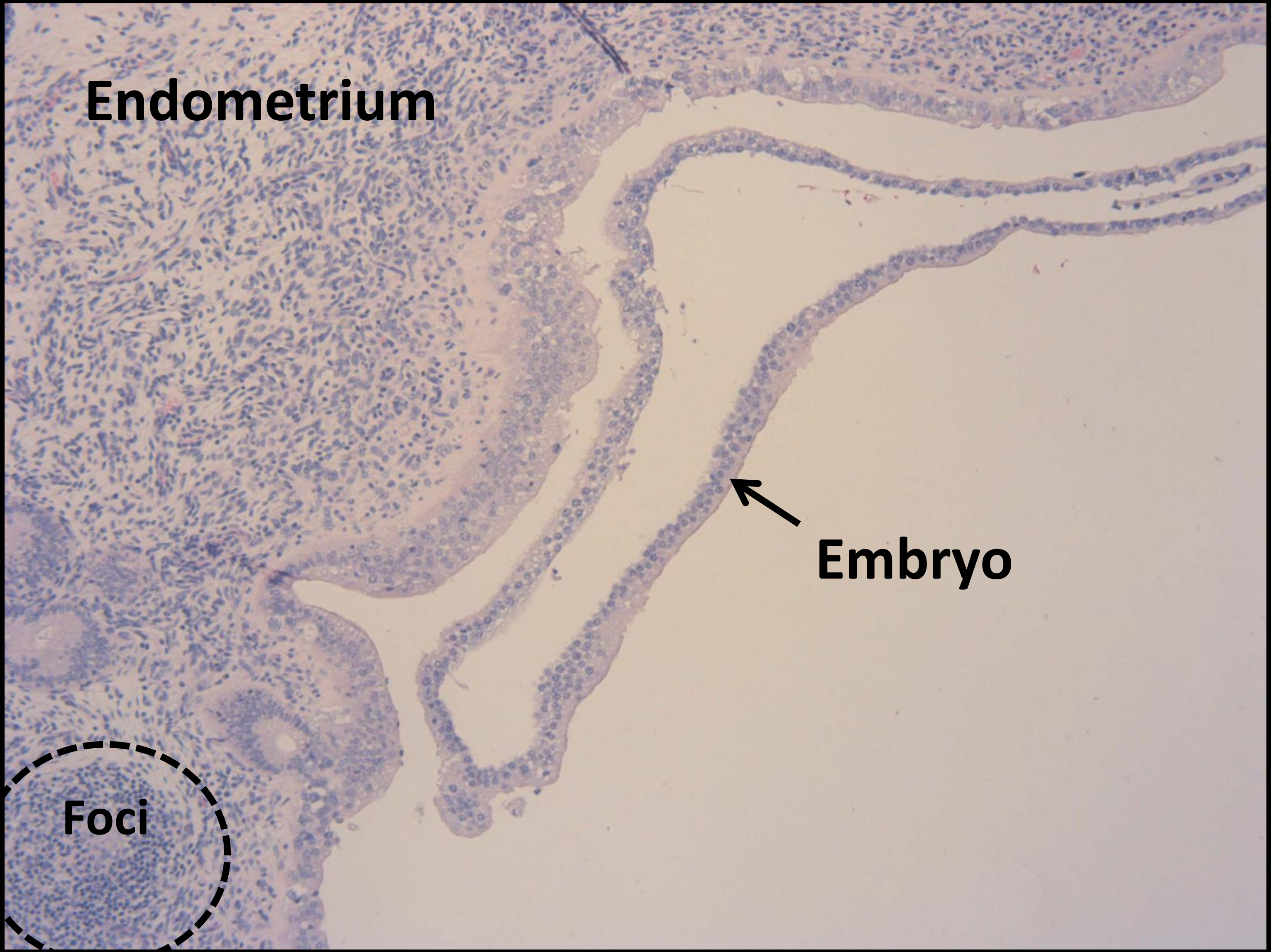
Foci and inflammation in the bovine uterus

Effect on placental weight

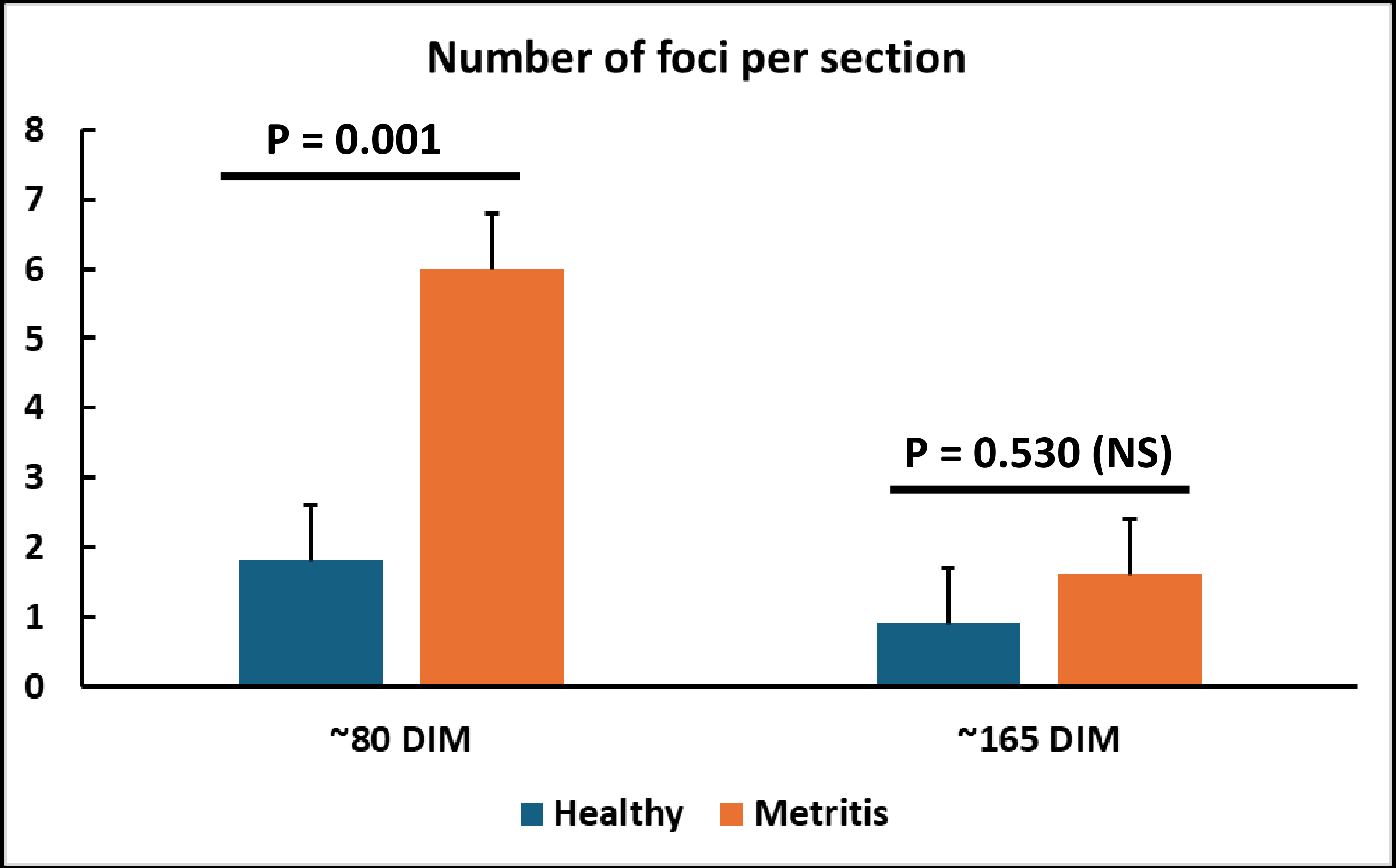


Lucy, Evans and Poock (2016)
Theriogenology 86:1711-9

Bovine embryo and endometrium with foci



Effect of DIM on the number of uterine foci in metritis versus healthy control cows



Lymphocytic foci go away over time

Dada and Lucy (unpublished)

Cellular memory

Cellular memory

Biology of Reproduction, 2024, **111(2)**, 332–350
https://doi.org/10.1093/biolre/ioae067
Advance access publication date 5 May 2024
Research Article

OXFORD

Metritis and the uterine disease microbiome are associated with long-term changes in the endometrium of dairy cows†

Josiane C. C. Silva[1], Monica O. Caldeira[1], Joao G. N. Moraes[2], Isabella Sellmer Ramos[1], Tamara Gull[3], Aaron C. Ericsson[3], Scott E. Poock[3], Thomas E. Spencer[1] and Matthew C. Lucy[1,*]

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† **Grant Support:** This study was supported by the National Institute of Child Health and Human Development of the National Institutes of Health under the award number R01HD092254.

J. Dairy Sci. 106:6593–6596
https://doi.org/10.3168/jds.2023-23445
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Perspective: Can early embryonic losses be reduced in lactating dairy cows?

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Yes!

- Through adopting best management practices for dairy reproduction
- Developing new and innovative tools based on sound science

While we wait for the science . . .
follow Fricke's rules for getting cows pregnant

Quotes from 2014 International Cow Fertility Conference (Westport, Ireland)

Paul Fricke (University of Wisconsin)

How to get cows pregnant

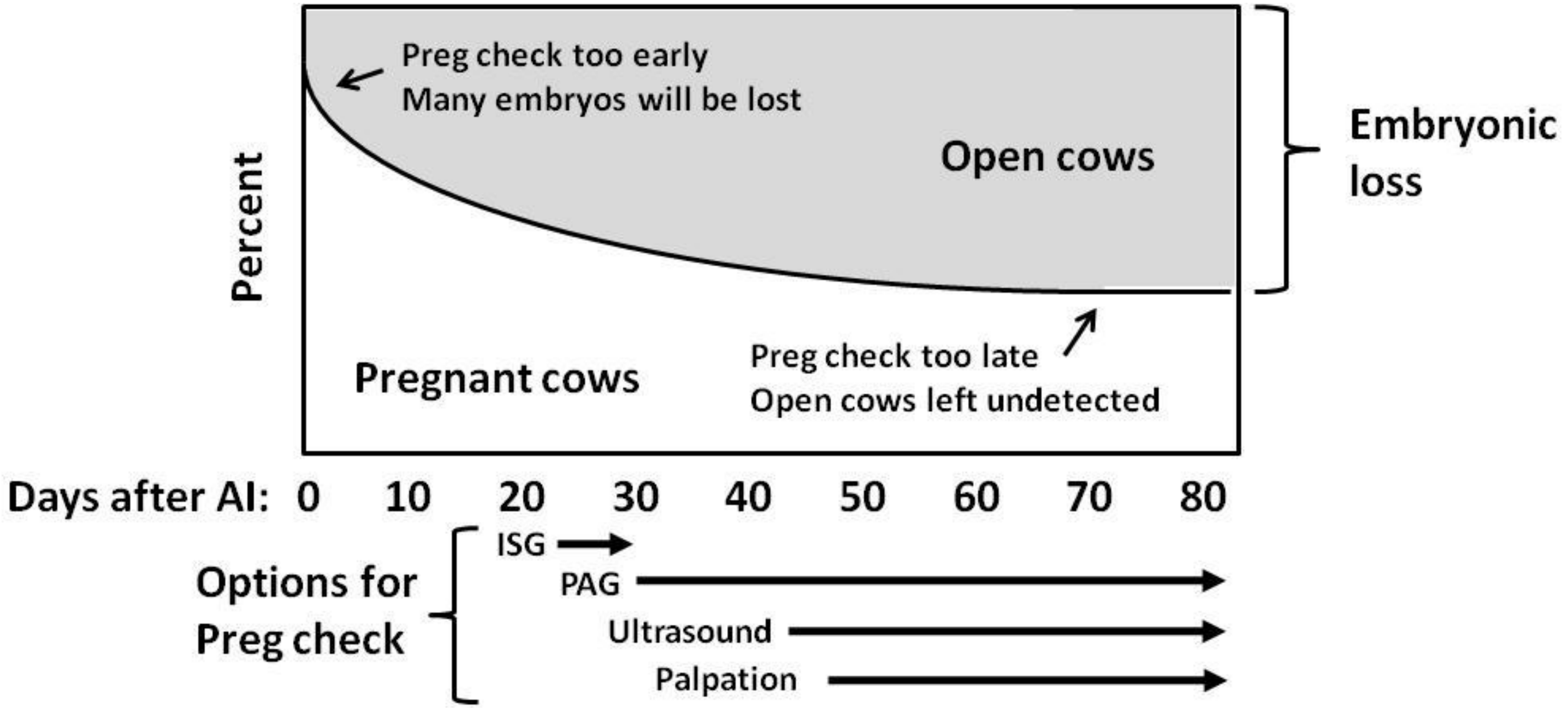
Rule 1: Put semen in the cow.

Rule 2: If she is not pregnant … refer to rule one.

Cows with embryonic loss are not pregnant!
You should apply Rule 2!

Decisions, decisions, decisions

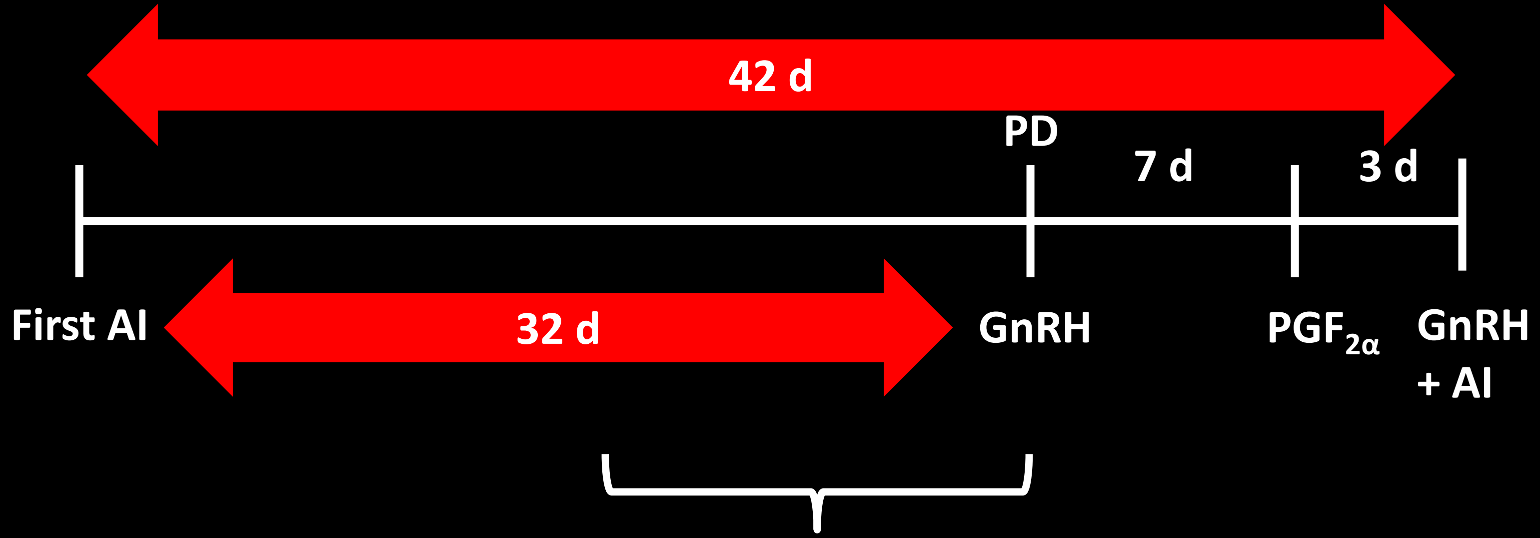
When should I put semen back into non-pregnant cows?



Lucy et al. DCRC Proceedings Fall 2009

Activity systems compliment resynchronization programs

Day 32 Ovsynch is popular for
second insemination resynch



Activity systems (neck collars, ear tags, etc.) are important for catching heats on “return to service” cows.

Biology of Reproduction, 2025, **112(5)**, 981–995
https://doi.org/10.1093/biolre/ioaf066
Advance access publication date 28 March 2025
Research Article

OXFORD

Early identification of bovine pregnancy status and embryonic mortality[†]

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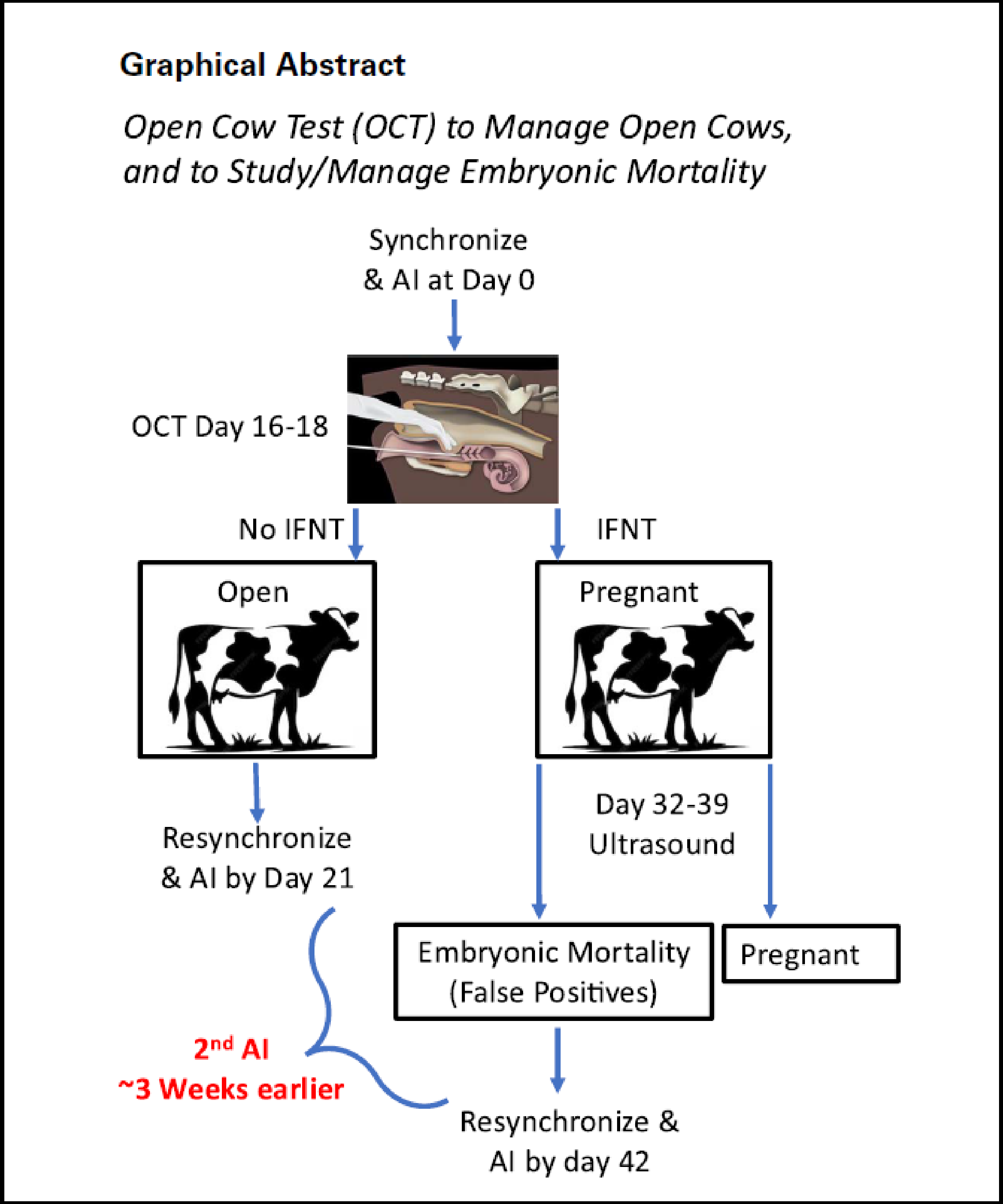
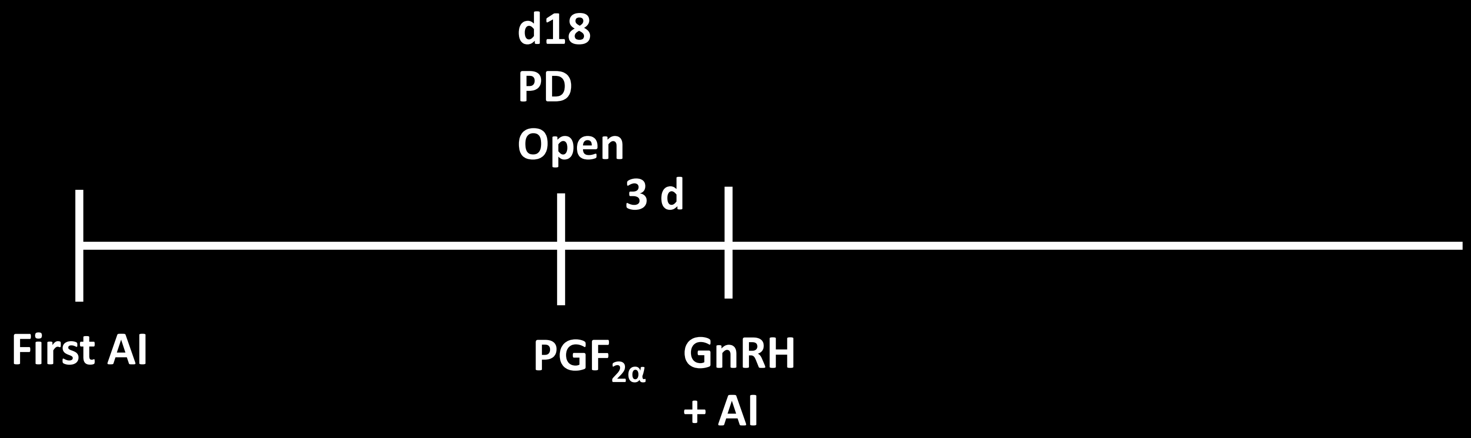
[†]**Grant Support**: Supported by the Hatch/Multi State Research Accession No. 1011648, W114 Project No. COLO0220C, Colorado Agricultural Experiment Station from the United States Department of Agriculture-National Institute of Food and Agriculture, Colorado Economic Development and International Trade Award number 010077-00002/Sponsor Award #DO 2021-2633, CSU STRATA Award 010077-00002/Sponsor Award #DO 2021-2693, and the Animal Reproduction and Biotechnology Laboratory (Translational Reproductive Biotechnology) grants program to TRH.
[‡]JVB and AG contributed equally to this work.

Day 16 to 18 Open Cow Test

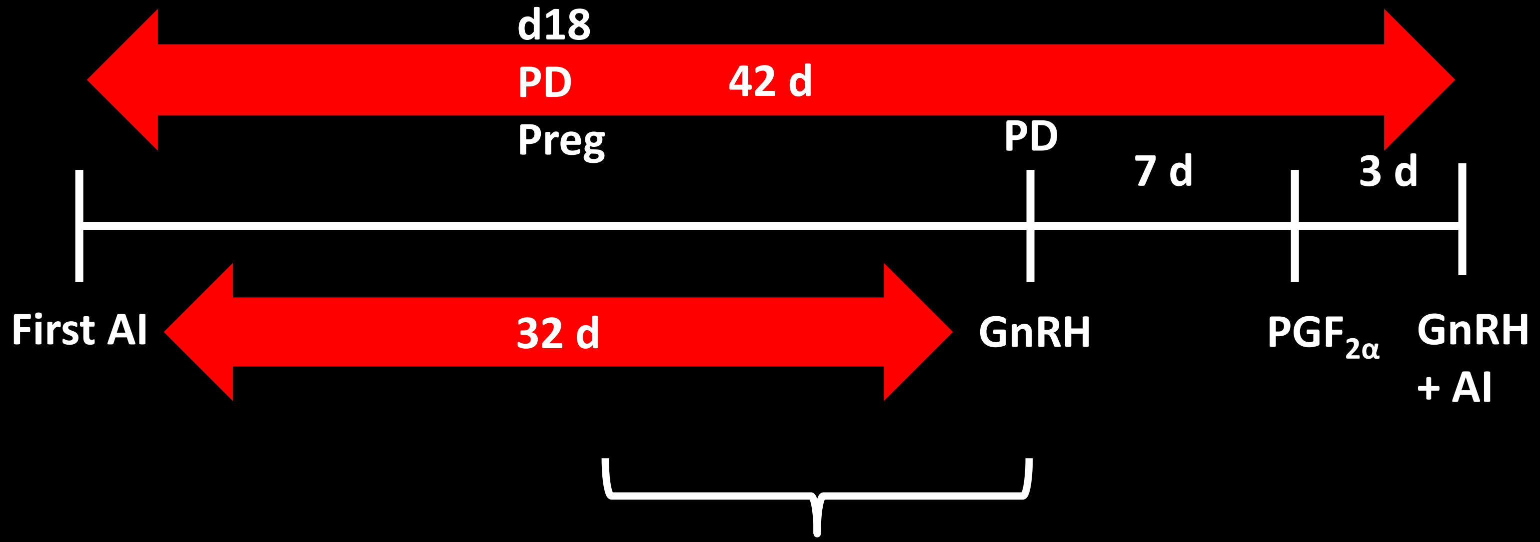
Bishop et al. (2025) *Biology of Reproduction* 112: 991-995



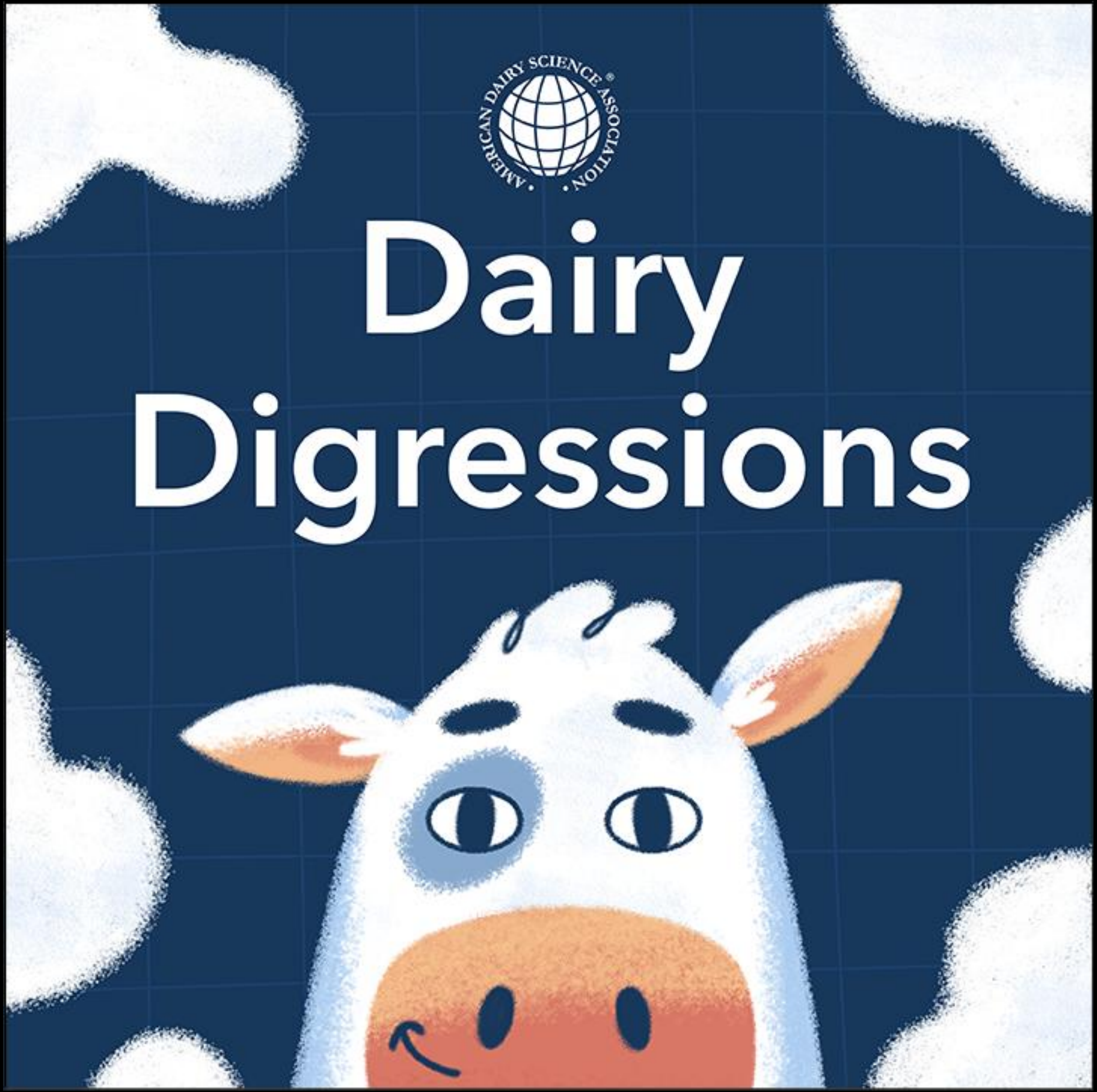
Future resynchronization programs



Future resynchronization programs



Activity systems are important for catching heats on “return to service” cows.



Thank you!

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