Faculty of Veterinary Medicine

1st International Symposium on Bison Health

Assisted Reproductive Technologies in Bison

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Assisted Reproductive Technologies

- Artificial Insemination
  - Frozen semen
  - Estrus synchronization
- Embryo Transfer
  - Superovulation
- In vitro Embryo Production
  - Oocyte collection
  - In vitro maturation
  - In vitro fertilization
  - In vitro culture
- Cloning

Bison Reproductive Physiology

- Male fertility
  - Puberty 16.5 ± 2.5 months (Helbig *et al*, 2007)
  - Effect of season
    - No differences in the proportion of normal and abnormal epididymal sperm between seasons
    - No change in sperm concentrations over the study period.
    - Increased testes weight and increased percentage normal sperm and individual sperm motility just prior to the rut. (Helbig *et al*, 2007)

Bison Reproductive Physiology

- Female fertility
  - Seasonally polyestrous (Rutley & Rajamahendran, 1995)
    - Spontaneous ovulation begins in August
    - Spontaneous ovulation ceased by the end of March
  - Follicle dynamics
    - Ovarian follicles develop in a wave-like pattern subsequent to a surge in FSH (McCorkell et al, 2013)

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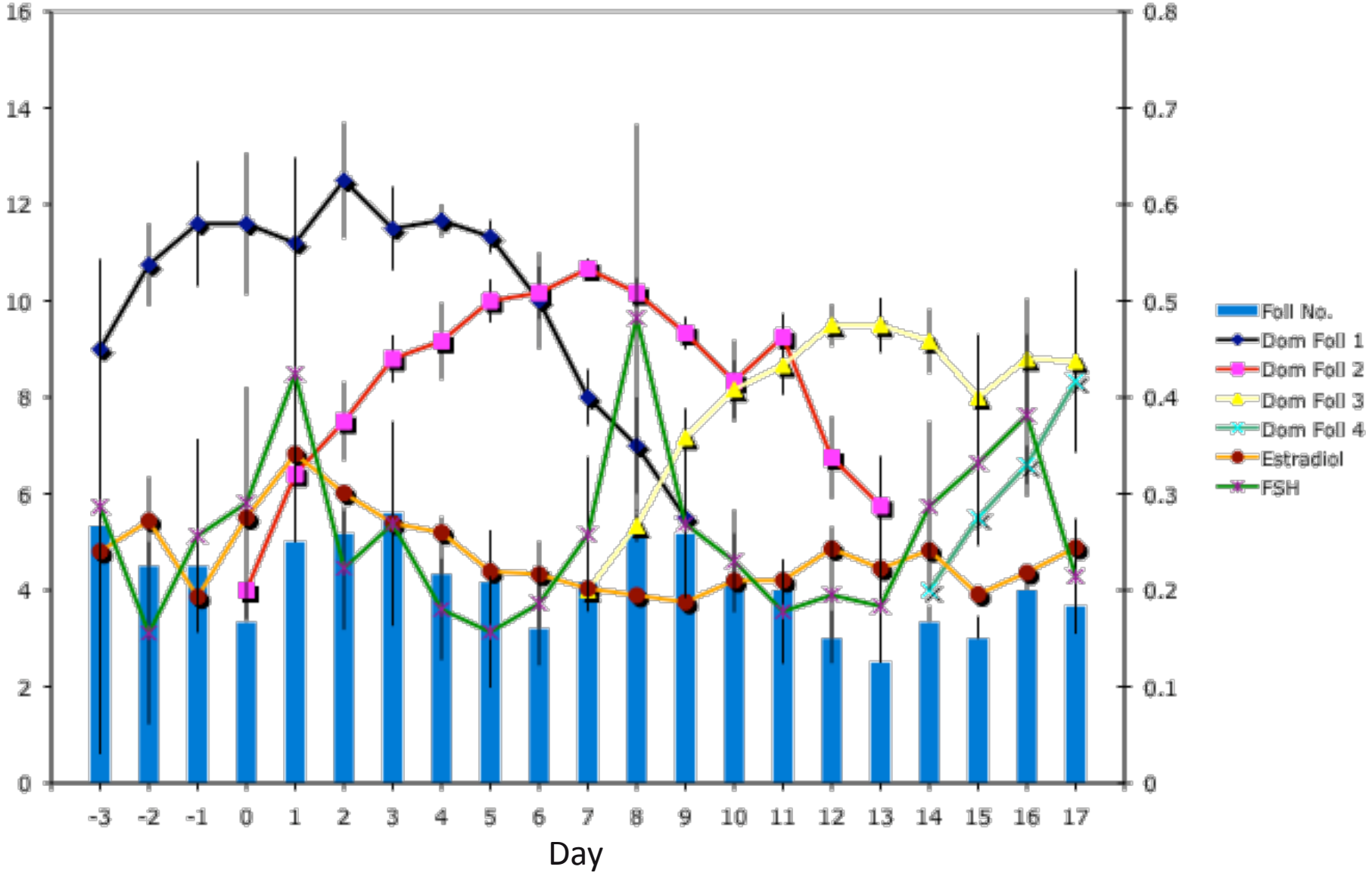
Ovarian Follicle Dynamics



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Ovarian Follicle Dynamics

Date of 1st ovulation (n=7)	August 24 ± 2.2 (range Aug. 11 to Aug. 28)
Diameter of 1st ovulatory follicle (n=8)	16.3 ± 0.5 mm
Maximum diamter of 1st CL (n=7)	17.5 ± 0.7 mm
Length of 1st IOI (n=6)	8.5 ± 0.2 days
No. of follicle waves in 1st IOI (n=6)	1 wave
Date of 2nd ovulation (n=8)	Sept. 4 ± 2.7 days (range Aug. 19 to Sept. 13)
Diameter of 2nd ovulatory follicle (n=8)	14.2 ± 0.7 mm
Maximum diamter of 2nd CL (n=6)	18.7 ± 0.6 mm

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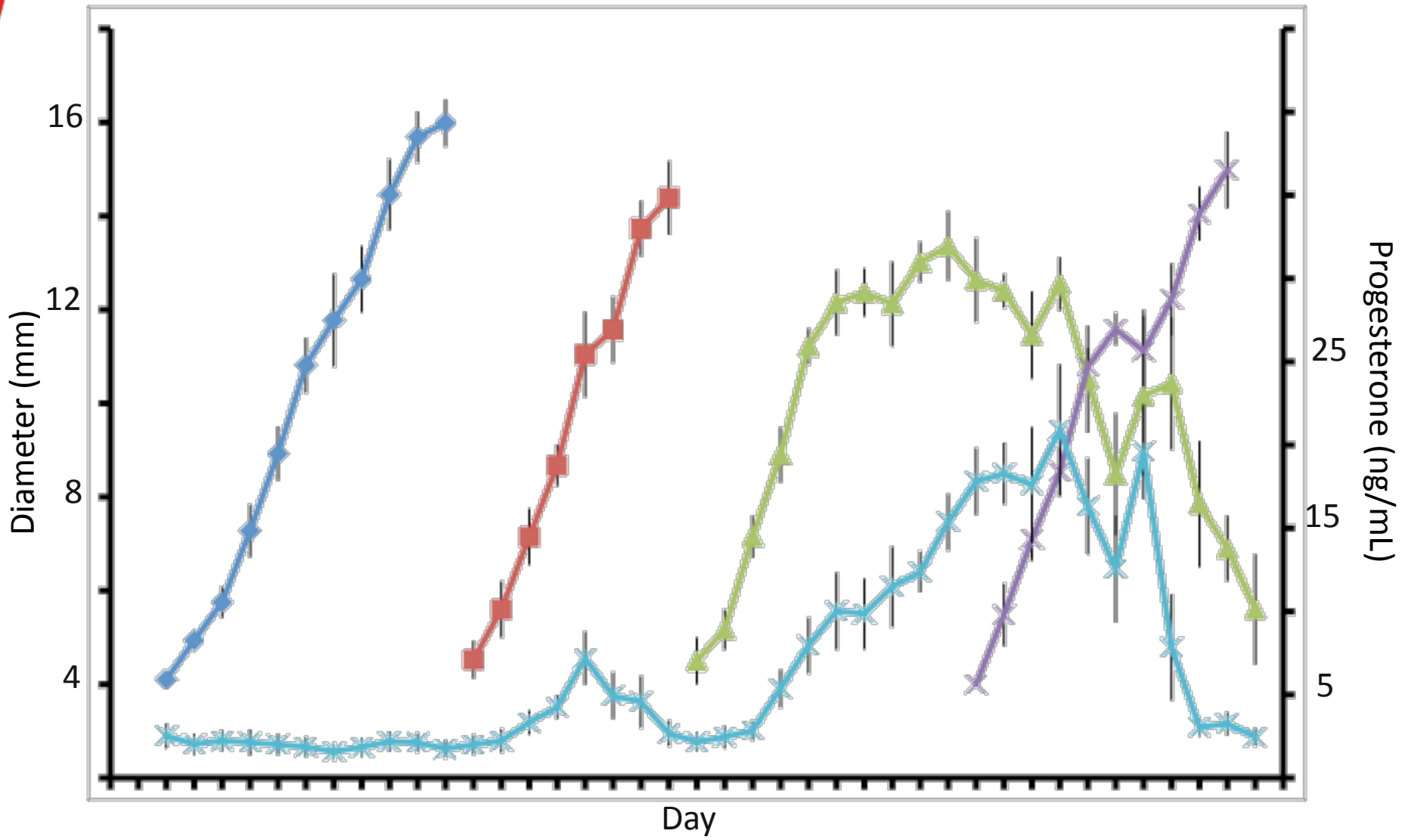
Ovarian Follicle Dynamics



Artificial Insemination

- 1st of the Assisted Reproductive Technologies used commercially, Mid 20th century
- Advantages
  - Genetic improvement
  - Disease control/biosecurity
  - Import and export friendly
  - Accurate breeding records
  - Reduced requirement for dangerous bulls
  - Genetic salvage
  - Marketable product

Artificial Insemination

- 1st bison calf from AI born 1994 (Dorn, 1995)
  - Estrus synchronized
    - 2 injections of $PG_{2\alpha}$ given 11 days apart
  - Frozen semen
    - Post thaw quality marginal
    - Further studies needed to determine optimum parameters for freezing of bison semen.
  - Insemination
    - 60 hours after 2nd $PG_{2\alpha}$
    - GnRH given at time of insemination
    - Estrus difficult to detect

Artificial Insemination

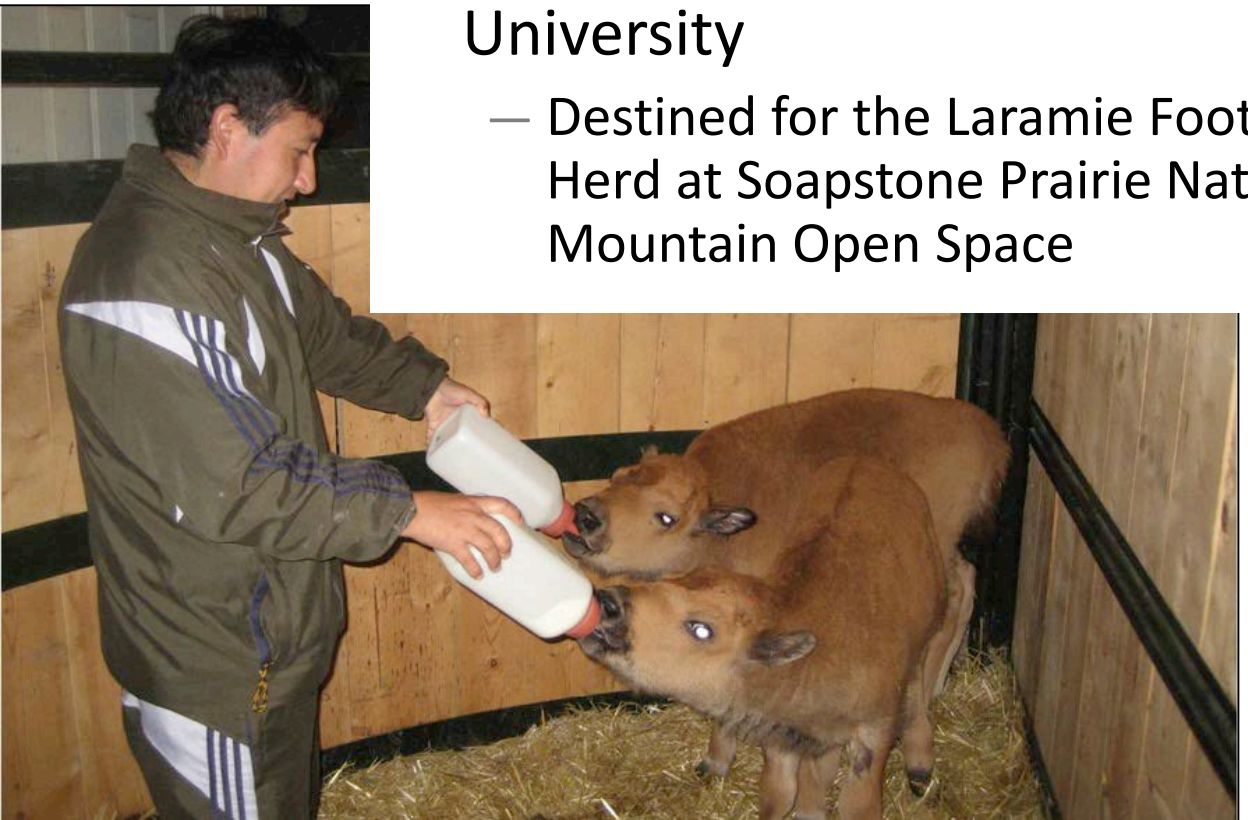
- Frozen semen
  - Maximum damage to bison sperm during freeze-thaw stage (Hussain *et al*, 2011)
  - Bison sperm pretreated with cholesterol loaded cyclodextrin and extended in Triladyl with glycerol should improve survival (Hussain *et al*, 2013)

Artificial Insemination

- Estrus synchronization
  - Day 0: Synchro-mate B (norgestomet implants) and injection of estradiol valerate. Day 8: Remove implant. Estrus 4.1 ±0.5 days after implant removal (Matsuda *et al*, 1996; Othen thesis, 1997)
  - Day 0: Estradiol 17β plus progesterone (50 mg) injection at the time of placement of an intravaginal progesterone-releasing device. Day 8: Device removed and $PGF_{2\alpha}$ given. Day 10: 5 mg pLH given. Ovulation 2.7 ± 0.6 days later. (Adams et al, 2010)
    - Fixed-timed AI

Artificial Insemination

- 2009 2 AI Wood Bison calves born Saskatoon
- 2011 1st AI Wood Bison calf born Toronto Zoo
  - Chilled semen from Saskatoon
  - AI calves born at Toronto Zoo in 2012, 2013, and 2014
- 2015 AI Plains Bison calves Colorado State University
  - Destined for the Laramie Foothills Bison Conservation Herd at Soapstone Prairie Natural Area and Red Mountain Open Space



Embryo transfer

- Commercial ET began in the late 1970's
- Advantages
  - Genetic material from dam as well as the sire
  - Import and export friendly
  - Disease control/biosecurity
  - Genetic salvage
  - Marketable product

Embryo transfer

- 1st ET bison calf born in 1993 (Dorn 1995)
  - Superovulation
    - Synchro-mate B (norgestomet implants) in place 5 days
    - 34 mg (Armour) FSH-P decreasing dose schedule over 4 days
    - AI at 60 and 72 hours after implant removal
    - 100 mcg GnRH given at 1st insemination
    - Results

Trial	Ovulations	Ova/embryos recovered	Transferable embryos
1	4.2 ± 1.0	3.1 ± 0.9	0.7 ± 0.3
2	3.6	3.3	1.6

Embryo transfer

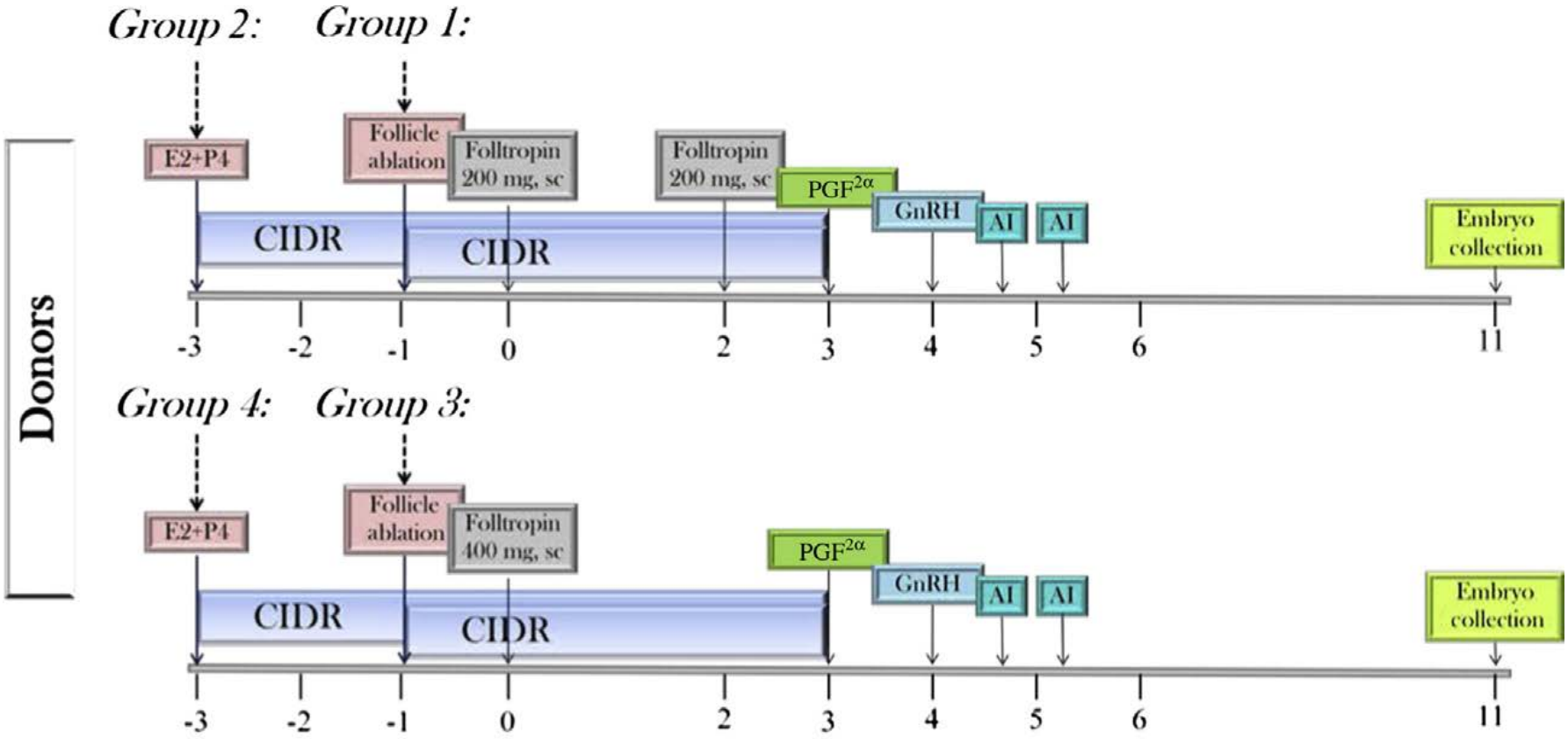
- Two ET bison calves negative for *Brucella abortus* born from infected donor cows (Robison *et al*, 1998)
  - Superovulation
    - Day 0: Synchro-Mate B implant
    - Day 3: 2500 PMSG + 8 mg FSH + 25 mg $PGF_{2\alpha}$
    - Day 5: Synchro-Mate B implant removed + 25 mg $PGF_{2\alpha}$
    - Day 7: 4,000 HCG, inseminate 36 and 48 hours post implant removal
    - Day 14: Non-surgical embryo collection
      - Collected embryos washed according to ET standards and transferred to synchronized recipients

Embryo transfer

- Results (Robison *et al*, 1998)
  - 33 attempts at superovulation of 16 cows
  - 28 attempts warrant proceeding with embryo collection
  - From 28 collections, 20 viable embryos, 11 degenerate embryos, and 63 unfertilized ova
  - 13 embryos transferred resulted in 2 pregnancies
  - 15% pregnancy rate
  - 2 calves born, 1 died during birth
- refinement of bison-specific super-ovulation procedures will be necessary to improve the efficiency of embryo transfer.
- Fixed-timed AI essential

Embryo transfer

- Two ET Wood bison calves born in Saskatoon 2011
  (Toosi *et al*, 2013)



Embryo transfer

Superovulatory response in wood bison in experiment 2.

End point	Folltropin dose		Combined
	2	4	
Number of ovulations			
CIDR	13.2 ± 3.9	7.2 ± 1.3	9.9 ± 2.1
No CIDR	8.8 ± 1.9	5.4 ± 0.7	6.9 ± 1.0
Combined	11.2 ± 2.4[a]	6.4 ± 0.8[b]	8.6 ± 1.2

(Toosi *et al*, 2013)

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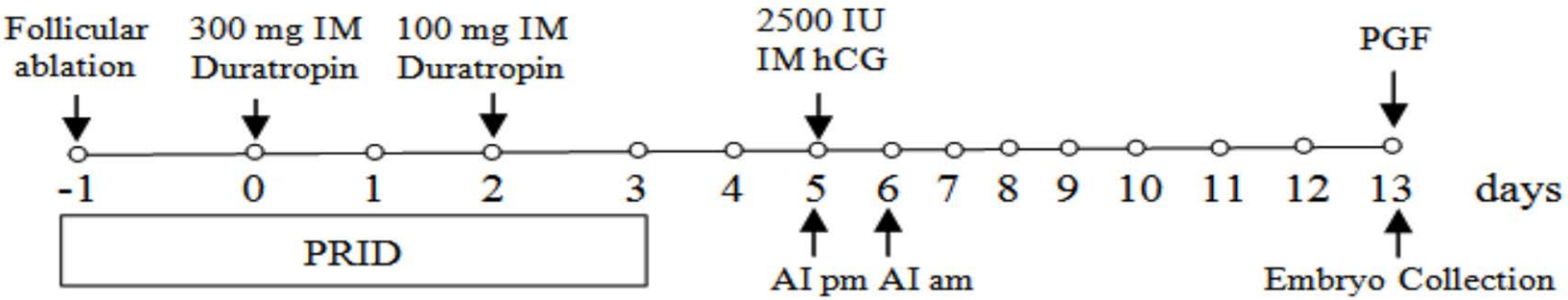
Embryo transfer

Embryo transfer

- Single dose of Folltropin in a slow-release formulation as effective as two doses of Folltropin
- Two doses of Folltropin was more effective than four doses
- Intravaginal progesterone-releasing device during superstimulatory treatment did not influence the superovulatory response
- ET not a specific objective
  - 4 bison flushed
    - 7 blastocysts and 3 UFO recovered

(Toosi *et al*, 2013)

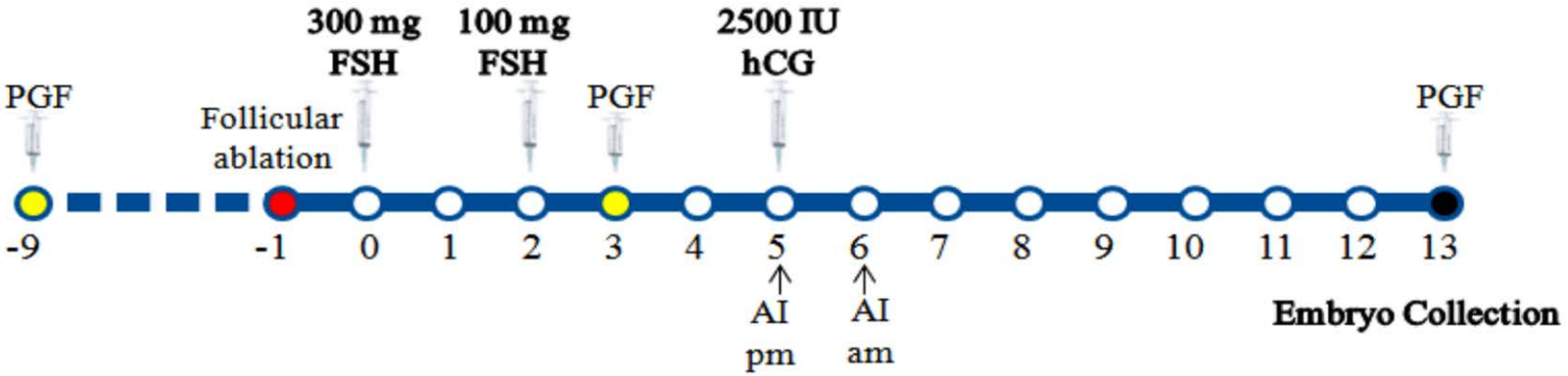
Embryo transfer anovulatory season



Number of bison	8
Number of follicles ≥9 mm on Day 5	14.8 ± 2.4
Ovulation rate (# ovulations/# follicles ≥9 mm)	80% [a]
Number of CL	10.8 ± 1.7
Total ova/embryos	5.4 ± 1.9
Transferable embryos	2.1 ± 1.0

(Palomino *et al*, unpublished)

Embryo transfer ovulatory season



n	# follicles ≥ 9 mm on Day 5	Ovulation rate (# ovulations/ # follicles ≥9 mm)	Number of CL	Number of ova/embryo	Number of Transferable embryos
12	8.2 ± 2.4	79%	6.9 ± 0.98	4.2 ± 0.46	2.9 ± 0.71

(Palomino *et al*, unpublished)

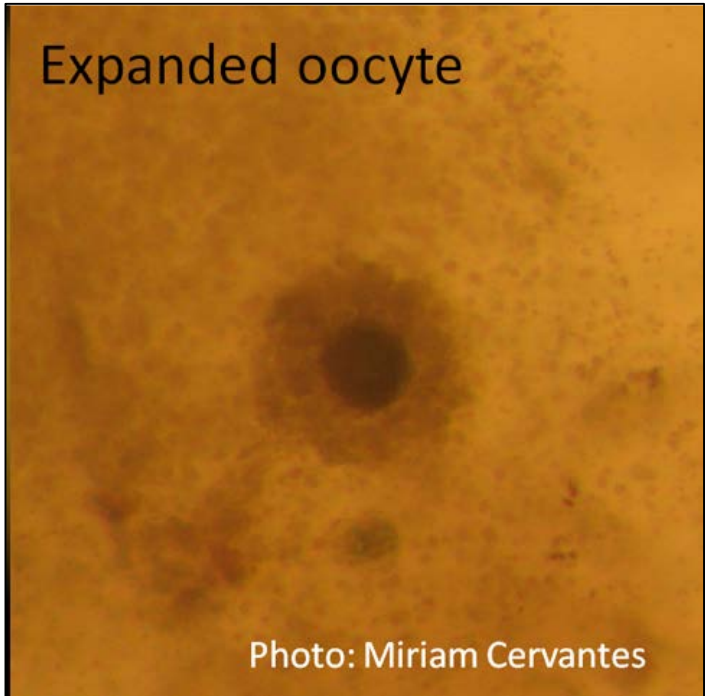
Embryo collection and cryopreservation in wood bison

	Number of Ova/embryo collected	Number of transferable embryos	Number of embryos frozen
Non breeding season 2011 - 2013	199	58	28*
Breeding season 2011 - 2013	151	89	46*
Total	350	147	74

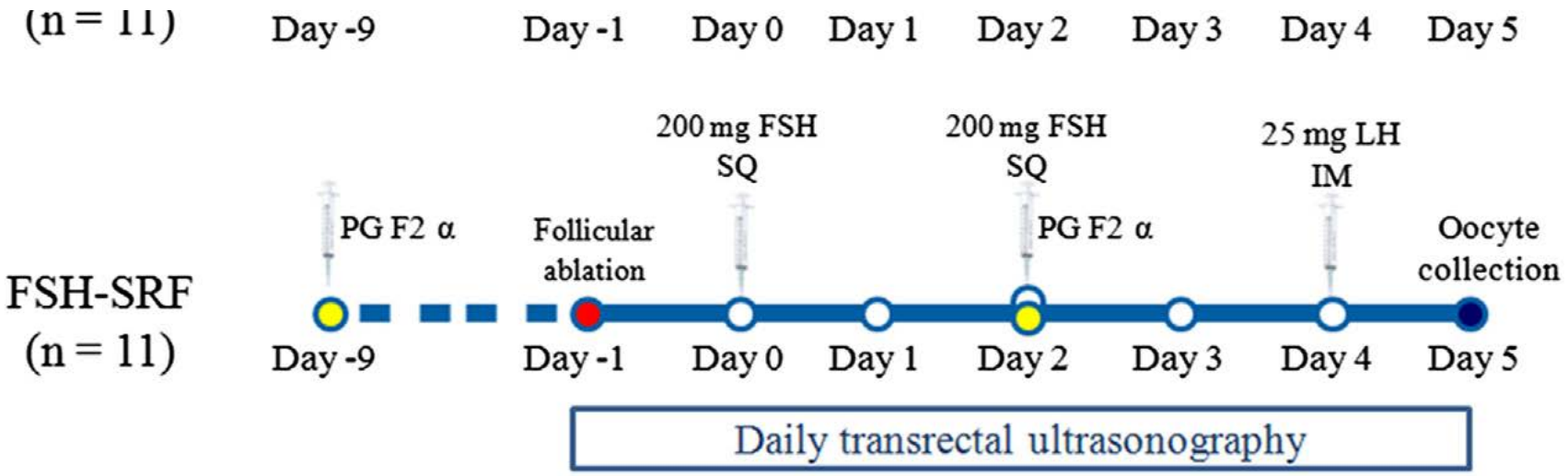
*Embryos from 2013 collection were not frozen

In vitro Embryo Production

- 1st IVF calf born in 1982
- No reported bison calves from IVF
- In vitro Embryo Production
  - Retrieval of oocytes from ovarian follicles
    - Ovarian follicle aspiration
    - Abattoir derived ovaries
  - In vitro maturation (IVM)
  - In vitro fertilization (IVF)
  - In vitro culture (IVC)



In vitro Embryo Production



Experiment 2

No. of follicles ≥5 mm	Diameter (mm) largest follicle	No. of follicles aspirated	No. of COC collected
12.4 ± 1.49	10.5 ± 0.59	11.0 ± 1.49	6.5 ± 1.13
13.8 ± 1.24	11.5 ± 0.76	11.4 ± 1.49	6.3 ± 0.96

(Palomino *et al*, 2014)

In vitro Embryo Production

- Ovarian superstimulation achieved in each of 22 wood bison in two experiments
- All developed between 6 to 12 follicles greater than 5 mm that were available for aspiration.
- Total of 431 follicles were aspirated transvaginally
- 237 COC collected (55% collection rate); comparable with collection rates reported in cattle (55%–70%)
- LH treatment 24 hours before COC collection to stimulate in vivo maturation of oocytes
  - More than 70% of COC collected were expanded

(Palomino *et al*, 2014)

In vitro Embryo Production

In vitro development of expanded wood bison COC after immediate fertilization or after additional 4 h of maturation

	Immediate fertilization	Additional 4 h of maturation	Total
Expanded COC (n)	38	38	76
Cleavage rate (Day 1)	21/38 (55%)	23/38 (61%)	44/76 (58%)
Blastocyst rate (Day 8)	7/38[a] (18%)	17/38[b] (45%)	24/76 (32%)

(Cervantes et al., 2015)

Wood Bison Reproductive Research Group

- Dr. Gregg Adams, WCVM
- Dr. Robert McCorkell, UCVM
- Dr. Murray Woodbury, WCVM
- Dr. Jacob Thundathil, UCVM
- Dr. Carl Lessard, Canadian Animal Genetics Resource Center, Agriculture Canada
- Dr. Douglas Whiteside, Calgary Zoo
- Dr. Gabriela Mastomonaco, Toronto Zoo
- Dr. Todd Shury, Parks Canada
- Dr. John Nishi, Government of Northwest Territories
- Dr. Brett Elkin, Government of Northwest Territories

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Supporting Partners

- AFIF, Specialized Livestock Chair WCVM
- Agriculture and Food Council (AFC)
- Agriculture and Agri-Food Canada (AAFC)
- Toronto Zoo
- World Wildlife Fund
- Parks Canada
- Government of the Northwest Territories
- Saskatchewan Bison Producers
- Bioniche