1st International Symposium on Bison Health

June 26th, 2015

In vitro embryo production in Wood bison (*Bison bison athabascae*)

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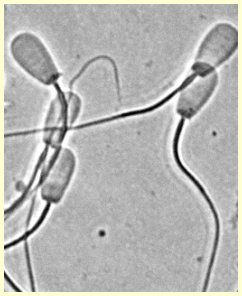
In vitro embryo production

- Applied in cattle with relative success

- Few studies in **wood bison→** slaughtered ovaries:
  - frozen-thawed semen: blastocyst rates 7.5%
  - chilled epididymal sperm: blastocyst rates 10%

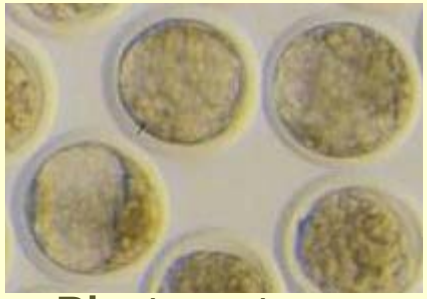
(Thundathil et al., 2007)

In vitro embryo production (IVP)



In vitro fertilization

In vitro culture



Blastocysts

Fresh or cryopreserved

In vitro maturation

COC: Cumulus-oocyte complexes

Slaughtered animals

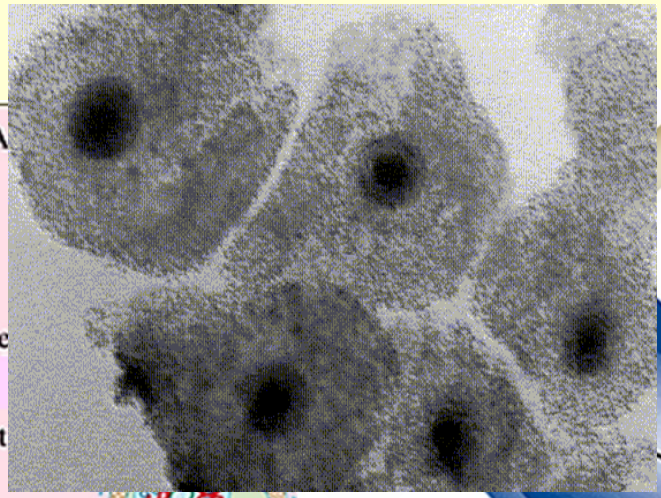
Live animals

Oocyte maturation

- Necessary to be competent for fertilization and embryo development

Oocyte maturation → ***in vitro*** / ***in vivo*** → *In vitro* embryo production

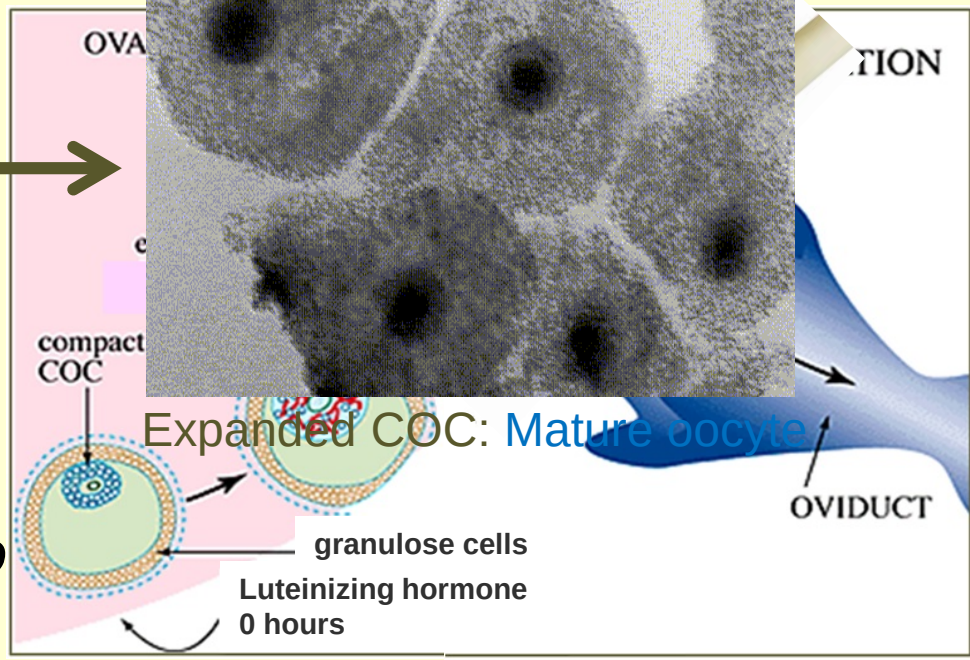
Compact COC



Expanded COC: Mature oocyte

In vitro

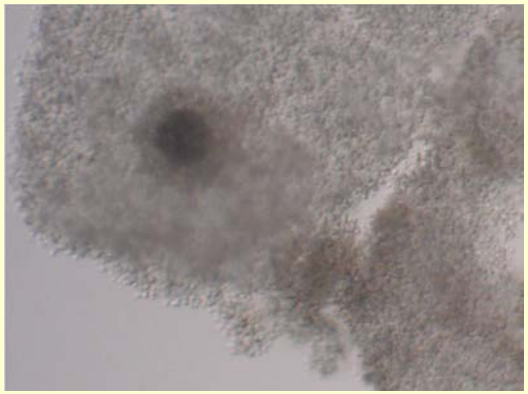
In vivo



Adapted from http://glycoforum.gr.jp

In vivo maturation

- In cattle: COC collected 24 h after GnRH treatment (Laurinciket al. 1993, Bordignon et al. 1997)
- In Wood bison: >70% expanded COC collected 24 h after LH treatment from superstimulated females (Palomino et al. 2014)



Can we produce wood bison embryos in vitro using in vivo matured oocytes?

Our first results

Does hCG induce in vivo maturation in wood bison?

✓ Yes, more than 33% of oocytes collected 30 h after hCG treatment were mature

Can bison oocytes collected after 30 h of hCG treatment be used for immediate IVF?

✓ Yes. After immediate fertilization: blastocyst rate 18%

Would additional maturation time be beneficial to embryo production in wood bison?

✓Yes. Additional 4 h of *in vitro* maturation: blastocyst rate 45%

Extending period of *in vivo* maturation?

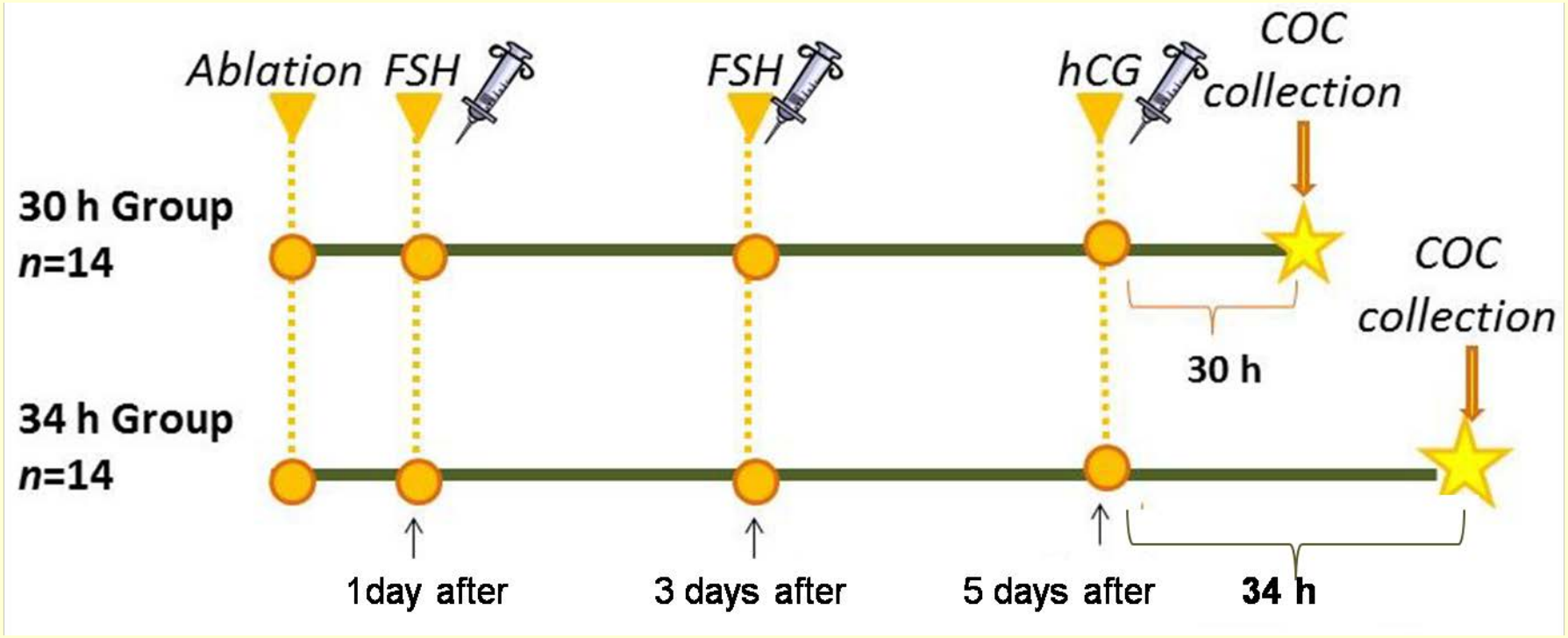
❖ Objective

- To determine the effect of an increased interval (34 h) from hCG treatment to cumulus-oocyte complexes (COC) collection on the number of expanded COC and on blastocyst rate

Animals

Adult wood bison females (n = 28) at the Native Hoofstock Centre, University of Saskatchewan

❖ Experimental Design

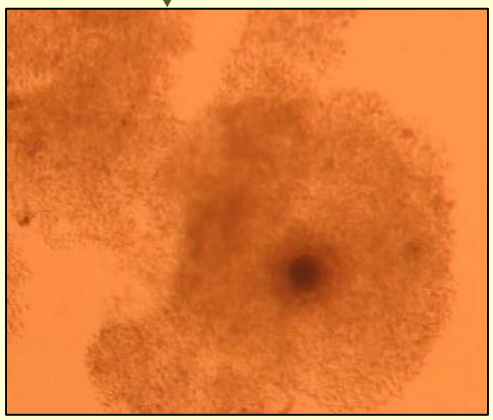


Transvaginal ultrasound-guided follicular aspiration

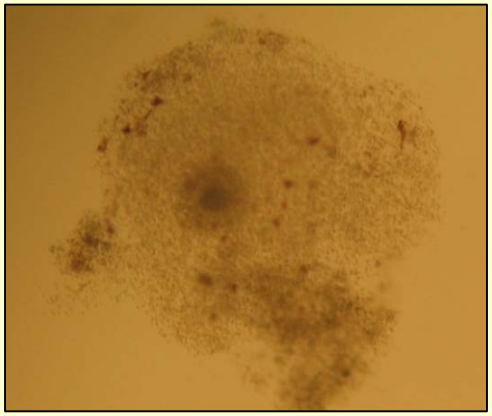
Expanded COC only:

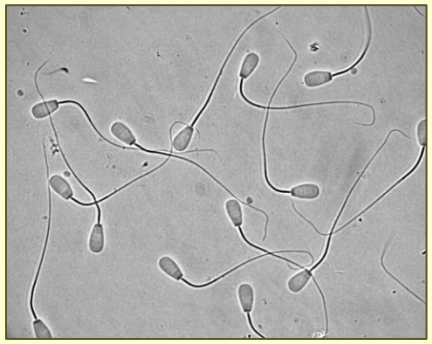
30 h Group
(additional maturation)

IVM 4 h
Incubated at
38.5°C, 5% CO2



34 h Group
(immediate fertilization)



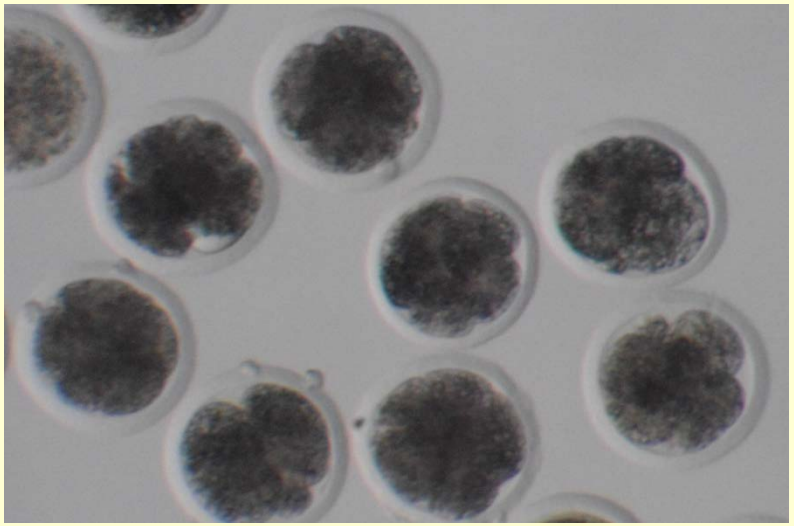


Frozen-thawed ejaculated wood bison sperm

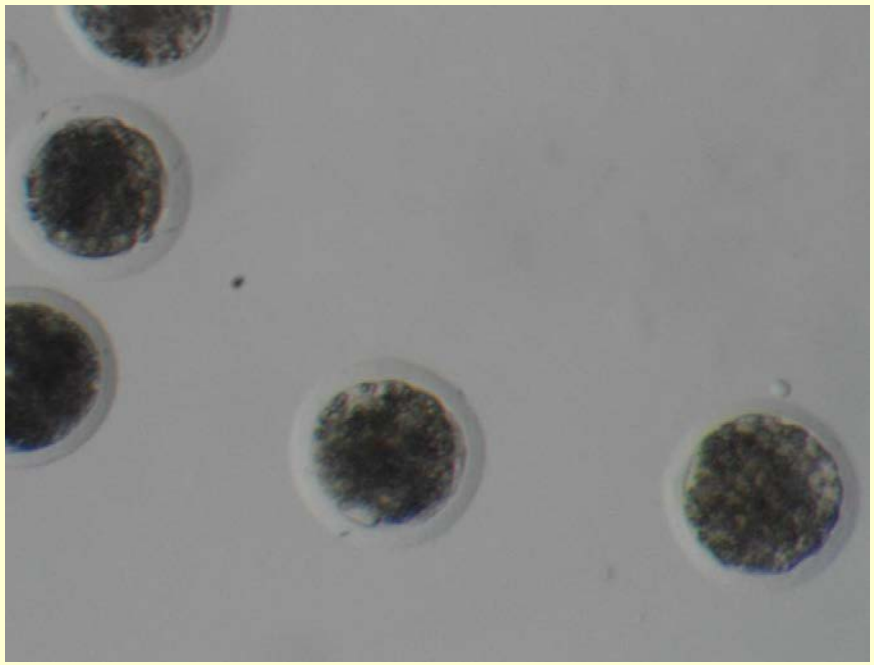
In vitro fertilization (Day 0)

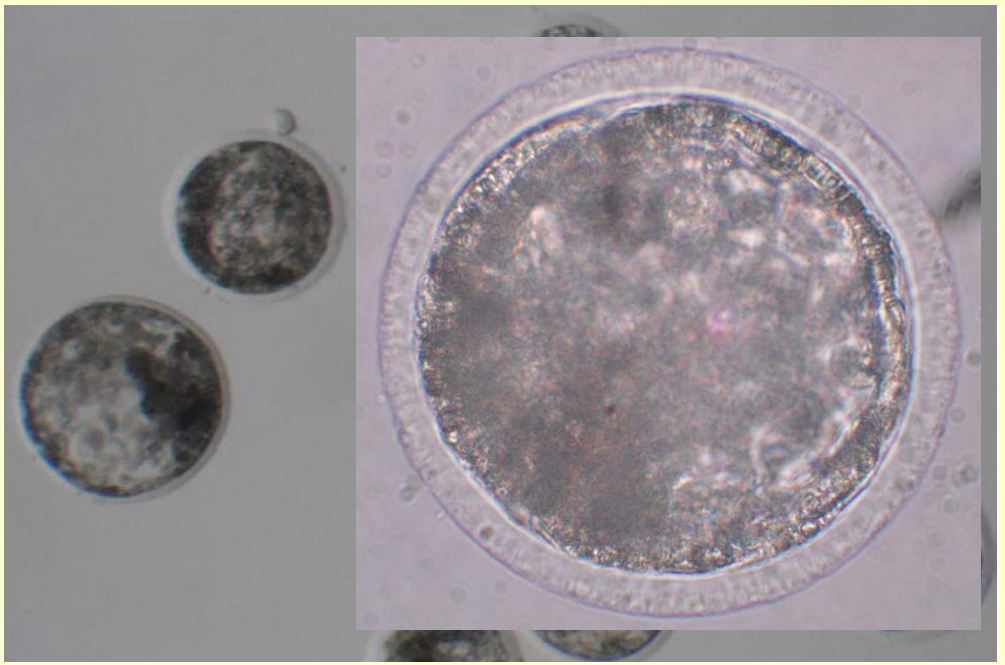
Oocytes and sperm co-incubated at 38.5°C in 5% CO_2 for about 18 h

***In vitro* culture** at 38.5°C in 5% CO_2, 5% O_2 and 90% N_2



Cleavage evaluation at Day 3





Embryo stage development evaluation at Day 7 & Day 8

Results

Table 1. Collection rate and number of *in vivo*-matured wood bison COC collected at 30 h or 34 h after hCG treatment

	30 h group	34 h group
No. of bison	14	14
No. of before-collection ovulations	12/89 (13.5%) [a]	17/147(32.0%) [b]
COC collection rate	148/230 (64%) [a]	147/188 (78%) [b]
Mean number of COC (±SEM) per bison	10.6±1.7	10.5±1.5
No. of expanded COC	104/148 (70.3%)	92/147(62.6%)

COC: cumulus-oocyte complexes

[ab]Within rows, values with no common superscripts are different (P<0.05, Chi-square test)

Results

Table 2. *In vitro* development of expanded *in vivo*-matured wood bison COC collected at 30 h or 34 h after hCG treatment

	30 h group	34 h group
No. of expanded COC	86	74
Cleavage rate at **Day 3** *	49/86 (57.0%) [a]	55/74 (74.3%) [b]
Day 7		
No. of morula*	20 (23.3%)	18 (24.3%)
No. of blastocyst *	9 (10.5%) [a]	25 (33.8%) [b]
Day 8		
No. of blastocyst *	32 (37.2%) [a]	40 (54.1%) [b]

COC: cumulus-oocyte complexes

[ab]Within rows, values with no common superscripts are different (P<0.05, Chi-square test)

*Calculated from the total numbers of oocytes submitted to IVF

Conclusion

- Extending the interval from hCG treatment to oocyte collection improved cleavage and blastocyst embryo production in wood bison

Acknowledgments

- Dr. Adams
- Dr. Anzar
- Dr. Mapletoft
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- Dr. Palomino
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Thank you!

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