SYNCHRONIZATION OF OVULATION AND FIXED TIME AI
IN WATER BUFFALOES: Recent Development and Insights
on Philippine Initiatives

Eufrocina P. Atabay
Supervising Science Research Specialist
Reproductive Biotechnology and Physiology Unit
Philippine Carabao Center

Paper presented during the International Training Course on Dairy Herd Improvement by
the Use of Reproductive Biotechnologies, Philippine Carabao Center, Science City of
Munoz, Nueva Ecija, Philippines, July 13 to 22, 2015
The Philippine Carabao Center:

- an attached agency of the Department of Agriculture (PCC-DA)
- started as an UNDP/FAO-assisted project “Strengthening of the Philippine Carabao Research and Development Center” coordinated by PCARRD from 1982 to 1992
- created by virtue of RA 7307, otherwise known as the Philippine Carabao Act of 1992 and became operational in 1993,
- a lead agency in Livestock Biotechnology in DA by virtue of AO No. 9 s. 2008
PCC’s Mission:

To conserve, propagate, and promote the water buffalo as important source of milk and meat towards better nutrition, higher levels of income, and improved general well-being of the rural farming families...
I. GENETIC IMPROVEMENT

GENE POOL
- Riverine Buffalo
- Swamp Buffalo

UPGRADING
- Bull Loan
- Artificial Insemination

II. Buffalo-Based Enterprise
- Cooperative Development
- Credit
- Market Assistance

III. Research and Development
- Technology Development
- Technical Training
- Policy
IN FOCUS
Philippine Carabao
A Traditional Draft Animal

Become the source of meat and milk
• REPRODUCTIVE BIOTECHNOLOGIES IN WATER BUFFALOES

• ARTIFICIAL INSEMINATION (1982)

• MULTIPLE OVULATION (MOET, 1986-1991)

• IN VITRO EMBRYO PRODUCTION (1996)

• CRYOPRESERVATION OF EMBRYOS (2002)

• SOMATIC CELL NUCLEAR TRANSFER (2005)

• ULTRA SOUND GUIDED OVUM PICK UP (2006)

• OOCYTE CRYOPRESERVATION (2009)

• INTRACYTOPLASMIC SPERM INJECTION (2011)

• Ovulation Synchronization and Timed AI (2014)
AI and ASSOCIATED CONCERNS:

- Artificial insemination (AI), is a reproductive tool used in the dissemination of superior genetics of paternal origin,

- the widely used reprobio technique for buffalo GIP

- Buffaloes exhibit “silent heat” with poor oestrus expression causing difficulties in estrus detection,

- They have a marked reproductive seasonality (non-breeding and breeding season)

- Therefore, an alternative reproductive method which eliminates the need for heat detection, and which can addresses problem on the inherent breeding seasonality of buffaloes is warranted.
What is Timed AI/ Fixed Time AI?

- Conventional AI is performed following synchronization of estrus with prostaglandin (PGF2α) and requires close heat detection of treated animals.
- Ovulation time is variable, until generally resulting in a lower pregnancy rate.
- Fixed Time AI is performed following synchronization of ovulation among animals,
- There is no need for estrus detection, but AI should be done at fixed time
- Tight synchrony of ovulation is observed, until 36 h after 2nd GnRH treatment.
- The original ovulation synchronization method called OVSYNCH PROTOCOL was developed to improve reproductive efficiency of post-partum diary cattle (Pursley et al, 1995),
- It basically uses the hormone: Gonadotropin-Releasing Hormone (GnRH) which is given 7 days before and 2 days after PGF2α injection.
- **OVSYNCH: GnRH (day 0), PGF2α (day 7), GnRH (day 9), AI (day 10)**
GENERAL APPLICATION OF FTAI IN REPRODUCTION MANAGEMENT

- **OVSYNCH** originated in the US, and numerous modifications of the original protocol since its publication (Theriogenology, 1995)
- FTAI works in buffaloes started to be reported in 2000: Brazil, Argentina, India,
- To date, FTAI is widely used in commercial scale production in livestock in many countries,
- In the US, Timed AI has revolutionized dairy cattle breeding program resulting in a **phenomenal change in reproductive performance and progress in dairy industry**.
- In Brazil, there is an **explosion in fertility program** with FTAI, leading to a vibrant beef industry,
- Inspite of these tremendous development, researches are actively carried out to further **increase its efficiency, simplify procedures** for its expanded and practical applications.
APPLICATIONS OF FTAI IN BUFFALO GIP

- Management of repeat breeders/dairy buffaloes particularly the at the National Genepool and Institutional Herds. 4 to 5 AIs
- Creating a contemporary herd of Philippine Native (Swamp) buffaloes at Philippine carabao Genepools,
- Facilitating a year-round calf production at the National Impact Zone to avoid the concentration milk supply in certain period of the year.
- Enhance related reproductive technologies such as OPU or MOET in buffaloes where ovulation synchronization is part of the application of technologies,
- Generally, to increase conception rates, reduce calving interval and improve reproductive efficiency following FTAI in buffaloes in heifers and cycling post-partum animals,
What are the Applications of FTAI in the current GIP in Buffaloes?

- **Note:** May not be applicable in National AI Program of PCC; due to the logistics required and economic implication
- FTAI protocol is being optimized/simplified for possible wide use in the field.
Furthering research efforts should be anchored in gaining deeper understanding of ovarian physiology thru:

- **Ultrasonography** enables us to better understand ovarian activities through follicular and luteal phase).

- **Hormone assay** to determine endocrine hormone profile at various stages of the estrus cycle,

- Strengthen understanding on the regulation and control of ovarian activity: Hypothalamus-Pituitary-ovarian axis.

- “Enlightened conduct of FTAI” is a must.
Main Project: Optimizing Assisted Reproductive Technologies (ART) in Water Buffaloes Through The Regulation Of Ovarian Function

- Enhancing Reproduction Efficiency through Synchronization of Ovulation and Fixed-Time AI in Water Buffaloes

Objective: To be able to enhance the outcome of AI thorough synchronized ovulation and timed AI in order to improve the reproductive performance and efficiency in water buffaloes

Part I. Validation and Optimization of the FTAI protocols in buffaloes

Parameters: Hormone Combinations:

- Follicle diameter
- Ovulation rate
- Corpus Luteum formation
- Pregnancy rates

Part II. Integration and Application of FTAI in buffalo GIP
FARM ACTIVITIES:

BCS
Ovary size
Ovarian structures
Ultrasonography of Sizes of Buffalo Ovaries

- **Pea size**
  
  (< 1.3 cm)

- **Bean size**
  
  (1.3 to 1.9 cm)

- **Thumb size**
  
  (>1.9 cm)
Ovulation Synchronization and FTAI Protocol

A. OVSYNCH: for Cyclic animals at Breeding season (Sept-April)

B. CIDR-SYNCH: Non-cycling animals at non breeding season (May to August)

(Controlled Internal Drug Release)
Physiological Events Underlying FTAI

- On day 0, the 1\textsuperscript{st} GnRH injection is given which induces the ovulation of DF follicle present, and a new follicular wave emerges 1-2 days later,
- On day 7, PGF2a injection results in CL regression, further follicular growth and estrus occurrence,
- On day 9, 2\textsuperscript{nd} GnRH administration induces the ovulation of DF, within 24 to 32 hrs, thereafter,
- On day 10, AI is performed at 14-16 hrs post 2\textsuperscript{nd} GnRH injection
- CIDR, an exogenous P4, inserted at 1\textsuperscript{st} GnRH injection, will prevent the formation of DF to cause more synchronous ovulation among animals following its removal on day 7.
PRELIMINARY RESULTS:

Ovarian response upon FTAI treatment (Ovsynch)
Follicular Development during Ovsynch Treatment

<table>
<thead>
<tr>
<th>Size (mm)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>8</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10am</th>
<th>10pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle</td>
<td>8.38</td>
<td>12</td>
<td>12.3</td>
<td>13.7</td>
<td>14</td>
<td>14.15</td>
<td>14.67</td>
<td>15.63</td>
<td>13.78</td>
</tr>
<tr>
<td>Corpus luteum</td>
<td>12.1</td>
<td>13.27</td>
<td>13.05</td>
<td>12.35</td>
<td>10.6</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ovarian response upon FTAI treatment (CIDR-synch)

SIZE (mm)

DAYS

CIDR (P+)

GnRH

PGF$_{2a}$

GnRH

10.1

10.6

13.2

12.3

9.85

9.11
Follicular Growth during CIDR-Synch Treatment

![Follicle size (mm) chart]

<table>
<thead>
<tr>
<th>Time</th>
<th>Follicle size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.9</td>
</tr>
<tr>
<td>7</td>
<td>12.21</td>
</tr>
<tr>
<td>8</td>
<td>13.33</td>
</tr>
<tr>
<td>9</td>
<td>14.2</td>
</tr>
<tr>
<td>10am</td>
<td>15.075</td>
</tr>
<tr>
<td>10pm</td>
<td>14.45</td>
</tr>
</tbody>
</table>

**Key Events**
- GnRH
- PGF2
Calves Produced 2014:
NGP   = 16/34 P+
CLSU  =  8/24 P+
Total  =  24/58
## Preliminary Results

Table I. Efficiency of Synchronization of Ovulation and FTAI at Institutional Genepool

<table>
<thead>
<tr>
<th>Synchronization Protocol</th>
<th>No. of Animals Treated</th>
<th>No. of Animals Pregnant</th>
<th>Pregnancy Rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovsynch</td>
<td>78</td>
<td>26/</td>
<td>33.33</td>
</tr>
<tr>
<td>2. CIDR-Ovsynch</td>
<td>27</td>
<td>8</td>
<td>29.63</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>105</strong></td>
<td><strong>34</strong></td>
<td><strong>32.38</strong></td>
</tr>
</tbody>
</table>

### 2014

<table>
<thead>
<tr>
<th>FTAI Protocol</th>
<th>No of Animal Treated</th>
<th>No. of Anim Pregnant</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIDR-Synch</td>
<td>30</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Hard breeders 3-5 AI failures</td>
<td>125 days open,</td>
<td>2/5=3-5AI (-)</td>
<td>Exposed to Bull, then subjected to FTAI</td>
</tr>
</tbody>
</table>
### Table II. The Effect of Body Condition Score on the Efficiency of Ovsynch Treatment and FTAI at the National Impact Zone/Farmers Level

<table>
<thead>
<tr>
<th>Body Condition Score</th>
<th>No. of Animals Treated</th>
<th>No. of Animals Pregnant</th>
<th>Pregnancy Rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 2.5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>b. 2.75</td>
<td>13</td>
<td>3/83</td>
<td>3.61</td>
</tr>
<tr>
<td>c. 3.0</td>
<td>54</td>
<td>27/83</td>
<td>32.53</td>
</tr>
<tr>
<td>d. 3.25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>e. 3.5</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>f. 3.75</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>g. 4.0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>83</strong></td>
<td><strong>30</strong></td>
<td><strong>36.14</strong></td>
</tr>
</tbody>
</table>

### Table IIb. The Influence of Ovarian Structures on the Efficiency of Ovsynch Treatment and FTAI at the National Impact Zone/Farmers Level

<table>
<thead>
<tr>
<th>Ovarian Structure Present</th>
<th>No. of Animals Treated</th>
<th>No. of Animals Pregnant</th>
<th>Pregnancy Rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Corpus luteum (CL)</td>
<td>53</td>
<td>19/95</td>
<td>20.00</td>
</tr>
<tr>
<td>b. Growing follicles</td>
<td>29</td>
<td>9/95</td>
<td>9.47</td>
</tr>
<tr>
<td>c. No structures</td>
<td>13</td>
<td>2/95</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td><strong>95</strong></td>
<td><strong>30</strong></td>
<td><strong>31.58</strong></td>
</tr>
</tbody>
</table>
Table IIa. Effect of Size of Ovaries on the Efficiency of Ovsynch Treatment and FTAI at the National Impact Zone/Farmers Level

<table>
<thead>
<tr>
<th>Size of Ovaries</th>
<th>No. of Animals Treated</th>
<th>No. of Animals Pregnant</th>
<th>Pregnancy Rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. &lt;13.0 mm</td>
<td>28</td>
<td>5/95</td>
<td>5.26</td>
</tr>
<tr>
<td>b. 13-19 mm</td>
<td>37</td>
<td>15/95</td>
<td>15.79</td>
</tr>
<tr>
<td>c. &gt;19.0 mm</td>
<td>30</td>
<td>10/95</td>
<td>10.53</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>30</td>
<td>31.58</td>
</tr>
</tbody>
</table>
PRELIMINARY RESULTS OF FTAI AT PCC-CLSU

<table>
<thead>
<tr>
<th>Protocol</th>
<th>No. of Animals Treated</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovsynch (only with CL are treated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Regular PGF2+ 2\textsuperscript{nd} GnRH on day 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Regular PGF2+ GnRH on day of AI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FTAI Method</td>
<td>No. of Animals Treated</td>
<td>No. of Animals Pregnant</td>
</tr>
<tr>
<td>1. Ovsynch</td>
<td>45</td>
<td>36</td>
</tr>
<tr>
<td>1\textsuperscript{st} Service</td>
<td>29</td>
<td>64.44</td>
</tr>
<tr>
<td>2\textsuperscript{nd} Service</td>
<td>7</td>
<td>15.56</td>
</tr>
</tbody>
</table>
IMPORTANT NOTES/HIGHLIGHTS:

- FTAI was performed following synchronized ovulation without the need to detect oestrus,
- It can be done at random stage of the estrus cycle (it could be at CL and follicular phase), after ovarian palpation for pregnancy.
- But, ovsynch yielded better results with cycling animals,
- There was a tight synchrony of ovulation and synchronous ovulation in treated animals,
- Ultrasonography is helpful and objective way of assessing the efficiency FTAI protocol,
- The size of dominant follicle at the end of the treatment period is indicative of successful ovulation and pregnancy.
• The **body condition score** of the animal is an important factor in the success of FTAI,
• The **size of the ovaries** at the initial treatment is an important criterion for animal selection to the FTAI program.
• The **structure present in the ovary** (CL, DF or NS) is highly determinant of FTAI efficiency.
• **Precise and right timing** of execution of activities is a key and critical factor in the successful FTAI outcome.
• Overall, our initial FTAI works resulted in acceptable pregnancy rates (36% at NIZ) is highly comparable with previous reports in buffaloes.
• Using Ovsynch only in animals with CL, resulted in a considerably high pregnancy rate (64%).
Intensify research on improving the technical efficiency of FTAl in buffaloes under specific application.

Conduct hormone assay along with ultrasonography for the coordination of ovarian activity.

Early pregnancy diagnosis using ultrasonography with pregnancy-associated glycoproteins (PAGs).
Comparisons between cattle and buffalo ovaries: Why is efficiency low? What can be done?

Size of CL = Short luteal phase???:
- early embryonic death
- fetal loss
Ultimate Goal of FTAI for Buffaloes:

1. FTAI protocol that is efficient in:
   a. induction of follicular wave
   b. Optimum size of ovulatory follicle
   c. Synchronized ovulation
   d. Ensure good CL formation after AI
      “to address the short luteal phase”

2. Simplified procedure of synchronized ovulation
   for its wider application in our National AI program
Thank you!!!
## Cost of Doing FTAI:

<table>
<thead>
<tr>
<th>FTAI Protocol</th>
<th>Hormones needed</th>
<th>Estimated Cost (PhP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovsynch</td>
<td>GnRH, PGF2, GnRH</td>
<td>650.00</td>
</tr>
<tr>
<td>2. PGF2 Synch’n</td>
<td>PGF2</td>
<td>250.00</td>
</tr>
<tr>
<td>5. CIDR-SYNCH</td>
<td>CIDR+OVSYNCH+eCG</td>
<td>2,000.00</td>
</tr>
<tr>
<td>(anestrus anim.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>