Reproductive endocrine profiles and follicular growth after estrus induction in the riverine water buffalo (*Bubalus bubalis*, 2n=50) and riverine-swamp hybrid buffalo (2n = 49)

J.L. Zambrano-Varón¹,²
R.H. BonDurant²

¹ Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional de Colombia
² Population Health & Reproduction, School of Veterinary Medicine, University of California. Davis

Corresponding author: J. Zambrano-Varón. Population Health & Reproduction, Room 1114 Tupper Hall. School of Veterinary Medicine. University of California. Davis. California. 95616 - Tel. (530)-752-1358 - Fax: (530)- 752- 4278 - Email: jzambrano@ucdavis.edu

**Abstract:**
Ten adult female water buffalo were used in the present study (5 x [2n = 50] and 5 x [2n= 49] hybrids). Ovarian activity was monitored daily by transrectal ultrasonography between two consecutive ovulations. Observed follicular wave numbers were: 1 (n=1), 2 (n=4), and 3 (n=5). The interovulatory interval ranged 17 to 23 days. Differences in mean follicular diameter between follicles of the normal karyotype (2n=50) and buffalo hybrids (2n=49) were found on the second subordinate group of follicles (P<0.05) but not between dominant and third subordinates. Differences in circulating concentrations of P4 and estradiol were not statistically significant (P>0.05), whereas inhibin profiles were significantly higher in the hybrid group (P<0.05). Understanding the biological meaning of the difference in inhibin concentrations in *B. bubalis* female reproductive performance will require further investigation.

**Key words:** Water Buffalo, Folliculogenesis, Endocrine profile, Ultrasound, *Bubalus bubalis*.

**INTRODUCTION**
The study of follicular dynamics and endocrine control of folliculogenesis has provided the basis for enhancing reproductive efficiency in many domestic species (Ginter et al., 2001). Although folliculogenesis studies have been done in the buffalo female (Baruselli *et al.*, 197, Taneja *et al.*, 1996), there is still the need for better understanding of some of these processes in order to improve conception rates following estrus synchronization, superovulation, and artificial insemination, and therefore to reduce the variability in ovulation rate and embryo loss. The objectives of the present study were to compare the endocrine profiles and the patterns of follicular development of hybrid (2n=49) and normo-karyotic (2n=50) *B. bubalis* females, during the breeding season (October-November).

**MATERIAL AND METHODS**
Ten adult female water buffalo were used in the present study (5 x [2n = 50] and 5 x [2n= 49] hybrids). The Ovsynch protocol was used to synchronize ovulation in all the animals. Briefly, each buffalo will receive 100 µg i.m.
of GnRH (Cystorelin; Merial Limited, Iselin, NJ), followed 7 days later by 25 mg i.m. of prostaglandin (PG) F₂α (Lutalyse; Pharmacia & Upjohn Co [Pfizer], Kalamazoo, MI), followed by a second i.m. treatment of 100 µg of GnRH 2 days later. The study was conducted during the fertile season (fall); buffalo females will be monitored from 3 days before the induced ovulation and will be followed up until 3 days after the second consecutive ovulation will occur. Ovarian activity was monitored daily by transrectal ultrasonography. Daily blood samples were also collected to assay for circulating levels of progesterone, estrogens, and inhibin; samples were collected five days before and after each of two consecutive ovulations. All samples for a given hormone were run in a single assay, to minimize inter-assay variability. An ELISA assay was performed for P₄ concentration, whereas E₂ and immunoreactive inhibin were measured by RIA. Follicular diameter, CL volume, and hormone concentrations, were compared by ANOVA. Differences between continuous data such as follicular diameter were compared between groups of animals (hybrids vs 50 chromosome animals) by Student t-test.

RESULTS AND CONCLUSIONS - Our preliminary results have confirmed some previously reported morphometric studies (Presicce et al., 2004); observed follicular wave numbers were: 1 (n=1), 2 (n=4), and 3 (n=5), and the interovulatory interval ranged 17 to 23 days. There was no statistical effect (P > 0.05) of karyotype on the number of follicular waves per cycle or on the inter-ovulatory interval however, mean follicular diameter of follicles in the second subordinated group were larger for a normal karyotype buffalo (2n=50) than for hybrids (2n=49) (Table 1). Interestingly, the smaller follicles of 2n=49 buffalo were associated with lower serum inhibin concentration.

Table 1. Mean follicular diameter (mm) in water buffalo carrying a normal karyotype (2n=50) and buffalo hybrids (2n=49).

| Follicle Category       | Chromosome number | Mean  | Standard Error | Minimum | Maximum | P-value |
|-------------------------|-------------------|-------|----------------|---------|---------|---------|
| Dominant                | 49                | 12.19 | 0.41           | 7.75    | 15.38   | 0.25    |
|                         | 50                | 11.59 | 0.31           | 8.58    | 15.08   |         |
| Second subordinates     | 49                | 8.83  | 0.29           | 6.10    | 11.03   | 0.01    |
|                         | 50                | 10.03 | 0.31           | 7.53    | 13.77   |         |
| Third Subordinates      | 49                | 5.23  | 0.16           | 3.10    | 6.90    | 0.12    |
|                         | 50                | 4.87  | 0.16           | 3.00    | 6.33    |         |

* Means are statistically different P < 0.05

We have found no statistically significant differences between normal karyotype (2n=50) and buffalo hybrids, in serum concentrations of progesterone and estradiol between two consecutive ovulations (2n=49) (P > 0.05). On the other hand, serum concentrations of inhibin were significantly different (P < 0.05) with hybrids showing consistently lower levels of immunoreactive inhibin than 2n=50 karyotypes (Figure 1).
Despite the fact that many studies have noted that swamp buffalo have a karyotype of 2n=48, while river buffalo have a 2n=50 karyotype (Manik et al., 2002), the two “species” readily hybridize, and will carry each other’s embryos. Further, the hybrids (2n=49) are apparently fertile (Guimaraes et al. 1995), a phenomenon which – if true – requires an explanation. Our findings suggest that hybrid follicles do not attain the size of normo-karyotype (2n=50) follicles, and they also secrete less inhibin. An important bioactivity of inhibin is to deliver negative feedback to the hypothalamic-pituitary-ovarian axis, thereby specifically modulating FSH release, a phenomenon that partly explains why only 1 follicle ovulates even though many are recruited. Higher levels of inhibin, or greater sensitivity to prevalent inhibin levels, could limit the success of superovulation (Takedomi et al., 2005; Gonzales-Bulnes et al., 2006).

Additional interesting findings of the present study include short estrus cycle presentation (< 10 days) following estrus synchronization, and anovulatory conditions such as retention of ovulatory follicles and follicular cyst development. The last two lead to an increase of the interovulatory interval; however, cyclicity was restore spontaneously within the next expected cycle. Although those animals were not included in the previous analyzes reported here, this clinical situation may certainly affect both conception and pregnancy rates following estrus synchronization.

ACKNOWLEDGMENTS - This project was funded by the Caroline and Mark Guidry Foundation.

REFERENCES - Ginther OJ, Beg MA, Bergfelt DR, Donadeu FX, Kot K. 2001. Follicle Selection in Monovular Species. Biol Reprod. 65: 638-647. Baruselli PS, Mucciolo RG, Viscintin JA, Viana WG, Arruda RP, Madureira EH, Molero-Filho JR. 1996. Ovarian follicular dynamics during the estrus cycle in buffalo (Bubalus bubalis). Preliminary research. Ann
Taneja M, Ali A, Singh G. 1996. Ovarian follicular dynamics in water buffalo. Theriogenology. 46: 121. Presicce GA, Senatore EM, Bella A, De Santis G, Barile VL, De Mauro GJ, Terzano GM, Stecco R, Parmeggiani A. 2004. Ovarian follicular dynamics and hormonal profiles in heifer and mixed-parity Mediterranean Italian buffaloes (Bubalus bubalis) following an estrus synchronization protocol. Theriogenology. 61: 1343-1355. Manik RS, Palta P, Singla SK, Sharma V. 2002. Folliculogenesis in buffalo (Bubalus bubalis): a review. Reprod Fertil Dev. 14: 315-325. Guimaraes SE, Pinheiro LE, Guimaraes JD. 1995. Meiotic peculiarities in hybrid buffalo. Theriogenology. 43: 579-583. Takedomi T, Kishi H, Medan MS, Aoyagi Y, Konishi M, Itoh T, Yazawa S, Watanabe G, Taya K. 2005. Active immunization against inhibin improves superovulatory response to exogenous FSH in cattle. J Reprod Dev. 51: 341-346. Gonzalez-Bulnes A, Garcia-Garcia RM, Carrizosa JA, Urrutia B, Souza CJ, Cocero MJ, Lopez-Sebastian A, McNeilly AS. 2004. Plasma inhibin A determination at start of superovulatory FSH treatments is predictive for embryo outcome in goats. Domest Anim Endocrinol. 26: 259-266.