

Ovarian morphometric characterization and *in vitro* maturation of oocytes obtained from buffalo (*Bubalus bubalis*) ovaries – partial results

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ABSTRACT: Buffalo ovaries were collected from a slaughterhouse (Frigol, Brazil) and transported to the laboratory in saline solution at 36° C. The ovaries were dissected to realize the evaluations (weight, length, width and height of the ovary; *corpus luteum* and dominant follicle diameters). The Cumulus-oocyte complexes (COCs) were recovered by aspiration of 2-8 mm follicles. Selected COCs were matured in TCM 199 supplemented with 10% fetal bovine serum, sodium pyruvate, LH, FSH, estradiol and gentamicin. *In vitro* maturation was carried out at 38.5° C for 22-24 h and 34-36 h. For the evaluation of the nuclear maturation the oocytes were placed in TCM 199 medium added with type v hialuronidase where the granulosa cells were extracted. The denuded oocytes were transferred to 10 µl of Hoescht 33342 and the chromosomic configuration was evaluated. The oocytes were classified according to meiosis stage in: Germinal Vesicle, Germinal Vesicle Breakdown, Metaphase I, Metaphase II and Degenerated. The means of weight, length, width and height of the ovary were 3.83 g, 2.27 cm, 1.08 cm and 1.56 cm, respectively. The means of *corpus luteum* and dominant follicle diameters were 1.40 cm and 7.77 mm. The proportion of oocytes that reached metaphase II stage was: 36.68%.

Key words: Buffaloes, *In vitro* maturation, Oocytes, Metaphase.

INTRODUCTION - Biotechnologies are applied to buffalo reproduction but poor fertility is observed. Reduced fertility is due to lower: responsiveness to hormonal stimulation, recovery of embryos, numbers of primordial follicles in ovary and a pregnancy rate, compared with cattle. Because of the low efficiency of MOET (Multiple Ovulation and Embryo Transfer) programs in this species, there is an increasing interest in large-scale *in vitro* production of buffalo embryos for faster propagation of superior germplasm (Gasparrini, 2002). Good progress has been achieved in relation to the maturation and *in vitro* oocyte fertilization (IVF) however in relation to the embryo culture is still necessary the determination of an adequate medium to the buffalo embryo (Ohashi *et al.*, 2003). The objectives of the present experiment were to study the ovarian morphometry and to realize *in vitro* maturation of oocytes obtained from buffalo ovaries collected from a slaughterhouse.

Figure 1. The evaluations (length, width and height) of the ovary were realized using a paquimeter.

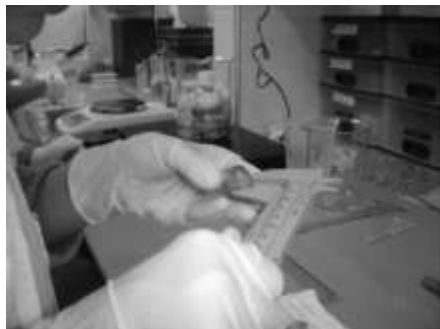


Figure 2. Buffalo oocytes after the period of IVM.

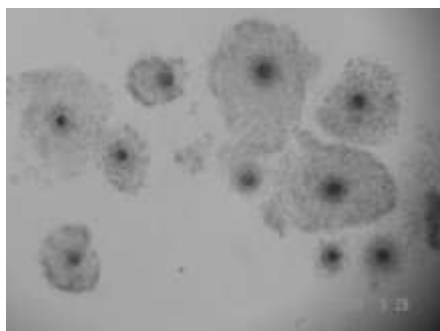
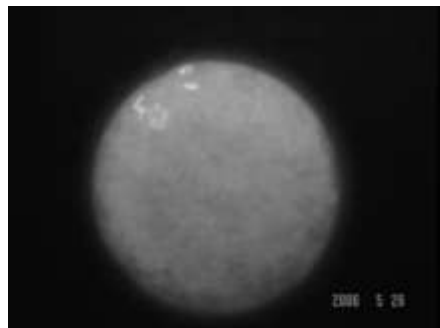


Figure 3. Buffalo oocyte that reached metaphase II stage stained with Hoescht 33342.



MATERIAL AND METHODS - Buffalo ovaries were collected from a slaughterhouse (Frigol, Lençóis Paulista – São Paulo – Brazil) and transported to the laboratory in saline solution (NaCl 0.9%) at 36° C. In laboratory, the ovaries were dissected to realize the evaluations (weight, length, width and height of the ovary; *corpus luteum* and dominant follicle diameters) using a digital balance and paquimeter (Figure 1). The Cumulus-oocyte complexes (COCs) were recovered by aspiration of 2 to 8 mm follicles and the selected COCs were matured. The *In vitro* Maturation (IVM) medium used was TCM 199 supplemented with 10% FBS (fetal bovine serum), 2.2 mg/ml sodium pyruvate, 1.0 µg/ml LH, 500 ng/ml FSH, 1.0 µg/ml estradiol and 25 µg/ml gentamicin. IVM was carried out at 38.5° C for 22-24 h and 34-36 h under a controlled gas atmosphere of 5% CO₂ in humidified air. For the evaluation of the nuclear maturation the oocytes were removed from medium after the period of IVM (Figure 2) and placed in TCM 199 medium added with type v hialuronidase where the granulosa cells were extracted by a glass micropipette. The denuded oocytes were transferred to 10 µl of Hoescht 33342 in glass slide and the chromosomic configuration was evaluated by on inverted fluorescent microscope (Leica DMIRB). The oocytes were classified according to meiosis stage in: GV (germinal vesicle), GVBD (germinal vesicle breakdown), MI (metaphase I), MII (metaphase II) and DEG (degenerated).

RESULTS AND CONCLUSIONS - The means of weight, length, width and height of the ovary were 3.83 g (n=84), 2.27 cm (n=84), 1.08 cm (n=84) and 1.56 cm (n=84), respectively. According to El-Wishy *et al.* (1971) the maximum and minimum weights of buffalo ovary are 2.90 and 6.10 g, respectively. The means of *corpus luteum* and dominant follicle diameters were 1.40 cm and 7.77 mm, respectively. The average recovery of total oocytes per ovary was: 4.24. This figure was higher than the figures of 1.70, 2.35 and 2.20 reported by Das *et al.* (1996), Kumar *et al.* (1997) and Ohashi *et al.* (2003), respectively. The proportion of oocytes that

reached metaphase II stage (Figure 3) was: 36.68%. This result is not comparable to high maturation rate (87% at 19 h) reported by Neglia *et al.* (2001) with using the same medium. The rate of buffalo oocytes that reached metaphase II stage varies according to the method of oocytes recuperation, IVM medium used, laboratory conditions and many factors. Although the use of a rich IVM medium, the preliminary results of this study were low.

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