Protocol of polyovulation to donor buffaloes
(Romanian Buffaloes)

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Abstract
Buffalo has a significant role in the agricultural economy of many developing countries by providing milk, meat and draught power. The breeding season for Romanian Buffaloes in the major buffalo rearing countries appear to extend from September to March. Compared to cattle, buffaloes exhibit delayed puberty in both males and females, poor estrus expression in a greater proportion of females. The main problem affecting the productivity of buffaloes is the long calving interval due to delayed postpartum estrus and delayed puberty. This is due to the difficulty in detecting estrus in this species on account of poor signs of estrus, such as silent heat, quiet ovulation or subestrus. Polyovulation is an important step in embryo transfer biotechnology. Over the years, techniques associated with embryo transfer have had many uses, especially in research. The widespread use of this technology in animal breeding schemes, however, is relatively recent. Successful embryo transfer requires manipulation of oestrus and ovulation of the donor and synchronization of these events with those of the recipients. A brief review of the physiological and endocrinological aspects of the oestrous cycle will contribute to more effective use of gonadotrophins (FSH-P or PMSG) for superovulation. Polyovulation research has been applied to a 6-year, clinically healthy and sexually-known donor buffalo. On the 11th day of the sexual cycle, the superovulation protocol was applied. PMSG was used in the 2500 IU dose. At the onset of oestrus, a number of 8 pre-ovulatory follicles have been identified. The monitoring of follicular evolution until ovulation was performed ultrasonographically. Of the 8 follicles increased 3 developed on the left ovary and 5 on the right ovary. After 7 days of ovulation have been identified six corpora lutea (2 ovary left and 4 right).
Key words*: buffalo, ostrus, poliovulation, MOET.*

Introduction

Buffalo usually attain puberty when they reach about 60% of their adult body weight (250 to 400) kg, but the age at which they attain puberty can be highly variable, ranging from 18 to 46 months (Jainudeen and Hafez, 1993). The factors that influence this are genotype, nutrition, management and climate. It could be attained under optimized conditions at 15 to 18 months in river buffalo and 21 to 24 months in swamp buffalo (Borghese, 2005).

High individual variability in follicular recruitment and hence in the number of embryos produced is a major factor limiting the application of reproductive technologies in buffalo. Therefore, the identification of reliable markers to select embryo donors is critical to enroll buffaloes in embryo production programs. Better understanding of factors involved in follicular growth is also necessary to improve the response to superovulation in this species.

Buffalo have a relatively high efficiency of feed utilization compared with cattle, which has important implications for resource use and the environment (Campanile G et al 2014, Neglia G 2014) .

Reproductive function in female river buffalo is closely linked to the environment. In equatorial aria and tropical regions, reproduction is more strongly influenced by nutrition. In the Mediterranean and higher latitudes, it is some distinct annual cycles in day length, female river buffalo are responsive to photoperiod. In Southern Europe (Romania), buffalo behave as a short-day breeder and show an increase in reproductive function during the summer-autumn transition (decreasing day length) and a decrease in reproductive function during the winter-spring transition (increasing day length). The latter period is characterized by reduced ovarian activity and function (Campanile G, 2010).

Similar to ruminants, buffalo have ovarian follicular waves and two waves in each estrous cycle is the most common pattern. Ovulation occurs between 26 and 33 hours after the LH surge (Baruselli P 1997, Presicce GA 2005, Neglia G, 2007, Seren E 1995, Porto-Filho RM 1999).

The ovaries of post-pubertal buffalo heifers have a reservoir of only 10,000–20,000 primordial follicles (Danell,1987) compared with over 100,000 in cattle. The mature ovaries are smaller than in cattle, weighing around 2.5 g when inactive and 4 g when active, with fewer tertiary follicles

Early studies on synchronization of time of oestrus in buffalo were based on those developed for cattle, aimed at either inducing premature luteolysis using prostaglandins or prolonging the luteal phase using progestagens (Perera, B. 2008.).

Follicular wave dynamics on the outcome of these procedures (De Rensis, F 2007) have prompted studies aimed at manipulating follicular development to achieve greater oestrous synchrony and improved fertility.

Gimenes et al. (2007) reported that follicles in Murrah×Mediterranean buffalo heifers acquired the capacity to ovulate in response to exogenous LH when they reach a diameter of 8.5–10.0mm. On the other hand follicles from multiparous Mediterranean cows with diameters ranging from 4.2 to 13.0mm ovulated when treated with hCG or GnRH agonist. In the latter studies, follicles ranging from 4.2 to 13.0mm did not consistently ovulate after treatment with hCG or GnRH agonist suggesting that size does not necessarily reflect stage of follicle development or functionality.

In buffaloes undergoing regular oestrous cycles dominant follicles attain a size of 13–15mm before ovulation (Neglia et al., 2007) and this could be interpreted to suggest that, under normal conditions, follicles in buffaloes acquire the capacity to ovulate at around 5–8 mm but must undergo further development in order to secrete sufficient oestradiol for a positive feedback action to induce the pre-ovulatory LH surge.

Follicular superstimulation. Follicular superstimulation is most successful when the initiation of exogenous FSH treatment is timed to coincide with the endogenous wave-inducing surge of FSH and emergence of the second follicular wave. This is difficult to achieve in randomly cycling buffaloes because of the rather large variation in duration of the first follicular wave. A second requirement in follicular superstimulation is the ability to control the time of ovulation in order to implement fixed-time AI. The injection of GnRH or porcine LH 24 h after the last treatment with FSH results in predictable and synchronous ovulation in buffaloes which removes the need for oestrous detection for AI (Baruselli et al., 2002).

In a superstimulation protocol that utilised a GnRH agonistto downregulate the anterior pituitary in buffaloes, the injection of porcine LH 24 h after the last injection of FSH is associated with greater ovulation rate, fertilisation and embryo recovery compared with injection of LH 12 h after the last FSH (Zicarelli, L. 2000).

The production of sufficient number of viable embryos through the use of gonadotropins for embryo transfer and multiple birth has been a subject of interest in buffaloes. In particular, follicle stimulating hormone (FSH) and pregnant mare serum gonadotropin (PMSG) have become the primary superovulatory drugs for donor buffalo cows. FSH is usually given within a 4- to 5-day period at midcycle as in cattle through a series of injections so that follicles are recruited immediately prior to lysis of the existing corpus luteum. A total dose of 40mg to 50mg is given to elicit optimal ovarian stimulation. The series of injections is required because of its short half-life in the circulation. The PMSG is used as a single injection of 2000-3000 IU.

Comparatively, FSH stimulation of ovaries results in more ovulation and recovery of embryos of better quality than with PMSG (13). A luteolytic dose of prostaglandin F 2 alpha (PGF 2 a) is usually given 48 h after initiation of the treatment. In all studies published to date, the superovulatory responses have varied, with low fertilization rates.

Rations with a higher level of protein influence the animal's level of luteinizing hormone (LH) and response to gonadotropin releasing hormone (GnRH), which would eventually affect the ovulation response of the donor. In addition, the season during superovulation treatment influences the rate of ovulation.

The use of either pure FSH or a controlled FSH -LH mixture plus GnRH and its analogues and/or an anti-PMSG, if PMSG is to be used, may improve the donor ovulation response.

Results, material and methods:

Polyovulation research has been applied to a 6-year, clinically healthy and sexually-known donor buffalo.

On the 11th day of the sexual cycle, the superovulation protocol was applied. PMSG was used in the 2500 IU dose. At the onset of oestrus, a number of 8 pre-ovulatory follicles have been identified. The monitoring of follicular evolution until ovulation was performed ultrasonographically. Of the 8 follicles increased 3 developed on the left ovary and 5 on the right ovary. After 7 days of ovulation have been identified six corpora lutea (2 ovary left and 4 right).

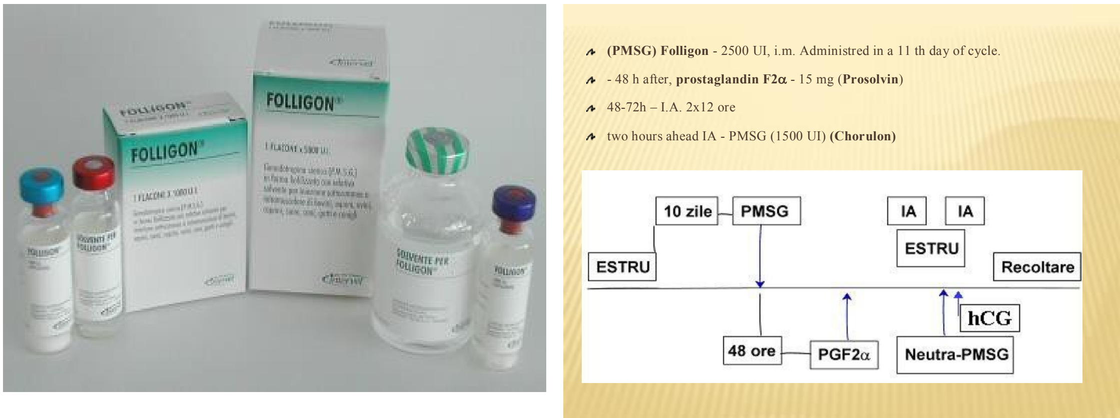

Fig. 1 Schedule the polyovulatory treatment in buffalo

Table 1.

<table>
<tr><th colspan="4">Poliovulation treatment and his effect</th></tr>
<tr><td>1</td><td>PMSG (Folligon)
On the 11th day of the sexual cycle</td><td colspan="2">25oo UI, im</td></tr>
<tr><td>2</td><td>Poliovulation effect</td><td>Left ovary</td><td>Right ovary</td></tr>
<tr><td>3</td><td>8 ovarian follicles</td><td>3</td><td>5</td></tr>
<tr><td>4</td><td>6 corpus luteum</td><td>2</td><td>4</td></tr>
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Fig. 2 . Buffalo, right ovary. Five follicles (F) identified by ultrasound, after 72 hours from the administration of PMSG

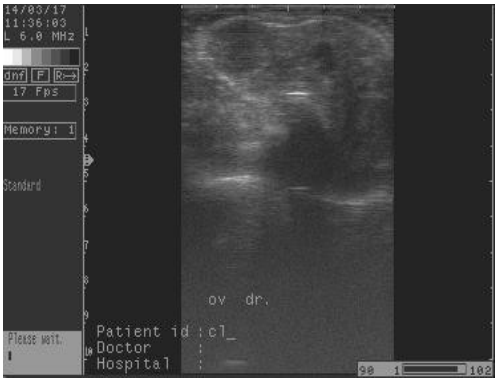

Fig. 3 . Buffalo, right ovary. Corpora lutea (CL), identified by ultrasound after 7 days from the administration of HCG

Discution

A review of studies using various treatment protocols (Borghese, A. 2005). shows that CR achieved with prostaglandins either alone or in combination with GnRH ranged from 7 to 56%, while CR following the use of a progesterone releasing devices either alone or in combination with eCG, and in some cases further supplemented with hCG or GnRH, ranged from 8 to 64%. The 'Ovsynch' protocol (GnRH followed by prostaglandin 7 days later and a second GnRH 2 days later) has been used successfully in buffalo, with synchronization of time of ovulation in 70–90% and CR of 33–60%.

In most studies the success rate was lower when treatment was done during the periods of low breeding activity or during seasonal anoestrus, and various modified protocols have been tried to overcome these problems. The two most effective approaches appear to be the Ovsynch protocol supplemented by administration of progesterone for 7 days between the first GnRH and prostaglandin treatments, and progesterone based regimens of 10–14 days with either GnRH or oestradiol treatment at the time of progesterone implant insertion and prostaglandin plus eCG treatment at implant removal (De Rensis and López-Gatius, 2007).

In addition to the type of protocol selected, the following factors must also be addressed when using any regimen in buffalo (Perera, 2008): (a) selection of animals that are in good body condition score and free from disease; (b) minimize stress during the treatment administration and AI, especially under tropical conditions, when animals may be herded together, tethered or moved to other locations; and (c) where seasonal differences exist, scheduling treatments for the more favourable periods or during the peak of the breeding season when the majority of animals are likely to be having oestrous cycles.

Is a worldwide increasing interest in largescale in vitro production of buffalo embryos to improve genetic progress through the maternal lineage. Indeed, the Ovum pick-up (OPU) and the in vitro embryo production (IVEP) are currently the most competitive technologies to produce transferable embryos (TE) over the long term.

Therefore, this procedure is a valid alternative to multiple ovulation/embryo transfer (MOET) programs because of the low response to hormonal stimulation, the poor embryo recovery, and the impossibility to repeat continuously the MOET treatments over the long-term. Indeed, multiple ovulations can be induced only in cyclic animals, whereas it is likely that buffalo cows enter seasonal anestrus (Zicarelli L 1997).

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