POLYOVULATORY RESPONSE AND *IN VIVO* EMBRYO PRODUCTION AT THE *ROMANIAN INDIGENOUS BUFFALO*, OUT OF BREEDING SEASON IN N-E OF ROMANIA

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Abstract

In recent years, by using Buffaloes breeding biotechnology, it has been found to improve the results obtained in the recovery of embryos produced in vivo. Following limited research in buffalo, the viable embryo production has increased significantly from less than 1 per flushing to 2.5-3.0 in general and over 4 in isolated cases and conception rate following embryo transfer improved from about 10% to about 30-40%. However, the response to superovulatory treatment and recovery of viable embryos following superovulation is still low compared to cattle due to various factors (Tyagi S. 2007). Through this study we want to make contributions regarding the obtaining of invivo embryos and their rate of recovery by the method of washing the uterus at the Romanian Indigenous Buffalo, located outside the breeding season. The breeding season for Romanian Buffaloes in the major buffalo rearing countries appear to extend from September to March. A polyovulation protocol, described by Hafez (2007), with PMSG at the single dose of 2500 IU administered on day 13, one day before the removal of progesterone vaginal devices (PRID), was used. On the 12th day, a dose of PG was also given. Ovarian response showed a rapid evolution of ovaries, follicle count and size, and ovulation. The ovary volume increased by 3 cm on the day of the oestrus and by another 0.5 cm on the flusing day (from 2 / 1.5 cm to 5.5 / 4 cm the right ovary, and from 1.5 / 1 cm to 4/3 cm left ovary). Five follicles were grown on the right ovary and 3 on the left ovary with a diameter of 0.7-0.9 cm. Of which ovulated only 6. Four corpus luteum were diagnosed on the right ovary and 2 on the left. After washing the uterus 6 days after insemination, a number of 4 embryos were recovered and identified in the laboratory. Three embryos came from the right horn and only one left.Depending on the embryonic stage of development and segmentation, two embryos were in the blastocyst stage and two were in the stage of compact morgue with non-homogeneous chromatin. Qualitative assessment of embryos was classified as two high quality transferable embryos and two non-transferable embryos

Introduction

Buffaloes are exploited in many countries (including traditionally non buffalo regions), and contribute very significantly in the rural economy, especially in South and South-East Asia. Some of the limitations of the buffalo reproduction include delayed puberty, higher age at first calving, long post partum anoestrus period, long intercalving period, poor detection of heat and low conception rate. Assisted reproductive technologies such as artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) have been introduced to overcome these inefficiencies and to accelerate genetic gain in this species in a planned manner.

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).

Poor response to superovulation treatment and low embryo recovery is attributed to low primordial follicle pool of 20% (in swamp) to 30% (in riverine) in buffaloes as compared to cattle and high rate of follicular atresia. As a consequence, 10% buffalo failed to respond (0-2 CL) and nearly half responded poorly (0-5 CL) to superovulation treatment (Misra, 1996).

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.

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).

Oogenesis in the cattle and buffaloes gonads begins in the early part of foetal/ neonatal life resulting in the formation of a pool of primordial follicles which would remain the only source of oocytes throughout the life of that female. These ovaries undergo continuous changes as a result of follicular growth, atresia/ovulation during the reproductive life.

The estrous cycle is characterized by a series of follicular waves. The numbers of waves differ between species and within species. In majority of cattle (~75%) 3 waves have been observed whereas 63.3% of buffalo exhibit 2 waves of follicular growth at an interval of about 11 days during the estrous cycle (Gasspari B. 2007).

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).

Equine chorionic gonadotropin: Because of easy availability, low cost and long biological half life, eCG has been widely used for inducing superovulation in cattle (Hammond Jr. and Bhattacharya, 1944; Fernandez et al., 1992) and buffalo (Karaivanov et al., 1990; Cruz et al., 1991, Misra, 1993). In cattle, eCG induces follicular growth primarily due to its FSH like activity by preventing or reversing the process of atresia in ≥ 1.7 mm diameter follicles and thereby increasing the number of follicles capable of responding to the gonadotropin (Moor et al., 1984). However, some of these follicles luteinize rather than ovulate (Monniaux et al., 1983). In eCG treated cows, the abnormal LH profiles may be associated with reduced ovulation and fertilization rate

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 . 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.

Through this study we want to make contributions regarding the obtaining of invivo embryos and their rate of recovery by the method of washing the uterus at the Romanian Indigenous Buffalo, located outside the breeding season.

Results, material and methods

A polyovulation protocol, described by Hafez (2007), with PMSG at the single dose of 2500 IU administered on day 13, one day before the removal of progesterone vaginal devices (PRID), was used. On the 12th day, a dose of PG was also given (fig no 1.).

Ovarian response showed a rapid evolution of ovaries, follicle count and size, and ovulation. The ovary volume increased by 3 cm on the day of the oestrus and by another 0.5 cm on the flusing day (from 2 / 1.5 cm to 5.5 / 4 cm the right ovary, and from 1.5 / 1 cm to 4/3 cm left ovary), (table no 1).

Five follicles were grown on the right ovary and 3 on the left ovary with a diameter of 0.7-0.9 cm. Of which ovulated only 6. Four corpus luteum were diagnosed on the right ovary and 2 on the left.

After washing the uterus 6 days after insemination, a number of 4 embryos were recovered and identified in the laboratory. Three embryos came from the right horn and only one left.Depending on the embryonic stage of development and segmentation, two embryos were in the blastocyst stage and two were in the stage of compact morgue with non-homogeneous chromatin.

Qualitative assessment of embryos was classified as two high quality transferable embryos and two non-transferable embryos (fig no 4)

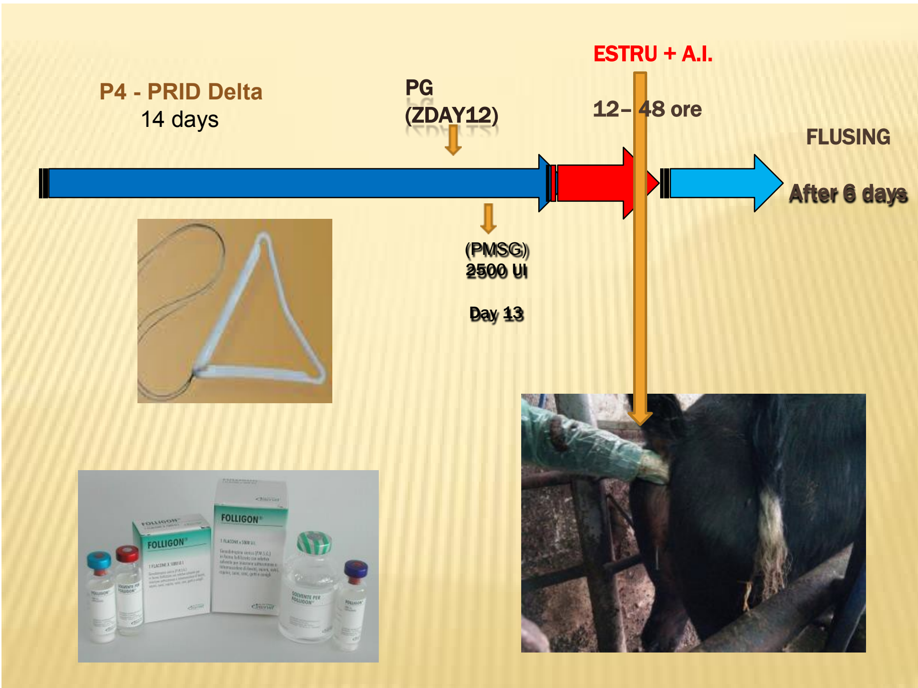

Fig. 1 Schedule the polyovulatory treatment in buffalo

Table 1.

Ovarian response after poliovulator treatment and embryo recovery rate in buffalo cow

		Day 0	Day 2	Day 4	Day 6 Oestrus (T.A.I.)	Day 12 Flusing	%
Volume Ovaries	**Right**	1,5/2	2,2	3/3	5/4	5,5/4	-
	Left	1/1,5	1,5/1,5	2,5/2,5	4/3	4/3	
Ovarian Follicles >0, 8cm	**Right**	0	0	0	5	1	-
	Left	0	0	0	3	1	
Corpus Luteum/ Ovulatory Rate	**Right Ovary**	0	0	0	0	4	75
	Left Ovary	0	0	0	0	2	
Embryo Recovery Rate	**Right uterine horn**	-	-	-	-	3	66.6
	Left uterine horn	-	-	-	-	1	

Fig. 2 . Buffalo, right ovary. Five follicles (F) identified by ultrasound, after 72 hours from the administration of PMSG

Fig. 3 . Buffalo after poliovulatory treatment and flushing

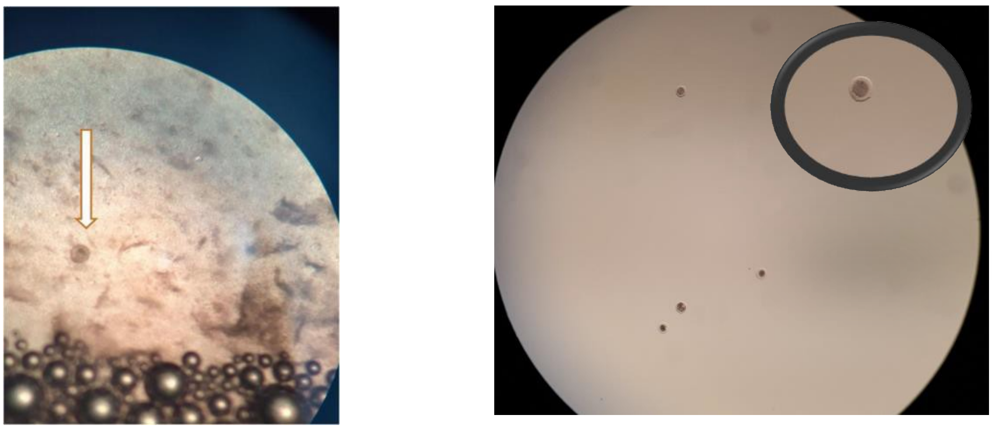

Fig. 4. Identification of the embryos harvested, and their qualitative assessment

Discution

The authors of the literature say that it has been found to improve the results obtained in the recovery of embryos produced in vivo. Following limited research in buffalo, the viable embryo production has increased significantly from less than 1 per flushing to 2.5-3.0 in general and over 4 in isolated cases and conception rate following embryo transfer improved from about 10% to about 30-40%. However, the response to superovulatory treatment and recovery of viable embryos following superovulation is still low compared to cattle due to various factors (Tyagi S. 2007).

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).

Acknowledgement

This article was the result of the project implementation ***PN-III-P1-1.1-MC-2018-2406***, and done in ***Terra de Buffalo*** farm, particularly like to thank for the biological material.

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