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CAN SOMATOTROPIN ENHANCE THE SUPEROVULATORY RESPONSE IN EGYPTIAN BUFFALO?

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ABSTRACT: The current study aimed to evaluate the follicular dynamics, superovulatory response, and embryo recovery in Egyptian buffaloes following superstimulatory treatment PMSG+rbst and its comparison with PMSG alone. Twelve buffalo cows, 60-120 days postpartum, 3-5 parities used and divided into two groups (6 animals each), the 1st group was superovulated with PMSG only, while the 2nd with PMSG+rBST. The ovarian response was followed using ultrasonography before treatments, at estrous day and before flushing process. All follicles with diameter < or ≥ 8mm, numbers of corpora lutea (CL) and unovulated follicles (UOF)/buffalo were recorded.

Obtained results showed that PMSG+rbst resulted a positive ovarian response and increased numbers of small and large follicles in the ovaries (3.0±0.52and 8.67±0.49) compared with those treated with PMSG alone (2.83±0.17and 5.83±0.4, respectively). Furthermore, it increased the number of luteal particles, unovulated follicles and ovulation rate% (5.83±0.31, 2.33±0.33 and 70.68±3.98) in G2 compared to that observed in G1 (3.67±0.33, 1.83±0.31 and 66.74±4.67, respectively). The average number of total embryos, transferable embryos and the %transferable embryos/head in G2 were (3.0±0.37., 2.0±0.37., 66.7±4.65, respectively) greater than that harvested from G1 (1.83±0.48, 1.17±0.31., 63.9±5.96., respectively). Also, embryos recovery rate harvested from the PMSG+rbst treated buffaloes was (51.5%) slightly higher than that obtained from the PMSG treated buffaloes (49.9%). In conclusion, the current study indicates that superovulation buffaloes with combination of PMSG + rbst can improve ovarian response, recovery rate and percentage of transferable embryos more than those treated with PMSG alone.

Key words: Buffaloes, PMSG, rbst, superovulation.

INTRODUCT ION

Buffaloes play a vital role in the agricultural economies of many developing countries due to their ability to adapt to harsh, stressful environments, convert low- quality bristles into meat and milk, and their ability to work (Condori et al., 2016). However, reproductive efficiency in buffaloes is still low (Drost,2007) as represented by low rate of conception following service (Sá Filho et al., 2009), a prolonged postpartum anoestrus period (El-Wishy, 2007) and late puberty (Kandasamy et al., 1989), as compared to those in cattle.

Reproductive techniques such as artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) have been deviced to overcome such reproductive problems and promote genetic advancement in this species in a planned manner.

Numerous research have been undertaken in buffaloes across the world to assess the efficacy of superovulation and embryo transfer (Madan et al., 1996; Campanile et al., 2010). The usage of these techniques on buffalo still has limitations, primarily due to the low rate of embryo recovery (1-3 viable embryos for harvest) as repoted by Misra et al., 1990; Baruselli, 1994; Madan et al., 1996; Baruselli et al., 2000; Misra and Yagi., 2007. According to Misra and Yagi (2007), buffaloes show a poor response to superovulation protocols because the primordial follicles numbers in river buffaloes is 30% lower than that recorded in cattle, poor ovarian follicle

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development (Vittoria, 1997), as well as low oocyte developmental competence, which leads to suboptimal corpus luteum formation and insufficient support to embryo development (Nandi et al., 2002). Also, Baruselli et al., 2000 and Carvalho et al., 2007 pointed that buffaloes have a lower embryo recovery rate than cattle, which might be due to certain failures in the collection and/or transfer of the oocyte throughout the oviduct. Furthermore, it is generally known that throughout the estrous cycle, buffaloes show lower plasmatic levels of estradiol than cows. (Batra and Pandey , 1983).

Injection of rbst into a timed AI synchronization protocol resulted increased the conception rate (Moreira et al., 2001) and oocyte quality in cows (Kuehner et al., 1993). In addition, Gong et al., 1996 and Lucy, 2000 reported that the use of rbst, either directly or indirectly - via the IGF system - increases the number of small antral follicles and enhances the quality of oocytes.

Izadyar et al., 1998 explained that the rbst treatment induces cumulus cells expansion which can contribute to oocytes adhesion to the fimbria and oviduct ciliated cells and consequently, increase the embryo recovery rate in superovulated buffaloes. While (Lucy, 2000) showed that rBST can be harmful in animals with weak metabolism, due to its indirect effect via the IGF system, so decreasing the rbst dosage in buffalo submitted to the MOET protocol can be effective. Thus, the aim of this study was to evaluate the efficacy of using rbst with the MOET protocols in buffaloes on follicle dynamics, , as well as quality and recovery rate of embryos

MATERIALS AND METHODS

The present study was conducted in the Animal Production Department, Faculty of Agriculture, Menoufia University in collaboration with Animal Production Research Institute, Egypt during the period from September 2019 to June 2020.

The experimental animals:

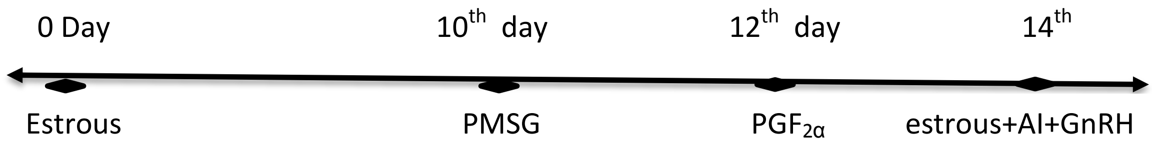
Twelve buffalo cows were randomly chosen from the experimental herd of the Animal Production Department, Faculty of Agriculture, Menoufia Univesity, Shibin El-kom, Egypt and used in the present study. These selected buffaloe donor dams were healthy, at 60-120 days postpartum and had 3-5 parities, divided into two groups (6 animals in each), the 1st group (G1) was superovulated with PMSG protocol only, while the 2nd group (G2) was superovulated with PMSG + rBST.

Superovulation protocols:

Two different ovulation protocols were applied in the current study as follows:

A. First Superovulation protocol (*PMSG protocol*):

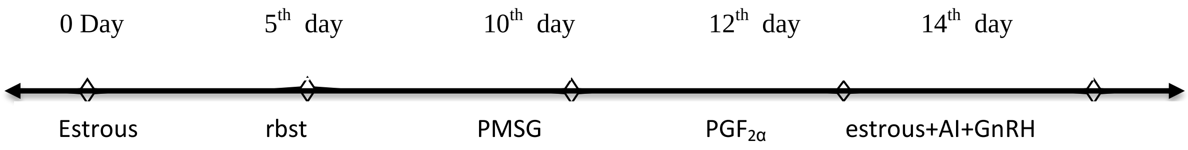
This protocol was performed as described by Ravindranatha and Reddy (1999). Each buffloe cows of the 1st group was injected intramuscularly (i.m.) with 2500 IU PMSG (Folligon, Intervit International BV. Boxmeer - Holland) at day tenth (10th) after a neutral estrous, then after 48 h each buffalo cow was intramuscularly injected with PGF2α analogue (2 ml Estrumate, Coopers Animal Health LTD, Berkhamsted–England, where each ml of Estrumate contained 263 µg of Cloporostenol sodium equivalent to 250 µg Cloporostenol). Buffalo Cows came in heat after 48 h after estrumate injection, after that each buffalo cow was artificially inseminated (A.I.) at onest of estrous (two inseminations were done at 12 hrs intervals), then injected with GnRH.



PMSG Protocol (G1)

B. Second Superovulation protocol (*PMSG + rBST protocol*):

This protocol was performed for buffaloe cows of the second group (G2) as: Each buffloe cows of the 2nd group was given a subcutaneous injection of 250 mg of continuous release formulation of rBST (Boostin- LG Chemical Ltd., Dai Jeon, Korea) at day fifth after signs of estrus, in addition to the PMSG protocol applied to cows of the 1st group (G1).



rbst + PMSG Protocol (G2)

Monitoring follicular dynamics:

The ovarian structures were monitored using real-time ultrasound (a 7.5 MHz transducer. LS-300A; Tokyo Keiki Co.Ltd., Tokyo, Japan) through the rectum on the following days: at the day of superovulation just before the treatments, at the day of the heat and before flushing process. All ovarian follicles < or ≥ 8mm were measured and recorded, as well as number of corpora lutea (CL) and unovulated follicles (UOF)/buffalo were recorded.

Embryo recovery:

Embryos were collected on day 6 following insemination by uterine flushing using modified phosphate buffered solution (mPBS) as described by Drost (1991).. The collected embryos were studied and classified using stereoscopic microscope as described by Reddy (1994), total number of recovered embryos, and numbers of transferable embryos were recorded.

Ovulation rate %, percentage of transferable embryos, recovery rate of transferable embryos and recovery rate of total embryos/ head were mathematically calculated as the following equations:

$$Ovulation\ rate\ \% = \frac{no.of\ copora\ lutea/\ head}{no.of total\ of\ ovarian\ response/buffalo} X100$$

$$Transferable\ embryos\ \% = \frac{No.of\ transferable\ embryos/\ head}{No.of\ \ total\ embryos/head} X100$$

$$Recovery\ rate\ of\ total\ embryo\ \% = \frac{No.of\ Total\ embryos/\ head}{No.of\ \ corpora\ lutea/head} X100$$

$$Recovery\ rate\ of\ transferabal\ embryo\% = \frac{No.of\ Transferable\ embryos/\ head}{No.of\ \ corpora\ lutea/head} X100$$

Statistical analyses:

The data are provided as means and standard errors for all variables. After confirming the data normality and homogeneity of variance, Student's t-test (two-tailed) was used to compare mean values of treatments in SPSS-16 (2016) Statistical program. A p=0.05 was considered as significant.

RESULTS AND DISCUSSION

1- Effect of superovulation proptcols (PMSG and PMSG+rbst) on Ovarian responses in Egyptian Buffaloes:

A- Ovarian follicles dynamics:

The data listed in Table (1) illustrate the dynamics of ovarian follicles in the two groups (G1 & G2) of the experimental buffaloes before and after the application of the superovulation protocols used in this study, namely: the PMSG protocol for G1 buffaloes, and the rbst + PMSG protocol in G2 buffaloes.

Table (1): Effect of combination of PMSG with rbst on the dynamics of ovarian follicles in buffaloes as compared to PMSG alone .

Items	Superovulation protocols		Significant
	PMSG	PMSG + rbst	
Just before superovulation treatment			
Number of follicles < 8mm	9±0.26	13.17±0.31	***
Number of follicles ≥ 8mm	1.17±0.17	1.33±0.21	NS
At oestrus day			
Number of follicles < 8mm	2.83±0.17	3.0±0.52	NS
Number of follicles ≥ 8mm	5.83±0.4	8.67±0.49	***

NS non-significant (p<0.05)
** ,*** highly significant (p<0.01)

Ultrasound examination of the ovaries of these buffaloes before treatments showed that the average number of small follicles (<8 mm) in the ovaries of G2 was significantly greater (p > 0.001) than that recorded for that of the first group (13.17 ± 0.31 vs. 9.0 ± 0.31, respectively) and the same was true for the average number of large follicles (≥8 mm) without significant differences animals (1.33 ± 0.21 in the 2nd group and 1.17 ± 0.17 in the 1st group). In addition that results indicated that the average number of large follicles (≥8 mm) recorded in the ovaries of the respective buffalo groups was lower than that of small ones (<8 mm) as shown in Table (1).

Regarding to the effect of treatment of the intended superovulation protocols on the ovarian response in the experimental buffaloes groups, the results obtained (Table 1) showed that the number of small and large follicles in the ovaries of the 2nd group treated with PMSG with rbst was greater (3.0 ± 0.52 and 8.67 ± 0.49, respectively) as compared to those of the first group treated with PMSG only (2.83 ± 0.17 and 5.83 ± 0.4, respectively) The statistical differences between the averages of large follicles were significant (p<0.01), while it was not significant among the averages of small follicles.

B- Numbers of corpora lutea and ovulation rate:

The results presented in Table (2) show that the combination of PMSG and rbst increased the number of luteal particles (CL), unovulated follicles (UOF) and ovulation rate (%) in buffalo ovaries (5.83 ± 0.31, 2.33 ± 0.33 and 70.68 ± 3.98, respectively)) compared to that observed in buffaloes treated with PMSG alone (3.67 ± 0.33, 1.83 ± 0.31 and 66.74 ± 4.67, respectively). The differences among the average of CL and Total no. of ovarian response / buffalo in the respective groups were significant (p<0.01), while it was not significant among the no. of Unovulated follicles (UOF)/ buffalo and Ovulation rate %.

These obtained results (Tables 1&2) could be concluded that superovulated buffaloes cows with PMSG+rbst resulted in a positive ovarian response, and increased the average number of the small (not significant) and large (significantly) follicles in the ovaries of these animals, compared with those treated with PMSG alone._ In accordance with findings of Gong et al., (1993) who revealed that using rbST can manipulate the size of the follicular population resulting in a greater number of small follicles, and follicular growth after recruitment (De la Sota et al., 1993; Lucy et al., 1994). In addition, Pavlok et al., (1996) and Lucy, (2000) pointed that rbST can enhance the population of small antral follicles by a direct and/or indirect influence of IGF-1. In this regard, it is concluded that recombinant bovine somatotropin (rbST) boosted the concentrations of intraovarian and blood insulin-like factors (IGFs), which supported oocyte maturation, fertilization rate, early embryonic development, CL function, and maternal pregnancy identification in dairy cows (Lucy 2000 and Ribeiro et al., 2014). Furthermore, rbST can stimulate the cumulus cells expansion, promote oocyte attachment to fimbria and ciliated cells of the endosalpinx, which may increase the proportion of good quality-oocytes in buffalo (Sá Filho et al., 2009) and the embryo recovery in superovulated animals (Izadyar et al., 1998). also Gong *et al.*, 1993 admitted that rbST appears to decrease donor superovulatory variability among donors by enhancing follicular dynamics and embryos production.

2- Effect of superovulation proptcols (PMSG and PMSG+rbst) on mean number of recovered embryos and recovery rate in Egyptian Buffaloes:

Regarding the effect of treated buffaloes with rbst combination with PMSG on the average of total embryos/ head, percentage of transferable embryos and recovery rate compared to PMSG alone, the results presented in Table (3) indicated that the mean total embryos, percentage of transferable embryos and recovery rate were relatively higher in buffalo ovaries treated with PMSG + rbst than those recorded in those buffaloes treated with PMSG only. In this regard, the average number of total embryos, transferable embryos and the Percentage of transferable embryos/head in the ovaries of treated buffaloes with rbst combination with PMSG were (3.0±0.37., 2.0±0.37., 66.7±4.65., respectively) greater than that harvested from the ovaries of treated buffaloes with PMSG only (1.83±0.48., 1.17±0.31., 63.9±5.96., respectively) with no significant differences.

Table (2): Effect of combination of PMSG and rbst on the numbers of Corpora lutea, unovulated follicles and ovulation rate in buffaloes as compared to using PMSG alone.

Ovarian structure's	Superovulation protocols		Significant
	PMSG	PMSG + rbst	
Corpora lutea (CL)/ buffalo (NO.)	3.67 ± 0.33	5.83 ± 0.31	***
NO. of Unovulated follicles (UOF)/ buffalo	1.83 ± 0.31	2.33± 0.33	NS
Total of ovarian response / buffalo	5.5 ± 0.34	8.33 ± 0.49	***
Ovulation rate %	66.74±4.67	70.68±3.98	NS

NS : non-significant (p<0.05)

Table (3): Mean number of recovered embryos and recovery rate (%) as affected by superovulation protocol (PMSG or PMSG+rbst) in Egyptian buffaloes.

Items	Superovulation protocols		Significant
	PMSG	PMSG + rbst	
Recovered embryos/ buffalo			
Average no. of embryos/head	1.83±0.48	3.0±0.37	NS
Average transferable embryos no./head	1.17±0.31	2.0±0.37	NS
(%) Transferable embryos	63.9±5.96	66.7±4.65	NS
Recovery rate (%) of			
Total embryos	49.9±14.14	51.5±7.25	NS
Transferable embryos	31.9±9.56	34.3±6.96	NS

NS: non-significant (p<0.05)

Additionally, results illustrated that the embryos recovery rate harvested from the ovaries of the treated buffaloes with PMSG combined with rbst (51.5%) was slightly higher than that obtained from the ovaries of the treated buffaloes with PMSG alone (49.9 %), these embryos recovery rates obtained in our study are higher than that reported in many studies (Boland et al., 1991 and Adams, 1994) who found that the buffaloes embryos recovery ranged approximately from 20 to 40%.

This discrepancy could be due to using various doses of rbst. In this respect, Baruselli et al., 2003 recorded that 500 mg of rbST enhanced embryo recovery (50.0 vs. 33.3%; P=0.06) and the number of recovered ovarian structures (5.1±6.8 vs. 1.6±1.7; P=0.18). Whenever, Carvalho et al., 2007 found no effects of any doses of rbST they used on the efficiency of MOET in buffalo. On the other hand, rbST appears to have a time-dependent influence on superovulatory response and embryo quality in bovines. Because providing a single injection seven (Kuehner *et al.,* 1993) or four (Neves *et al.,* 2005) days before and three days after (Márquez-Hernández, 2011) the start of the superstimulatory treatment enhanced embryo quality. However, Donors who received rbST five days before (Gong et al., 1993b) or at the start of the superstimulatory treatment (Kuehner et al., 1993) demonstrated an increase in superovulatory response but not in embryo quality.

It is worth noting that the results of the current study in addition to several previous researches showed that the rate of embryo recovery in buffaloes treated with various superovulated protocols is still lower compared to cattle. It has been hypothesized that this differentiation in embryo recovery rates results from oocyte capture failure and/or oocyte transit through the oviduct (Baruselli et al., 2000). On the other hand, the lower quality of oocytes in buffaloes could be due to the fragile contact between oocytes and granulosa cells, as opposed to what happen in bovine species as pointed out by Gasparrini (2002).

Conclusion:

The current study indicates that superovulated buffaloes with PMSG + rbst can improved the ovarian response , recovery rate and transferable embryos percentage more than those received PMSG only.

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هل يستطيع السوماتوستاتروبين أن يحسن الاستجابة للتبويض الفائق في الجاموس المصرى ؟

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الملخص العربى

تهدف الدراسة الحالية إلى تقييم ديناميكيات الحويصلات المبيضية ، والاستجابة الفائقة للتبويض ، واسترداد الأجنة في الجاموس المصري بعد المعاملة بجونادوتروبين مصل الفرس الحامل بالإضافة إلى السوماتوتروبين البقري (PMSG + rbst) ومقارنتها مع بروتوكول التحفيز الفائق التقليدي بال PMSG فقط .

وقامت الدراسه الحالية على استخدام اثني عشر بقرة جاموس في فترة 60 -120 يومًا بعد الولادة وذات مواسم 3-5 تم تقسيمها إلى مجموعتين (6 حيوانات في كل منهما) ، المجموعة الأولى (G1) تمت إباضتها باستخدام بروتوكول PMSG فقط ، بينما المجموعة الثانية (G2) تمت إباضتها باستخدام PMSG + rBST، تمت متابعة استجابة المبيض باستخدام الموجات فوق الصوتية عبر المستقيم في الفترات التالية : قبل المعامله و في يوم الشبق وقبل استرداد الأجنة.و تم تسجيل جميع الحويصلات المبيضية التي يبلغ قطرها أصغرأوأكبر من 8 مم ، وكذلك عدد الأجسام الصفراء (CL) والحويصلات المبيضة التي لم يحدث لها تبويض (UOF) / الجاموس.

أظهرت النتائج المتحصل عليها أن معاملة الجاموس بال PMSG + rbst أدت إلى استجابة مبيضية إيجابية وأدت إلى زيادة متوسط عدد الحويصلات المبيضية الصغيرة والكبيرة في المبايض (3.0 ± 0.52 و 8.67 ± 0.49) مقارنة بتلك تعامل مع PMSG وحده (2.83 ± 0.17 و 5.83 ± 0.4 على التوالي).بالإضافة إلى زيادة عدد الأجسام الصفراء (CL) والحويصلات التي لم يحدث لها تبويض (UOF) ومعدل التبويض (٪) (5.83 ± 0.31 ، 2.33 ± 0.33 و 70.68 ± 3.98 على التوالي) في مبيض الجاموس المعالج بـ PMSG + rbst بالمقارنة مع تلك التي لوحظت في الجاموس المعالج بـ PMSG وحده (3.67 ± 0.33 ، 1.83 ± 0.31 و 66.74 ± 4.67 ، على التوالي).

بالإضافة إلى ذلك ، كان متوسط العدد الإجمالي للأجنة والأجنة القابلة للنقل ونسبة الأجنة القابلة للنقل / الرأس في مبايض الجاموس المعالج مع مزيج rbst مع PMSG (3.0 ± 0.37، 2.0± 0.37، 66.7 ± 4.65 ، على التوالي) أكبر من ذلك الذي تم حصاده من مبيض الجاموس المعالج بـ PMSG فقط (1.83 ± 0.48. ، 1.17 ± 0.31. ، 63.9 ± 5.96. ، على التوالي) مع عدم وجود فروق ذات دلالة إحصائية.وكان معدل استرداد الأجنة المتحصل عليها من مبيض الجاموس المعالج بالـ PMSG مع rbst أعلى بقليل (51.5٪) من ذلك الذي تم الحصول عليه من مبيض الجاموس المعالج بالـ PMSG وحده (49.9٪).

في الختام ، تشير الدراسة الحالية إلى أن معاملة الجاموس ببروتوكول التبويض الفائق (مزيج PMSG + rbst) يحسن استجابة المبيض ومعدل الأجنة والنسبة المئوية للأجنة القابلة للنقل أكثر من تلك التي عومل بـ PMSG وحده.