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IMPROVEMENT OF THE CONCEPTION RATE IN EGYPTIAN BUFFALOES DURING SUMMER SEASON

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ABSTRACT

The present study aimed to improving the conception rate in Egyptian buffaloes during hot summer season via increasing the number of buffaloes that are service by implementing the fixed-time natural service protocol of ovsynch without the need of estrus detection with or without injection of antioxidant (vit E+Sel). A total of 40 buffalo cows suffering from postpartum anoestrus for 4-6 months with inactive ovaries, 3-8 years old and 1-6 parities were used. Animals were divided into four comparable groups, each group of 10 buffalo cows, and treated by GPG-GS, GPG-VS, GPG-S and GPG-W protocols of fixed time service. The conception rate was 70, 50, 30 and 70% for GPG-GS, GPG-VS, GPG-S and GPG-W, respectively. Also, the present study did not reveal any significant difference in concentration of serum progesterone between different groups on days 0, 7 and 9 before natural service, but the difference appeared more prominent on days 8 and 18 after natual service. From the present study, it can be concluded that the GPG protocol of fixed time service induces more obvious improvement in the conception rate of buffaloes with the reinjection of GnRH on the day 8 after natural service during the summer months and without reinjection of GnRH during the winter months; injection of Vit.E-Selenium induces little improvement the conception rate when compared to the non-injected group.

***Key words:** Conception Rate, Egyptian buffaloes, Summer season.*

INTRODUCTION

Heat stress can be defined as external force acting on an animal to raise the body temperature over the normal state (Hansen & Arechiga, 1999). Cows that are exposed to heat stress suffer from decreased feed intake, reduced milk yield, poor signs of estrus and infertility (Hansen, 2005). In domestic buffaloes, heat stress during summer results in late maturity, long post parturient anestrus ,long intercalving interval, poor expression of estrus and low conception rate (Singh and Madan 1989; Shah, 1990 and Singh *et al.,* 2013). Heat stress leads to elongation of postpartum period extended for months during the hot season than cold season (Perera *et al.,* 1987 and Qureshi *et al.,* 1998). Even if fertilization occur, heat stress will lead to an increase in rates of the embryonic mortality (Hansen, 2007), mostly in association with the hot season in cattle and buffaloes (Ryan *et al.,* 1993). The survival of embryo in the uterus is impaired due to the deficiency of progesterone in the hot season, in addition to this, heat stress has a drastic effect on the sperm quantity and quality, thus, reducing the males fertilizing capacity (Gordon, 2005).

During the follicular recruitment, heat stress suppresses the subsequent follicular growth to ovulation accompanied by a decrease in the LH receptor level and estradiol synthesis in the follicles (Roth, 2008). Therefore, the decrease in estrous intensity may be induced by a decline in the follicular estradiol secretion during pro-estrus (Gwazdauskas *et al.,* 1981), an evidence which results in shortness of the oestrus behavior period (Pinda and Dooley, 2004), poor detection of oestrus (Thatcher and Collier, 1986) and high incidence of silent ovulations (Gordon, 2005).

Throughout the summer season, the female river buffaloes seemed to have a high concentration of prolactin (hyperprolactinemia) and low concentration of progesterone and oestradiol-17beta (Roy and Prakash, 2007 and Singh *et al.,* 2013), a finding which may be partially responsible for the low sexual activities and low fertility in the buffaloes during the hot season.

The present study aimed to improving the conception rate in Egyptian buffaloes during hot summer season via increasing the number of buffaloes that are service by implementing the fixed-time natural service protocol of ovsynch without the need of estrus detection with or without injection of antioxidant.

MATERIALS and METHODS

A total of 40 buffalo cows, 3-8 years old and 1-6 parities, kept under the village system in Dakahlia Province, were used in the present study. Animals were allowed to have the available daily ration which consisted of adlib barseem in Winter and darawa in Summer as well as about 10 kg wheat straw and 4-6 kg concentrate mixture. All animals were vaccinated against the common epidemic diseases and dewormed against the common parasitic diseases. These animals had normal parturition with a history of 4–6 monthes postparturient anoestrus characterized by normal genital tract and inactive ovaries diagnosed by rectal examination.

Animals were divided into four comparable groups, each group of 10 buffalo cows and treated as shown in Table (1):

Group 1 (GPG-GS): The number of animals in these group 10 buffaloes treated during the Summer season in the first day (0day) by GnRH (Receptal), in a dose 2.5 ml injected Intramuscularly followed in the day 7 by PGF2 alpha (Estumate), in a dose 2 ml injected Intramuscularly followed after 48 hours by second dose from GnRH (known as Ovsynch) and naturally service 12 hours after the second dose of GnRH followed by a dose from GnRH 8 days after natural service.

Group 2 (GPG-VS): The number of animals in these group 10 buffaloes treated during the Summer season in the first day (0day) by GnRH in a dose 2.5 ml injected Intramuscularly plus Vit E+ Se (Vitacillin), in a dose of 15 ml injected Intramuscularly followed in the day 7 by PGF2 alpha in a dose 2 ml plus Vit E+ Se followed after 48 hours by second dose from GnRH and naturally service 12 hours after the second dose of GnRH followed by a dose from Vit E +Se 8 days after natural service.

Group 3 (GPG-S): The number of animals in these group 10 buffaloes treated during the Summer season in the first day (0day) by GnRH in a dose 2.5 ml injected Intramuscularly followed in the 7 day by PGF2 alpha in a dose 2 ml injected Intramuscularly followed after 48 hours by second dose from GnRH and naturally service 12 hours after the second dose of GnRH.

Group 4 (GPG-W): The number of animals in these group 10 buffaloes treated during the Winter Season in the first day (0day) by GnRH in a dose 2.5 ml injected Intramuscularly followed in the day 7 by PGF2 alpha in a dose 2 ml injected Intramuscularly followed after 48 hours by second dose from GnRH and naturally service 12 hours after the second dose of GnRH.

Table 1: Different treatment groups of buffalo cows during Summer and Winter season by different protocol.

Time of Treatment	1st group (S)	2nd group (S)	3rd group (S)	4th group (W)
Protocol	GPG-GS	GPG-VS	GPG-S	GPG-W
Zero day	GnRH[1]	GnRH + Vit E-Se[2]	GnRH	GnRH
7 days	$PGF_2\alpha$[3]	$PGF_2\alpha$ + Vit E-Se	$PGF_2\alpha$	$PGF_2\alpha$
9 days	GnRH[1]	GnRH	GnRH	GnRH
+ 12 hrs	NS	NS	NS	NS
+ 8 days	GnRH	Vit E-Se	-	-

1:Gonadotropin releasing hormone(Receptal[R]), Intervet International GmbH –Germany.
2:VitE+Sel(Vitacillin[R]), Adweia Co., ARE.
3: Prostaglandin F2 alpha (Estrumate[R]), Schering Plough USA.
(S): Summer ; (W): Winter
NS :Normal service.

A blood serum sample of 10 ml was taken in sterile tubes from each animal via the jugular vein on days zero, 7, 9 & 10 immediately before treatment, and days 8 & 18 after natural service. Samples were centrifuged at 3000 rpm for 20 minute and the serum was stored at -20^{o} C till progesterone analysis by Tietz (1995). After two monthes of the natural service, all animal groups were checked to confirm pregnancy by rectal examination.

The obtained data were tabulated and statistically analyzed to estimate percentages, Mean ± S.E. and the analysis of variance according to SPSS (2007).

RESULTS

Table 2: Mean ± S.E. of age, parity number and conception rate for the different treated groups of buffalo cows.

group	Age	Parity	CR
1	6.10 ±.41[a]	4.00 ±.47[a]	70.00 ±15.28[a]
2	5.50 ±.50[a]	3.20 ±.51[a]	50.00 ±16.67[a]
3	5.50 ±.43[a]	3.00 ±.42[a]	30.00 ±15.28[a]
4	5.40 ±.48[a]	3.20 ±.44[a]	70.00 ±15.28[a]

Symbol (a) within the same column mean no significant difference.

Table 3: Mean ± S.E. of progesterone level (ng/ml) throughout the period of treatment for the different treated groups of buffalo cows.

Days	Gr.1	Gr.2	Gr.3	Gr.4
0	0.312 ± 0.027[d]	0.326 ± 0.032[b]	0.326 ± 0.018[c]	0.353 ± 0.023[a]
7	2.904 ± 0.076[c]	3.003 ± 0.279[a]	3.113 ± 0.181[a]	2.794 ± 0.189[c]
9	0.577 ± 0.032[d]	0.544 ± 0.028[b]	0.584 ± 0.039[c]	0.564 ± 0.029[a]
10	0..465 ± 0.024[d]	0.437 ± 0.022[b]	0.454 ± 0.024[c]	0.461 ± [0.020[a]
+8	3.585 ± 0.117[b]	3.094 ± 0.061[a]	2.638 ± 0.158[b]	3.673 ± 0.149[b]
+18	4.313 ± 0.126[a]	3.530 ± 0.148[a]	2.619 ± 0.185[b]	4.348 ± 0.103[a]

Values with different letters within the same column differed significantly at least at $P < 0.05$.

DISCUSSION

Summer heat stress is a major contributing factor to low the fertility rate among Egyptian buffaloes. Dairy cows that are exposed to heat stress were noticed to suffer from a decrease in the feed intake, a reduction in milk production, poor expression of the signs of estrus, and infertility (Hansen, 2005). By using a protocol of fixed-time insemination, without oestrus detection, De-Rensis and Scaramuzzi (2003) was able to reduce summer inferltility in Holstein cows, a finding which came in association with the enhancement of folliculogenesis and oocyte quality after hormonal treatment. In the present study, inspite of the non-significance, the conception rate seemed to differe between the different groups (Table, 2). It was much better in buffaloes having the GPG-G (70.00 ±15.28%) than those having the GPG (30.00 ±15.28%) during the hot summer. This finding came in agreement with some previous reports indicating improvement of the conception rate after reinjection of GnRH 7days in cows (Yılmazbas-Mecitoglu *et al.,* 2012), 11 days in heifers (Willard *et al.,* 2003) and 11-12 days in buffaloes (Mandal *et al.,* 2004 and Lattoo *et al.,* 2013) from fixed time insemination during the summer season.

The highly significant increase of conception rate in protocol one than that in protocol three could be attributed to significant increase in serum progesterone level during the early stages of pregnancy (Table, 3), a finding which promotes the embryonic development and controls the luteolysis (Stevenson *et al.,* 1993 and Lopez-Gatius *et al.,* 2006). The elevated serum progesterone after GnRH treatment (Beltran and Vasconcellos, 2008 and Paksoy and Kalkan, 2010) might be due to the formation of accessory corpus lutum (Thatcher *et al*., 1993; Howard *et al.,* 2006 and Gaja *et al.,* 2008), besides that GnRH administration apparently provides a protective effect within the uterine environment to improve embryo survival (Gordon, 2005). The lowered conception rate in buffaloes having the protocol of GPG during the summer season (Table, 2), came in parallel to that obtained in some previous studies, where it was 20 - 30% (Cavestany *et al.,* 1985; Baruselli *et al.,* 2002; De Rensis *et al.,* 2002; Ahmadi and Ghaisari, 2007). This finding could be attributed to the early embryonic loss during the heat season with low progesterone levels during the first week after fixed time insemination (Stevenson *et al.,* 1993 and Lopez-Gatius *et al.,* 2006).

In the present study, there was much better improvement in the conception rate of buffaloes having GPG protocol with Vit. E-Selenium supplementation (50.00±16.67%) when compared to those having GPG only (Table, 2). This finding came in agreement with that earlier reports indicating improvement of fertility after selenium supplementation in dairy cows (Gleed *et al.,* 1983 and McClure *et al.,* 1986) and in buffaloes (Hala-Abou Zeina *et al.,* 2009). Also, cows, in heat stressed, having vitamin E with/ without Selenum supplementation were noticed to have more fertility rate (Shubin, 1986 and Arechiga *et al.,* 1994). The increased fertility rate after injection of vit E and selenium was attributed to initiation of the processes of folliculogenesis, steroidogenesis and stimulation of the anterior pituitary to secret and release gonadotropin hormones (Shahat and Abdel Monem, 2011).

When buffalo cows were allotted on the GPG protocol, there was more obvious improvement in the conception rate during the winter (70.00 ±15.28) when compared to that during the summer (Table, 2). This finding came in consistent with some previous studies indicating the presence of lower conception rate in summer months comparing to that in winter months (Almier *et al.,* 2002 & Ahmadi and Ghaisari, 2007). Such difference could be attributed to the reduction in the duration and intensity of behavioural oestrus by heat stress, so a smaller proportion of cows are detected in oestrus under heat stress conditions (Thatcher and Collier, 1986). However, in some other previous investigations, it was concluded that the GnRH administration at days 11–15 after anticipated ovulation or estrus did not consistently increase pregnancy rates in either cool or warm seasons (Jubb *et al.,* 1990; Stevenson *et al.,* 1993 and Franco *et al.,* 2006).

As shown in table (3), the present study did not reveal any significant difference in concentration of serum progesterone between different groups on days 0, 7 and 9 of natural service, but the difference appeared more prominent on days 8 and 18 after natual service. This finding came in comparable to some earlier reports indicating the increased level of serum progesterone after GnRH injection on days 11-14 of the fixed time insemination (Stevenson *et al.,* 1993; Teferra *et al.,* 2001 and Howard *et al.,* 2006). On the other hand, treatment of dairy heifers with GnRH during the days 9-12 of the estrous cycle was noticed to have no significant affect on the serum progesterone level (Young and Swanson, 1988 and Coleman *et al.,* 1991). In the present study, the increased progesterone level in different groups after injection of GnRH (Table 3) could be explained firstly by inducing the GnRH an additional LH surge to enhance active luetinization of granulose and theca cells to ensure adequate production of progesterone in developing the corpus lutum; secondly by the probable action of the GnRH on the developing CL to promote the conversion of small luteal cells to large luteal cells, which are responsible for about 85% of basal progesterone secretion at luteal phase (Kaim *et al.,* 2003 & Shahat and Abdel Monem, 2011).

From the present study, it can be concluded that the GPG protocol of fixed time insemination induces more obvious improvement in the conception rate of buffaloes with the reinjection of GnRH on the day 8 after natural service during the summer months and without reinjection of GnRH during the winter months; injection of Vit.E-Selenium induces little improvement the conception rate when compared to the non-injected group.

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تحسين معدل الحمل فى الجاموس المصرى أثناء فصل الصيف

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تهدف هذه الدراسة إلى تحسين معدل الحمل في الجاموس المصري خلال موسم الصيف الساخن عن طريق زيادة عدد الجاموس الملقحه طبيعيا بنظام التلقيح الثابت التوقيت(GPG) عن طريق تنفيذ بروتوكول ovsynch دون الحاجة إلى الكشف عن شبق مع أو بدون حقن مضادات الأكسدة. تم استخدام عدد ٤٠ من الجاموس المصرى يعاني من عدم ظهور الشبق بعد الولادة لمدة ٤-٦ أشهر مع خمول فى المبايض، وأعمارها تتراوح من ٣-٨ سنوات وعدد الولادات من ١-٦ ولاده. تم تقسيم الحيوانات إلى أربع مجموعات متماثله ، كل مجموعة من عدد ١٠ من الجاموس، وتم التعامل مع المجاميع المختلفه كالتالى GPG- GS، GPG -VS، GPG -S و GPG -W. وكان معدل الحمل ٧٠ ، ٥٠ ، ٣٠ و ٧٠ ٪ للبروتوكولات المعتمدة GPG -GS، GPG -VS، GPG -S و GPG -W على التوالي. أيضا ، فإن الدراسة الحالية لم تكشف عن أي اختلاف كبير في تركيز هرمون البروجسترون في الدم بين المجموعات المختلفة في الأيام صفر و ٧ و ٩ قبل التلقيح الطبيعى، ولكن الفرق يبدو أكثر بروزا في أيام ٨ و ١٨ بعد التلقيح الطبيعى. يستنتج من هذه الدراسة ، زيادة معدل الحمل فى الجاموس المصرى بنظام التلقيح الثابت التوقيت (GPG) مع إعادة حقن هرمون GnRH في اليوم ٨ بعد التلقيح الطبيعى خلال أشهر الصيف، ودون إعادة حقن هرمون GnRH خلال أشهر الشتاء , حقن Vit.E - السيلينيوم خلال شهر الصيف يؤدي الى تحسن طفيف في معدل الحمل عند مقارنة بالمجموعة غير المحقونة.