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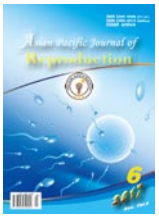

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Factors affecting *in-vivo* fertility of crossbred Egyptian - Italian buffalo semen

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ABSTRACT

Objective: To assess the effect of region, season and year of insemination on *in-vivo* fertility of Italian-Egyptian crossbred buffalo semen. **Methods:** A total number of 4 799 female buffaloes were inseminated by frozen semen with at least 50% post-thaw motility of Egyptian-Italian crossbred bulls in three localities in Delta, lower Egypt (El-Behira, El-Sharkia and Damietta) during the period of 2013, 2014 and 2015. The pregnancy rate after two months was evaluated during the four seasons. **Results:** The rate of pregnancy was significantly (P<0.000 1) differ among the three localities. The effect of year of insemination on pregnancy rate was significantly higher during 2014 and 2015 than 2013 in El-Sharkia and El-Behira. But in Damietta, the rate of pregnancy was significantly higher in 2014 than 2013 and 2015. There were no significant differences among seasons in El-Behira and Damietta governorates but there was significant (P<0.05) differences in pregnancy rate in El-Sharkia. It was higher in summer, spring and autumn than in winter. **Conclusions:** Localities, year of insemination and season of the year have effects on fertility of crossbred Egyptian-Italian buffalo semen.

1. Introduction

Buffaloes are playing essential role in agricultural economy in Egypt. Reproductive efficiency is one of the important aspects of animal breeding. Long calving intervals[1], due to irregular ovarian activity, poor estrus expression and detection[2] are the major constraints in Egyptian buffalo reproduction. The animal productivity can be enhanced by genetic improvement through application of assisted reproductive techniques, such as artificial insemination (AI) and embryo transfer. Unfortunately, keeping buffaloes under the small holder farming system in Egypt constitutes a major challenge against using such technologies. Using of AI with frozen semen exhibited lower fertility in buffalo than in cattle spermatozoa[3-10]. Moreover, the application of AI in buffaloes faces many difficulties due to low estrous expression and difficult to predict ovulation time[11]. Problems in the field related to AI service include poor awareness of farmer, unskilled technician, irregular supply of nitrogen, long distance, improper detection of estrous and time of insemination[12]. In buffaloes, various causes of lower fertility range from inadequate management in female to poor quality of frozen semen. The overall conception rate through application of artificial insemination technique in cattle and buffalo ranges from 38% to 62%[13] and 29%[12], respectively. To improve productivity of Egyptian buffaloes, there is an essential need to use semen from genetically selected bulls. The Ministry of Agriculture in Egypt accepted the importation of Italian buffalo's semen since 2003 for private farms, as practice needs for improving milk production and reproduction traits[14,15]. This study aimed to explore the major factors that may affect fertility rates of local breeds of Egyptian buffalo after AI with Egyptian-Italian crossbred bulls under field conditions. The effects of region, years of insemination

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and season on pregnancy rate were studied.

2. Materials and methods

2.1. Buffalo and geographical location

Total number of 4 799 female buffalo from different governorates (El-Behira, El-Sharkia and Damietta) in north of Delta Egypt were admitted to AI using Egyptian-Italian crossbred bulls frozen semen under the supervision of General Organization for Veterinary Services (GOVS) during the period of 2013, 2014 and 2015. The female buffaloes were inseminated in four seasons of a year by semen with at least 50% post-thaw motility. Weather parameters in studied governorates were illustrated in Table 1 according to Khalil *et al*[16].

Table 1

Weather parameters in studied governorates.

Governorates	Elevation above sea level (m)	Mean temperature (℃)	Relative humidity (%)	Wind speed (km/day)	Potential sunshine (h)
El-Behira (West Delta)	6.7	21.2	57	350.6	9.4
El-Sharkia (Middle Delta)	13.0	23.6	61	144.7	9.3
Domietta (North Delta)	5.0	19.5	65	221.0	9.2

2.2. Bulls and semen

Crossbred Egyptian-Italian buffalo bulls reared in Abbasia semen freezing center, which belongs to GOVS semen source for cryopreservation and insemination program, were used for this study. The cryopreservation extender used in freezing was Bioxcell (IMV, France).

Semen samples were diluted with extender at 37 ℃ to reach 60 million spermatozoa per milliliter concentrations. After that the semen was cooled to 4 ℃ slowly for 2 h and equilibrated for 4 h, then put into 0.25 mL straws. After equilibration, the straws were frozen in a vapour above liquid nitrogen by 4 cm for 10 min then were stored in liquid nitrogen.

2.3. In vivo fertility

Female buffalos were artificially inseminated with crossbred Egyptian-Italian semen under the supervision of GOVS. Pregnancy was diagnosed at 60-70 d after insemination by rectal palpation. The pregnancy rates of crossbreed Italian-Egyptian buffalo were recorded by our team in Damietta governorate. Data from other governorates were obtained from AI records of GOVS.

2.4. Statistical analysis

Data were statistically analyzed by ANOVA using SPSS version 16.0. Means of pregnancy rates for different localities, years and seasons were compared by Duncan's test. Differences at $P<0.05$ were considered to be significant.

3. Results

The pregnancy rates in three different localities of El-Behira, El-Sharkia and Damietta were reported in Table 2. The results showed that the rate of pregnancy was significantly ($P<0.000\ 1$) different among three localities. It was higher in El-Behira and El-Sharkia than Damietta governorate.

Table 2

Effect of localities on pregnancy rate of crossbred (Egyptian-Italian) buffalo bulls.

Localities	Number of inseminated buffalo	Pregnancy number	Pregnancy rate (%)
El-Behira	3 348	2 221	65.7±0.6[a]
El-Sharkia	680	416	62.7±1.6[a]
Damietta	771	410	53.5±1.4[b]
Total	4 799	3 047	60.6±0.9

[a,b]values differ significantly ($P<0.000\ 1$).

The effect of year of insemination on pregnancy rate was illustrated in Table 3. The pregnancy rate was recorded during 2013, 2014 and 2015. In El-Behira, the pregnancy rate was significantly higher during 2014 ($P<0.01$) and 2015 ($P<0.05$) than 2013. Also, in El-Sharkia, it was significantly increased in 2014 ($P<0.001$) and 2015 ($P<0.000\ 1$) compared with 2013. But in Damietta, the rate of pregnancy was significantly higher in 2014 ($P<0.01$) than 2013 and 2015.

The effect of season on pregnancy rate of Egyptian-Italian crossbred buffalo was presented in Table 4. There were no significant differences among seasons in El-Behira and Damietta governorates. But, the difference in pregnancy rate was significant in El-Sharkia ($P<0.05$). It was higher in summer, spring and autumn than winter season.

Table 3

Effect of year of insemination on pregnancy rate of crossbred (Egyptian-Italian) buffalo bull.

Years	El-Behira			El-Sharkia			El-Damietta		
	Number of inseminated buffalo	Pregnancy		Number of inseminated buffalo	Pregnancy		Number of inseminated buffalo	Pregnancy	
		No	Rate (%)		No	Rate (%)		No	Rate (%)
2013	537	347	63.4±1.3[b]	299	161	54.5±3.3[b]	223	110	48.9±3.7[b]
2014	751	505	67.3±0.8[a]	170	112	65.7±1.6[a]	140	81	57.2±1.2[a]
2015	2 060	1 369	66.4±0.7[a]	211	143	67.8±1.0[a]	408	219	54.3±0.5[b]

[a,b]Values within the same column differ significantly ($P<0.05$).

Table 4

Effect of season on pregnancy rate of crossbred (Egyptian-Italian) buffalo bulls.

Season	El-Behira			El-Sharkia			El-Damietta		
	Number of inseminated buffalo	Pregnancy		Number of inseminated buffalo	Pregnancy		Number of inseminated buffalo	Pregnancy	
		No	Rate (%)		No	Rate (%)		No	Rate (%)
Summer	808	541	67.2±0.9	102	68	63.5±4.4[a]	243	131	55.5±2.1
Spring	1 050	679	64.9±0.7	216	140	65.8±2.5[a]	163	92	57.1±1.9
Autumn	828	565	66.4±1.5	115	75	65.4±1.7[a]	201	107	50.7±2.1
Winter	662	436	64.5±1.7	247	133	56.1±2.9[b]	164	80	50.5±4.2

[a,b]Values within the same column differ significantly (P<0.05).

4. Discussion

Genetic improvement through crossbreeding and better management should go hand-in-hand to improve the animal reproduction. In Egypt, genetic improvement of the indigenous buffalos focusing on cross breeding has been practiced by GOVS since 2012. The success of crossbreeding programmes in particular needs to be monitored regularly by assessing the *in vivo* fertility. However, information about evaluation of crossbred buffalo bull is limited particularly in Egypt[17]. The fertility rate is the most important parameter to evaluate the frozen-thawed semen quality[18] and affected by sperm quality parameters[19].

Our results showed pregnancy rate of crossbred semen was 65.7% in El-Behira, 62.7% in El-Sharkia and 53.5% in Damietta. The pregnancy rate was higher in El-Behira and El-Sharkia than Damietta governorates. This variation may be attributed to the difference in AI technicians rather than temperature as the three governorates had a minimal temperature fluctuation. In the same time, we have not any information about the age, parity and nutritional status, condition score and health of inseminated animals. These factors affect the conception/pregnancy rate. In this respect, Den Daas[20] documented that non-return rates can be improved by interaction of different factors such as season, management and AI technician. Moreover, Foote[21] reported that many environmental factors affect fertility of inseminating bull while one single bull enhanced fertility by a factor of 4.7[22].

The percentage of pregnancy rate in our study was higher than 45.2% recorded by Barile *et al*[23] in Italian buffalo inseminated with frozen semen and 55.2% by Mahmoud *et al*[17] for Egyptian-Italian crossbred. Al Naib *et al*[24] reported that bulls with 50% pregnancy rate are categorized as high fertile.

In our study, the effect of year of insemination on pregnancy rate was significantly higher during 2014 and 2015 than 2013 in El-Sharkia and El-Behira. But in Damietta, the rate of pregnancy was significantly higher in 2014 than 2013 and 2015. The variation between the results in different localities may be attributed to differences in technicians who performed insemination. Moreover, various factors affecting fertility as genetic and environment may be put in consideration[25]. The variations in pregnancy rates are attributed to various bull factors[26] as spermatozoa from different bulls have different ability to fertilize buffalo oocytes *in vitro*[27,28] due to differences in metabolic activity of sperm cells[29] and lots of semen from the same bull[30].

With respect to the effect of season on pregnancy rate of Egyptian-Italian crossbred buffalo, there were no significant differences among seasons in El-Behira and Damietta governorates. Also, Bhattacharyya *et al*[31] recorded no significant variation on conception rate among seasons in crossbred Jersy. But in El-Sharkia, pregnancy rate was higher in summer, autumn and spring than winter season. This could be due to the presence of high environmental temperature fluctuation at different season of the year. In this respect, Robinson[32] stated that the cold stress of winter reduce the conception rate in cows. In contrast, Hamam *et al*[33] in buffalo and Mahmoud and Eashra[34] in cattle found that the quality and percentage of mature oocytes were decreased in hot seasons than in cooled seasons. Moreover, Ono *et al*[35] in cows stated that the pregnancy rates were significantly decrease (P<0.05) in summer (June-August) comparing with spring (March-May) and winter (December-February).

In conclusion, *in vivo* fertility was affected by the localities, year of insemination and season and more studies to evaluate other factors affecting the fertility of crossbred are needed.

Conflict of interest statement

We declare that we have no conflict of interest.

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