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Comparison of Semen Freezability and *In-vivo* Fertility of Egyptian and Egyptian-Italian Crossbred Buffalo Bulls

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Abstract: Veterinary Organization trys to improve Egyptian buffalo breed by program using semen from crossbred Egyptian-Italian bulls in artificial insemination center since 2012. Our investigation aimed to evaluate the freezability and *in vivo* fertility of the crossbred and Egyptian bull semen through comparative study. Three mature Egyptian -Italian buffalo crossbred and three Egyptian bulls maintained at Buffalo Semen Freezing Center were used as a source of semen. The semen was evaluated after freezing for semen characteristics and pregnancy rates. The percentage of post thawed sperm motility, live sperm, abnormalities, fragmented DNA and membrane integrity did not differ between Egyptian and Egyptian-Italian crossbred buffalo bulls. But, the percentage of acrosome integrity ($p < 0.05$) and pregnancy rate ($p < 0.01$) were significantly higher in Egyptian Italian buffalo crosses (1/2EG.1/2IT).In conclusion, the percentages of acrosome integrity and pregnancy rate were significantly higher in Egyptian- Italian crossbred than Egyptian buffalo bulls. Further studies on productive and reproductive traits of offspring produced from Egyptian buffaloes crossing with Italian buffaloes are needed.

Key words: Buffalo bull · Semen characteristics · Egyptian- Italian Crossbred · *in vivo* fertility

INTRODUCTION

Buffaloes constitute an essential part of the domestic stock and are playing important role in Egypt's agriculture economy. The Egyptian buffaloes are nearly about 4 million[1]. In Egypt, the increasing importance of buffaloes in the dairy industry has made the technology of artificial insemination a requisite to improve productivity of this species. Cryopreservation of semen is a widely used technique in farm animals, especially buffaloes. However, in this species the fertility of frozen semen still poorer compared to fresh semen [2].

To improve buffaloes productivity and continue with the genetic improvement program in Egypt, there is an urgent need to have quality frozen semen from genetically superior foreign bulls.

In 2003, Egyptian Ministry of Agriculture approved the commercial importation and utilization of Italian buffalo's semen, which is being uncontrollably spread in the country; a practice needs prior genetic assessment for milk production and reproduction traits [3,4].

Egyptian private farms of buffalo imported frozen semen straws for artificial insemination to improve their buffalo reproduction. Veterinary organization selected Egyptian–Italian crossbred bulls produced by private sectors for artificial insemination of indigenous buffalo.

Cross-bred Egyptian-Italian buffalo bulls were maintained at Abbasia buffalo semen freezing center which belong to General Organization for Veterinary Services as semen source for cryopresevation and insemination program.

The fertility rate is considered the best parameter to evaluate the quality of frozen–thawed semen [5]. No previous assessment, for semen freezability and fertility, has been performed for crossbreeding. Therefore, the present work was carried out to compare frozen–thawed semen quality parameters and the pregnancy rates through performing artificial insemination at field level in Domaite province for both Egyptian and Egyptian-Italian crossbred buffalo bulls.

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MATERIALS AND METHODS

Semen Collection and Initial Evaluation: A total number of three mature Egyptian -Italian buffalo crossbred and three Egyptian bulls maintained at Buffalo Semen Freezing Center, General Organization for Veterinary Services, Ministry of Agriculture, Abbasia, Egypt, was used as donors for the semen. Ejaculates were collected by artificial vagina. Semen samples were initially assessed for motility and sperm concentration. Ejaculates fulfilling 70% minimum standard of sperm motility and 80% sperm morphology were processed for freezing. Semen samples were kept in the water bath for 10 minute at 37°C before dilution.

Semen Processing: The reference cryopreservation extender was Bioxcell, obtained from IMV, France. Semen samples were diluted at 37°C with each extender to provide concentration of 60 million sperm/ml. extended semen was cooled slowly for 2 hrs to 4°C and equilibrated for 4 hrs. Semen was packed into 0.25 ml French straws. After equilibrium time, the straws were placed on a rack and frozen in a vapour 4 cm above liquid nitrogen for 10 minutes and were kept in liquid nitrogen.

Assessment of Semen Quality: Frozen straws were thawed at 37°C/ 1 minute. The studied parameters were sperm motility, sperm membrane integrity, alive sperm, total sperm abnormalities, DNA damage and acrosome integrity percentage.

Sperm Motility: Sperm motility percentage was assessed subjectively using microscope at magnification of 400 and equipped with a heating plate (37°C). Visual motility was evaluated microscopically according to Graham *et al.* [6].

Sperm Membrane Integrity: Sperm membrane integrity % was assessed using the hypo-osmotic swelling test (HOST) as outlined by Jeyendran *et al.* [7]. Two hundreds spermatozoa were assessed and the percentage of spermatozoa with swollen/ intact plasma membrane (curled tails) was calculated.

Acrosome Integrity: The acrosome integrity % was evaluated under x 1000, using Giemsa staining, according to Watson [8].

Live Sperm Percent: Semen samples were evaluated using Eosin-Nigrosin staining method [9].

DNA Fragmentation by Acridin Orange Test (AOT): Glass slides smears were prepared according to Liu and Baker [10]. The slides were air dried and then fixed overnight in methanol/acetic acid, 3: 1(Carnoy's solution). Once air dried, the slides were stained for 5 min with acridine orange (AO) stain. The slides were examined under blue light fluorescent microscope (Leitz, Germany; excitation of 450–490 nm). Sperm with normal DNA content had green fluorescence, where sperm with an abnormal DNA content emitted fluorescence varying from yellow-green to red.

***In vivo* Fertility:** Cyclic buffalo cows were observed for estrus twice daily. Buffalo cows would be inseminated once standing heat is observed, although the best time for insemination is traditionally considered to be 12 hours after detection of heat. Buffalo cows were inseminated with frozen semen from the Egyptian and Egyptian- Italian crossbred buffalo bulls. Pregnancy was diagnosed by rectal palpation at 60 days after insemination. The data of pregnancy rates were collected over an interval of two years (2014 and 2015) from Domiate province.

Statistical Analysis: Data were analyzed using the SPSS computerized program v. 16.0 [11]. Results were tabulated in a way to indicate the mean values of the various parameters studied and their standard errors. Student's t test was performed to compare between Egyptian and Egyptian- Italian crossbred buffalo bulls. Differences were considered to be significant at $P < 0.05$.

RESULTS

The post thawing semen characteristics in Egyptian and Egyptian-Italian crossbred buffalo bulls are shown in Table (1). The percentages of post thaw sperm motility, live sperm, abnormalities, fragmented DNA and membrane integrity were not differ between Egyptian and Egyptian-Italian crossbred buffalo bulls. But, the percentage of acrosome integrity was significantly higher ($p < 0.05$) in crossbred than Egyptian buffalo bulls.

A total number of 1182 buffalo cows was used for insemination by the two types of semen. Pregnancy rate from Egyptian and Egyptian-Italian crossbred buffalo bulls frozen semen was recorded in Table (2). The pregnancy rate was significantly ($p < 0.01$) differ between the two bull breeds. The overall pregnancy rate was 55.2% for crossbred and 50.1 % for Egyptian semen.

Table 1: Post-thawing semen characteristics in Egyptian and crossbred (Egyptian-Italian) buffalo bulls (Means ± SE)

Bull breed	Motility %	Live sperm %	Sperm abnormalities %	Acrosome integrity %	Fragmented DNA	Membrane integrity %
Crossbred (Egyptian-Italian)	47.3±09	65.3±3	12.40±0.6	80.8±0.4*	2.7 ±0.1	63.1±1.7
Egyptian	46.9±1.3	64.5±1.4	14.1±0.6	78.9±0.7	2.7±0.2	60.3±1.8

* = P< 0.05(t-Test)

Table 2: Pregnancy rate after artificial insemination in Egyptian and crossbred (Egyptian-Italian) buffalo bulls (Means ± SE)

		Pregnancy rate	
Bull breed	Number of inseminated Buffaloes	NO	%
Crossbred (Egyptian- Italian)	548	300	55.2±1.1*
Egyptian	634	284	50.1±1.4

* = P< 0.01(t-Test).

DISCUSSION

The post-thawing semen characteristics including motility, live sperm percentage abnormalities, fragmented DNA and membrane integrity in the current study were within the range reported in Egyptian buffalo bulls [12-15]. In contrast, Lemma and Shemsu [16] reported that volume, concentration of sperm per unit, total count, mass motility and pre-freeze individual motility were significantly affected by bull breeds of Holstein Friesian, Jersey, Borana and Cross breeds (Borana X Holstein Frisian).

In the present study, the percentage of acrosome integrity was significantly differ between the two groups of bulls. It was higher in the Egyptian-Italian buffalo crosses (1/2EG.1/2IT) than Egyptian semen. Similarly the same range of acrosomal integrity was reported in Indian Murrah buffalo [17]. It is known that sperm membrane/acrosome integrity is important for adhesion, penetration for the oocytes [18]. Therefore, a sufficient number of motile acrosome-intact sperm is necessary for fertilising the oocyte [19].

Our results showed that the pregnancy rate ranged from 55.2% for crossbred semen and 50.1 % for Egyptian. In this respect, Barile *et al*. [20] recorded 45.2% pregnancy rate in Italian buffalo after artificial insemination with frozen semen while El-Sisy *et al.* [21] recorded a range of 51-63% in different bulls in Egyptian buffalo. On the contrary, in Pakistani buffalo, the fertility rate in Nili Ravi buffaloes inseminated with frozen semen was 33% [22] and 30% [23]. In this respect, Al Naib *et al.* [24] stated that bulls with pregnancy rate of about 50% are considered of high fertility and more effective in penetrating artificial mucus.

In the context of our study, few investigations were performed in Egypt on milk productivity and showed higher least square means estimates for total milk yield in Italian crosses than the Egyptian buffaloes [3]. Also, study on reproductive traits indicated that the Egyptian buffaloes had better reproductive performance than the crossbred for number of service per conception, calving interval, days open and service period traits [4]. With respect to male reproduction, no previous assessment on semen parameters and pregnancy rates has been performed for this crossbreeding. So our work should be follow by others before application the crossbred semen.

In conclusion, the percentage of acrosome integrity and pregnancy rate was significantly higher in Egyptian- Italian crossbred buffalo than Egyptian semen. Further studies on other productive and reproductive traits were needed before the application the semen of crossbreeding on national level.

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