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Influence of Season of Calving, Parity and Flunixin Meglumine Administration on Conception Rate in Repeat Breeder Egyptian Baladi Cows and their Crosses

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ABSTRACT

This work was conducted to evaluate the effect of season of calving, parity and treatment with Flunixin Meglumine (NSAID) on conception rate in repeat breeder Egyptian Baladi cows and their crosses (Baladi x Friesian). Season of calving was divided into two season cold season (from November to April) and hot season (May to October). A total of 120 cows (n= 60 Baladi cows, n= 60 crossbred cows) were used in the study. 30 Baladi cows were taken and another crossbred was calved during the cold season and was divided into four groups of each group consisting of 15 cows. A total of 30 Baladi cows were also taken and another crossbred was calved during the hot season. It was divided into four groups, each consisting of 15 cows. Fifteen Baladi cows and another crossbred Flunixin Meglumine were treated (1.1 mg / kg BW intramuscular) at fourteenth day from mating in both cold and hot seasons and the other group was as control. The main results showed that the overall average conception rate in Baladi cows was 43.3% higher than that of crossbred cows (30%). Conception rate was higher in Baladi cows calved during the cold season (46.7%) than in cows calved during the hot season (40%). Conception rate of crossbred cows calved during the season was 33.3% higher than those born during the hot season (26.7%). Conception rate in cows in the fourth parity was higher in both Baladi and crossbred cows (83.3 and 50%) respectively compared to the second and third parity. Administration with Flunixin Meglumine resulted in an increase in conception rate of 13.3% in both Baladi and crossbred cows. Concentration of P4 was higher during the first three weeks of conception in the treated Flunixin Meglumine cows than in non-treated cows in both Baladi and crossbred cows. The current study showed the negative effect of the hot season on the rate of conception in cows raised under the conditions of the hot zones, as well as the positive effect of treatment with Flunixin Meglumine on the conception rate in cows. Conception rate was higher during the 4th parity in Baladi and their crosses. The current study recommends the synchronization of estrous in cows to arrange the births to be during the cold season to avoid the problems of the hot season, especially in southern Egypt, as well as treated with Flunixin Meglumine on the fourteenth day of mating in cows. Future studies on the treatment of Flunixin Meglumine in cows.

Keywords: Baladi and crossbred cows, season, parity, Flunixin Megluminen ,conception rate.

INTRODUCTION

Most of small holders in Aswan governorate reared Baladi and cross bred (Baladi x Friesian) cows, because these breeds are more adapted to the environmental conditions than pure breeds. In small holders, it was observed (personal communication) that cows are characterized by reduce of conception rate (CR) and repeat breeding. This is considered one of the important problems, which may impair the reproductive efficiency of herds in Upper Egypt. Animals reared in Aswan governorate are suffered from the high ambient temperature in most months of the year, being ≥ (40.0 ^{o} C) during the hot months. There are several factors affecting reproductive efficiency of cows, including season of calving, parity order and Flunixin Meglumine treatment. In this respect Wilard *et al.* (2003) found that the ambient temperature affects reproductive performance. Also, several authors reported that higher environmental temperature and relative humidity lead to decline in endocrine activity and lower reproductive efficiency (De-Rensis *et al.,* 2002; Sartori *et al.,* 2002; Sonmez *et al.,* 2005). Recently, Hossein-Zadeh *et al*. (2013), Potdar *et al*. (2016) and De Souza *et al*. (2016) found that the pregnancy rate (PR) of dairy cows was lower in summer than in winter. In addition, Ono *et al*. (2016) reported that PR was lower in summer than winter. On the other hand, Quintela *et al*. (2004), Balendran *et al*. (2008) and Hossain *et al.* (2016) found marked decrease in PR with increasing parity order in cows. Flunixin Meglumine (FM) is considered as a potent non-steroidal, anti-inflammatory agent that inhibits cyclo-oxygenase, thus prevent conversion of arachadonic acid to prostaglandins (Anderson *et al*., 1990; Odensvik, 1995). Administration of FM to cows is associated with increase in PR as compared to control cows (Merrill *et al.,* 2007; Guzeloglu et *al*., 2007; Pfeifer *et al*., 2007). Little information are available about the effect of season of calving, parity and Flunixin Meglumine treatment on conception rate of Baladi cows and their crosses under Upper Egypt conditions, and for these reasons, the investigation was planned.

MATERIALS AND METHODS

Location and climatic conditions:

This investigation was carried out in Aswan governorate; it is far from Cairo city by about 890 km. The animal farm located in Abasia village in Kom Ombou city (32°, 31' 23" East and 22°, 28' 09" North). The climatic conditions of Aswan governorate are famous for high ambient temperature and lower relative humidity. The year were divided into two seasons cold season (extended from November to April) and hot season (from May to October). Ambient temperature and relative humidity during cold and hot seasons are presented in Table (1).

Table 1. Ambient temperature (^{o} C) and relative humidity (%) during cold and hot seasons.

Season	Ambient temperature (^{o}C)				Relative humidity (%)	Av.
	Max.	Av.	Min.	Av.		
Hot (May –October)	36.3 – 46.2	40.0±1.9	20.3 - 27.2	24.3±1.8	15 – 27	19.7±3.5
Cold (November– April)	21.7 - 34.5	27.7±4.0	7.8 - 18.2	12.9±3.5	17 – 40	30.8±8.1

Animals and management:

A total number of 120 repeat breeder (3> services) Baladi (BC) and crossbred cows (CC) (n= 60 cows for each) were used in the present work. All experimental cows did not appear any pathological signs. The parity of cows ranged between the 2nd and the 7th. order. Average live body weight at mating of Baladi and crossbred cows are presented in Table (2). Animals were reared in traditional farm as semi-shaded yards. Beside the concentrate ration (corn grains and wheat bran), animals were offered roughage corn fodder and hay wheat during the period from May to the end of November. Egyptian clover (*Trifolium alexandrinum*), wheat hay and concentrate feed mixture were offered from December to the end of April. The animals were fed *ad-libitum*. All cows were kept under the same managerial and environmental conditions according the farm routine work.

Table 2. Mean ± SE of body weight (kg) at mating of Baladi and crossbred cows during the cold and hot season.

Breed	Body weight (kg)		Overall mean
	Cold season	Hot season	
Baladi cows	369.8± 45.7	368.8± 41.8	369.3±43.8
crossbred cows	411.7±34.2	397.5± 36.9	404.6± 36.3

Treatment of animals:

Animals from each breeds (Baladi and crossbred cows) were divided into two groups (n=30 cows) calved in the cold season and thirty cows calved in the hot season. Fifteen cows from thirty in each group were intramuscularly injected with Flunixin Meglumine (1.1 mg/kg BW) on day fourteen after mating according to Geary *et al*. (2010), while the second group was untreated and served as a control group.

Heat detection and pregnancy diagnosis:

Visual observation of cows was conformed at the morning and night. When any symptoms were manifested like vaginal mucus discharge, standing behavior, cows were considered in heat. Pregnancy was diagnosed applying rectal palpation 60 days (without any heat sings) after mating as described by Arthur (1964). Conception rate: was calculated as the percentage of cows which conceived throughout 120 days post-partum. Conception rate = (Number of pregnant cows/number of mated cows) x 100.

Blood sampling and analysis:

Blood samples, 10 ml, were collected on day 7, 14 and 21 after mating in heparinized tubes from the jugular vein. Blood samples were centrifuged at 3000 rpm for 15 minutes for plasma harvesting. Plasma was separated and stored at -18 ºC until time of progesterone (P4) hormone determination using radioimmunoassay technique. Quantitative assessments of plasma progesterone concentration were carried out using readymade kit (Immunotech, France). Sensitivity value was reported to be 0.03 ng/ml according to manufacturer information. The intra- and inter-assay variation coefficients were 6.3 and 10.2%, respectively

Statistical analysis:

The statistical model including (season, parity and Flunixin Meglumine treatment were analyzed) the analysis was confirmed by SAS (2002) using the following model:

$$Yijkl = u + si + pj + tk + eijkl$$

Where:

Yijkl: the observation trait
μ = overall mean
si = effect of season (cold=1 and hot=2)
pj = effect of parity (2,3,4,…)
tk = effect of treatment (treatment =1, control=2)
eijkl= experimental error

Duncan's Multiple Range test (Duncan, 1955) was used to test the significance of difference among means. Chi Squire was performed.

RESULTS AND DISCUSSION

Effect of season of calving on conception rate

Conception rate (CR, %) of Baladi and crossbred cows is presented in Table (3). The obtained CR of Baladi cows is within a range from 43.2 to 57.7% that reported by Oloufa (1968); El-Wardany *et al*. (2000) and Damarany *et al*. (2013), but it was lower than 66.3 – 76.5% that found by Barkawi *et al*. (2001) and Zahed *et al*. (2001) on Baladi cows. However, the obtained CR of crossbred cows is nearly similar to that reported by Swiefy (1997) and Potdar *et al*. (2016), being from 20 to 41.4% in Holstein Friesian. Higher CR was reported by Sharifuzzaman *et al*. (2015) and Hossain *et al*. (2016) who found the conception rate was 57.2 and 48.75% in Friesian cross and crossbred (Sahiwal x Holstein Friesian), respectively.

Conception rate of Baladi cows was higher in cows that served in the cold season (46.7%) than that in hot season (40%), but the difference was insignificant ($P \geq 0.05$, Table 3). Similar trend was observed in crossbred cows during the two seasons. The obtained results agreed with pervious researches (Hossein-Zadeh *et al*., 2013; Potdar*et al*.,2016;De Souza *et al*.,2016), who found the pregnancy rate was lower ($P<0.05$) in summer than in winter season in dairy cows. In addition, Ono *et al*. (2016) reported that pregnancy rates were lower in summer (17.1%) than in winter (40.9%).

The observed higher CR of Baladi and crossbred cows in cold than in hot season may be due to the effect of increment air temperature on feed intake and blood supply of reproductive oranges. In this respect, Wolfenson *et al*. (2000) explained many processes may be caused a decline in the CR of cows during the hot season such as the oocyte, corpus luteum and early embryonic development, as well as the endometrium and hypothalamic-pituitary axis functionality, are sensitive to hyperthermia caused by heat stress. Demétrio *et al*. (2007) and Beltran and Vasconcelos (2008) found a reduction in pregnancy rate conjugated with rectal temperature below. The decline in the conception rate may be associated with endocrine changes and the follicular microenvironment (Roth, 2012). Khalil *et al*. (2010) found that crossbred cows had lower heat tolerance than Baladi cows under heat stress climate.

Table 3. Conception rate (%)[1] of Baladi and crossbred cows during the cold and hot season.

Season Breed	Cold season	Hot season	Overall mean
Baladi cows	46.7	40	43.3
	14/30	12/30	26/60
Crossbred cows	33.3	26.7	30
	10/30	8/30	18/60

Generally, the present results concerning the effect of season are in agreement with the findings of several authors (Jochle, 1972; Pires *et al*., 2002; Miah *et al*., 2004), who found positive impact of cold season on conception rate of cows. Similar trend was observed by Nabenishi *et al*. (2011) and Mellado *et al.* (2013) in dairy cows.

Effect of parity on conception rate

In both Baladi and crossbred breeds, CR of cows within the 4th parity was significantly (P<0.05) the highest (Table 4). There was increment in CR from the2nd parity to the4th parity in crossbred cows (P <0.05). High CR in cows at 4th may be due to that cows reached to somatic maturity and decline the competence between growth and reproductive process. There was decrease in conception rate in the two breeds during the subsequent parities (Table 4).

The present results are in agreement with those reported by Quintela *et al*. (2004) and Balendran *et al*. (2008), who found decrease in pregnancy rate with increasing parity order in cows. In this way, Barkawi *et al.* (2006) reported a gradual increase in CR fromthe1st tothe2nd parity and a decrease in subsequent parities in Baladi cows. Similar trend was reported by Sharifuzzaman *et al*. (2015), who found higher conception rate between 4 and 5 years of age, being72.31 and 70.51% and lower in cows with 9 years or more.

Recently, Hossain *et al.* (2016) found that PR was gradually increased from parity 1st, 2nd, 3rd, 4th and 5th were 48.72, 56.45, 62.03, 67.74 and 61.54%, respectively in cows. In accordance with the present results, the pregnancy rate was found to decrease after the 4th parity due to the nutritional stress to maintain the reproductive physiology as well as the subnormal body condition scores (Paul *et al*., 2015).

Table 4. Conception rate (%)[1] of Baladi and crossbred cows during the subsequent parities.

Breeds	Parities			
	2	3	4	≥ 5
Baladi cows	37.5 [ba]	12.5 [ca]	83.3 [ab]	37.5 [bc]
	3/8	1/8	10/12	12/32
crossbred cows	25.0[a]	33.3[a]	50.0[a]	27.8[c]
	2/8	4/12	2/4	10/36

[a, b]: values within the same row having different superscripts are significantly different at (*P <0.05*)

The present result is in agreement with that reported by Xu Fengxum (1997) and Bhagat and Gokhale (1999), who showed higher CR in the first fourth parities than the subsequent parities in cows. Similar, trend was observed by Muller *et al*. (2014), who found that the CR of cows within 100 and 200 days post-partum a curvilinear downward trend from 1st to 6th parity.

Effect of Flunixin Meglumine on conception rate

Obviously the CR was insignificantly higher in treated than in non-treated cows in both breeds (Table, 5). Administration of Flunixin Meglumine in Baladi and crossbred cows lead to increase in CR in the two breeds by 13.3%. The obtained results are in agreement with that reported by Schrick *et al*. (2001), who found that using Flunixin Meglumine leads to increase in pregnancy rate of beef cows by 12.7%. Also, Merrill *et al.* (2007) found that administration of Flunixin Meglumine in transported animals conjugated with increase in pregnancy rates compared to the control group (70% vs. 59%). Similar results were reported by Guzeloglu *et al*. (2007), who found marked increase in pregnancy rates, ranging between 23 and 26.9%, when the Holstein heifers were treated with Flunixin Meglumine. Additionally, Pfeifer *et al*. (2007) found similar trend. Kim *et al*. (2014) found that the rate of *in vitro* development of bovine embryos was higher when Flunixin Meglumine was added to culture medium than in control medium.

On the other hands, Lucacin *et al.* (2010) and Rossetti *et al*. (2011) reported no effects of Flunixin Meglumine on conception rate in beef and Nelore cows. Thatcher *et al.* (2001) suggested that embryonic loss may be due to some embryos fails to produce sufficient interferon *tau* (IFN-τ) to initiate maternal recognition of pregnancy approximately fourteen day after mating that inhibit uterine secretion of PGF2*α*. Guilbault *et al*. (1987) found that treatment of beef cows with Flunixin Meglumine was associated with decrease in PGF2*α* secretion. Administration of Flunixin Meglumine to cows leads to decrease PGF2*α* concentration in the blood serum and increased the pregnancy rate (Merrill et al. (2007). Recently, Kim *et al*. (2014) reported that the developmental rate of embryos was greater in cows that treated with the Flunixin Meglumine than in control cows.

Table 5. Conception rate (%)[1] of Baladi and crossbred cows as affected by FM administration.

Breed	Treated cows	Control cows	Overall mean
Baladi cows	50	36.7	43.3
	15/30	11/30	26/60
Crossbred cows	36.7	23.4	30.0
	11/30	7/30	18/60

Generally, the present results agreed with that reported by previous studies (Guzeloglu *et al*., 2007; Dogruer *et al.,* 2007; Tek *et al*., 2010) indicating a positive effect of Flunixin Meglumine treatment on conception rate of cows.

Progesterone concentrations in blood plasma during the post-estrus period

Table (6) indicated that concentration of P4 remained higher in pregnant cows to twenty one day compared to in non-pregnant cows. Progesterone concentration was significantly higher (*P <0.05*) in treated pregnant cows during the 7th , 14th and 21th days from estrous cycle than non- treated pregnant cows Table (6). These findings may be due to the effect of administration by (Flunixin Meglumine) on the lifespan of the corpus luteum.

The present result agreed with that reported by Rossetti *et al*. (2011), who found that concentration of P4 was higher in treated cows (Flunixin Meglumine) (5.15 and 9.11 ng/ml) than in untreated cows (4.7 and 8.6 ng/ml) on seven and sixteen days post estrus, respectively. Similar trend was reported by Lucacin *et al*. (2010), who found that concentration of P4during days seventh to fourteenth of the estrous cycle was higher in treated pregnant cows treated with Flunixin Meglumine than in untreated pregnant cows. Green *et al*. (2005) found relationships between increase of maternal P4 concentration during the estrous cycle and embryo development in dairy cows. Feliciano *et al.* (2003) reported that concentrations of P4 during the fine of luteal phase were higher in pregnant than non-pregnant cows.

Spencer *et al.* (2016) found that P4 concentration was higher in cows administrated the aspirin (non-steroidal, anti-inflammatory agent) compared with control cows. Administration of aspirin may suppress $PGF_{2\alpha}$ secretion during days from 14 to15 post- estrus and may be prevent early corpus luteum regression.

Table 6. Mean ± SE of progesterone concentrations (ng/ml) in treated and untreated Baladi and crossbred cows during estrous cycle.

Days of estrous cycle	Control group		Treated group	
	pregnant	non-pregnant	pregnant	non-pregnant
		Baladi cows		
7	2.04±0.17[a]	3.3±0.21	4.6±0.52[b]	3.8±0.43
14	5.57±0.66[a]	4.44±0.58	8.44±0.55[b]	5.11±0.65
21	4.13±0.61[a]	0.63±0.28	6.41±0.79[b]	0.89±0.09
		crossbred cows		
7	2.27±0.18[a]	3.8±0.31	4.48±0.31[b]	4.27±0.36
14	5.41±0.62[a]	4.5±0.55	8.9±1.1[b]	5.83±0.87
21	4.76±0.57[a]	0.7±0.19	6.28±0.78[b]	0.74±0.19

[a, b]: values within the same row having different superscripts are significantly different at (*P <0.05*).

CONCLUSION

The current study showed that the negative effect of the hot season on the rate of conception in cows kept under the hot conditions could be eliminate the positive effect of treatment with Flunixin Meglumine (1.1 mg/kg BW intramuscular) on the fourteenth day of mating in cows on the conception rate of cows. Conception rate was higher during the 4th parity in Baladi and their crosses (Baladi x Friesian). The current study recommends the synchronize the calvings in cows to arrange the births to be during the cold season to avoid the problems of the hot season, especially in southern Egypt, as well as treatment with Flunixin Meglumine in cows. Future studies on the treatment of Flunixin Meglumine in cows.

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تأثير موسم الولادة ، عدد مواسم الحليب والمعاملة بالفلونكسين مجلومين على معدل الاخصاب في الأبقار البلدية المصرية وخلطانها متكررة التلقيح.

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تم إجراء هذا البحث لتقييم تأثير موسم الولادة , عدد مواسم الحليب و االمعاملة بالفلونكسين مجلومين (مضاد التهاب غير استرويدى) على معدل الاخصاب فى الأبقار البلدية المصرية وخلطانها متكررة التلقيح. تم تقسيم موسم الولادة الى موسمين موسم بارد (يمتد من نوفمبر حتى ابريل) والاخرموسم حار (من مايو حتى أكتوبر). استخدم في هذه الدراسة عدد مائة وعشرون من الأبقار البلدية والخليطة (ستون بقرة بلدي وستون خليطة). تم اخذ ٣٠ بقرة بلدى واخرى خليط ولدت خلال الموسم البارد وتم تقسيمها الى اربعة مجاميع كل مجموعة تتكون من ١٥ بقرة. كذلك تم اخذ ٣٠ بقرة بلدى واخرى خليط ولدت خلال الموسم الحار قسمت الى اربعة مجاميع كل مجموعة تتكون من ١٥ بقرة. تم معاملة ١٥ بقرة بلدى واخرى خليط فى اليوم الرابع عشر من التلقيح بالفلونكسين مجلومين بمعدل (١.١ مليجرام/ كجم من وزن الجسم فى العضل) فى كل من الموسمين البارد والحار والمجموعة الاخرى كانت كنترول. اظهرت اهم النتائج ان المتوسط العام لمعدل الاخصاب اعلى فى الابقار البلدية (٤٣.٣%) منه فى الابقار الخليطة (٣٠%). كان معدل الاخصاب اعلى فى الابقار البلدية التى ولدت خلال الموسم البارد (٤٦.٧%) عنه فى الابقار التى ولدت خلال الموسم الحار (٤٠%). كذلك كان معدل الاخصاب اعلى فى الابقار الخليطة التى ولدت خلال الموسم البااارد (٣٣.٣ %) منه فى الابقار التى ولدت خلال الموسم الحار (٢٦.٧%). كان معدل الاخصاب اعلى فى الابقار التى فى موسم الحليب الرابع فى كل من الابقار البلدية والخليطة (٨٣.٣ و ٥٠%) على التوالى بالمقارنة بالموسم الثانى والثالث. أدت المعاملة بالفلونكسين مجلومين الى زيادة معدل الاخصاب ١٣.٣% فى كل من الابقار البلدية والخليطة. كان تركيز هرمون البروجستيرون اعلى معنويا خلال الاسابيع الثلاثة الاولى من الاخصاب فى الابقار المعاملة بالفلونكسين مجلومين عنه فى الابقار غير المعاملة فى كل من الابقار البلدية والخليطة. اظهرت الدراسة الحالية التأثير السلبى للموسم الحار على معدل الاخصاب فى الابقار التى تربى تحت ظروف المناطق الحارة وكذلك التأثير الايجابى للمعاملة بالفلونكسين مجلومين على معدل الاخصاب فى الابقار. ارتفاع معدل الاخصاب فى الابقار البلدية والخليطة خلال موسم الحليب الرابع. توصى الدراسة الحالية باجراء ترتيب الولادات فى الابقار حتى تكون خلال الموسم البارد لتجنب مشاكل الموسم الحار خصوصا فى جنوب مصر كذلك المعاملة بالفلونكسين مجلومين فى الابقار. اجراء دراسات مستقبلية على المعاملة بالفلونكسين مجلومين فى الابقار.