

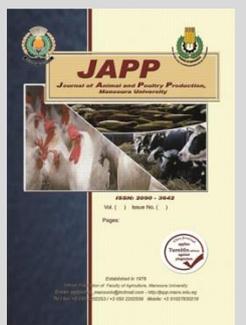
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Distribution of Nulliparous Fertility Traits

Anas A. A. Badr*

Animal Production Research Institute, Ministry of Agriculture and Land Reclamation, Nadi El-Said Str., Dokki, Giza, Egypt



ABSTRACT

Reproductive data of Egyptian Friesian heifers were collected from 1979 to 2013 to study the normality of fertility traits (age at first breeding, age at successful breeding, age at first calving, number of services per conception, conception rate and service period) and to examine environmental factors affecting these traits. A secondary objective was to compare different age at first breeding and its effect on fertility traits of heifers. Age at first breeding, age at successful breeding and age at first calving exhibited symmetrical distribution (bell shape), however number of services per conception, conception rate and service period exhibited lack of symmetrical distribution (L shape). Square root transformation decreased the variability for number of services per conception and conception rate however logarithmic transformation was the best for service period. Youngest heifer at first breeding (13 mo.) needed only one service to be pregnant with zero SP and CR was near to 100%.

Keywords: Heifer fertility traits, data transformation.

INTRODUCTION

Statistical model used to describe the data and the assumptions inherent in chosen method of analysis (Kominakis *et al.*, 1998). All methods of statistical analysis (Paternal half-sib, dam-daughter regression, Henderson's methods or REML procedures) assume that the data are normally distributed (Casu *et al.*, 1975, Barillet and Boichard, 1987). Deviations from a normal distribution increase the error variance estimates and in consequence the functions of variance components are biased (Banks *et al.*, 1985). The accuracy of genetic evaluation of (co)variance components depends on how well the assumptions match the data (Varkoohi *et al.*, 2007). Nonnormality has been mentioned as a possible source of error when variance components and genetic parameters are estimated (Banks *et al.*, 1985 and Westfall, 1987). Measurements that represent a percentage, such as percentage of white coat color, often follow a nonnormal distribution (Becerril and Wilcox, 1994). To approach normality for percentage, empirical transformations (arcsine, square root, log) have been used with little success to date (Briquet and Lush (1947). Jenko *et al.*, (2015) reported that records for lifetime milk production (LMP) and length of productive life (PL) in which distribution was positively skewed were normalized using square root transformation. The same authors added that square root transformation is a proper solution for LMP and PL, as well as the data are higher than 1. The convergence was not achieved if data on the original scale were used, however convergence was achieved with the normalized data used in the model (Jenko *et al.*, 2015).

Replacement heifers require a lot financial expenses, with no returns until the animals enter the milking herd. Heifers do not normally become profitable until their second lactation. Rearing heifers to join the herd

at an age and body weight that will enable them to achieve their full lifetime potential, in terms of both yield and longevity, is also fundamental. Gestation length in fixed, therefor age at first calving is a function of the age at the commencement of first breeding, combined with the reproductive efficiency of the heifer. The decision on when to start breeding is primarily a management one. It is usually based mainly on the age of the heifer, but is also influenced by nutrition and health. Poor growth during the rearing period due to underfeeding and/or disease has been associated with delayed first breeding and first calving (Brickell, *et al.*, 2009 and Johnson *et al.*, 2011). Once heifers have been bred, their fertility during the breeding period will also affect the age at which they calve. Poor fertility of heifers can lead to a large spread in age at first calving in practice there may be large differences between the target and actual age at first calving achieved (Cook *et al.*, 2013). Objectives of the present study were (1) determine which traits are normally distributed and which are not, (2) estimate statistical properties and compare between untransformed and transformed data and (3) determine the effect of age at first breeding on NSC, SP and CR.

MATERIALS AND METHODS

Data were provided by the Animal Research Institute, Ministry of Agriculture, Egypt. A total of 3081 reproductive records of Friesian heifers covering the period from 1979 to 2013 were used. Heifers were inseminated by professional artificial insemination technicians at 18-22 months of age (about 350 kg body weights). Mating that was less than 6 day a part, only the later mating was kept. A maximum of 5 services per heifer was imposed; services beyond 5 were excluded. Management of the two

* Corresponding author.

E-mail address: enas_emad22@yahoo.com
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experimental farms (Saka and El-Karada) was described in more details by Zahed *et al.*, (2020).

Fertility traits under study were age at first breeding (AFB), age at successful breeding (ASB), age at first calving (AFC), number of services per conception (NSC), conception rate (CR) defined as the percentage of successful inseminations ($CR=(1/NSC)*100$) and days from first to last service (service period, SP). All heifers with missing sire or dam were discarded. The ranges for AFB, ASB, AFC, NSC and SP were 11-32 mo., 11-32 mo., 20-42 mo., 1-5 and 15-200 d. The records that included abortion were eliminated from heifer's data set. Heifer records were analyzed according to the following fixed model using MIXED procedure of SAS software (SAS, 2011).

$$Y_{ijkl} = F_i + R_j + M_k + FR_{ij} + RM_{jk} + e_{ijkl}$$

Where:

Y_{ijkl} was the individual observation, F_i was the fixed effect of farm, R_j was the fixed effect of year of first breeding, M_k was the fixed effect of month of first breeding, FR_{ij} was the fixed effect of farm by year of first breeding, RM_{jk} was the fixed effect of year by month of first breeding and e_{ijkl} was random residual term. All non-significant interactions were removed from the model. AFB was added as a fixed effect to the previous model to study its effect on the NSC, CR and SP.

Test of Normality

As the sample size was greater than 2000 record, raw and transformed data were tested against a normal distribution with the Kolmogorov test of normality (Kominakis *et al.*, 1998). In this test, the Kolmogorov D statistic value and the probability associated with the test statistic is obtained. If the D value is sufficiently large or small, the probability of rejecting the null hypothesis of normality appears less than 0.01 or greater than 0.15, respectively (SAS, 2011).

RESULTS AND DISCUSSION

Descriptive statistics for different measures of heifer fertility are presented in Table 1. The symmetry or lack of symmetry of a distribution may be expressed in terms of whatever difference there is between the mean, the median or the mean and the mode (Table 1). The mean values of AFB, ASB and AFC (21.7, 23.4 and 32.2 mo., respectively) were nearly the same as median values of the same traits (21.0, 23.0 and 32.0 mo., respectively). However, the mean of NSC, CR and SP (2.07, 0.70 and 43.9 respectively) were generally exceeding the median (1.0, 0 and 1.0, respectively). Eastham *et al.*, (2018) reported that mean and median of AFC across all heifers were 29.1 and 28 mo., respectively.

Coefficient of variation (CV%) for AFB, ASB and AFC (17.0, 18.1 and 13.6%, respectively) were smaller than the corresponding estimates for NSC, CR and SP (69.8, 48.2% and 146.2, respectively). Similar results have been reported in literature (Raheja *et al.*, 1989 and Jagusiak, 2005).

The frequency of heifers in different ages at first breeding, at successful breeding and at first calving are presented in Figure 1. The distribution is symmetrical (bell shape) for AFB, ASB and AFC. However, the frequency of heifers in different NSC, CR and SP classes are presented in Figure 2. The distribution of untransformed NSC, CR and SP data having a tail on the right (positive skewness shape, L shape) and skewness were 1.07, 0.358

and 1.362, respectively (Table 1). Becerril and Wilcox (1994) reported that percentage of white coat color trait take asymmetric L-shaped distribution skewed to the right (skewness=1.03), however strict normality was not achieved, but distribution was somewhat more symmetric after Box-Cox power transformation (BC). Jenko *et al.*, (2015) found that square root transformation changed the distribution of lifetime milk production (LMP) from leptokurtic to platykurtic, and made the distribution of length of productive life (PL) even more platykurtic.

Table 1. Descriptive statistics² for heifer fertility traits.

Trait ¹	Mean±SE	Median	CV%	SK	KU	D
AFB (d)	21.7±0.07	21.0	17.0	0.48	0.41	0.099**
ASB (d)	23.4±0.08	23.0	18.1	0.29	-0.38	0.089**
AFC (d)	32.2±0.08	32.0	13.6	0.33	-0.17	0.085**
NSC(No.)	2.07±0.03	1.0	69.8	1.070	-0.35	0.313**
CR (%)	0.70±0.006	1.0	48.2	0.358	-1.65	0.354**
SP(d)	43.9±1.2	0.0	146.2	1.362	0.56	0.295**

¹: AFB = age at first breeding , ASB = age at successful breeding, AFC= age at first calving, NSC= number of services per conception, CR= conception rate, SP= service period.

²: CV%= coefficient of variation, SK= skewness , KU= kurtosis.

Statistical properties of non-transformed (NT), logarithmic (LT) and square root transformation (ST) for NSC, CR and SP were shown in table 2. ST showed lowest variability (33.79%) for NSC compared with NT and LT (69.8% and 119.9%, respectively). The same trend was observed for CR (17.56% for ST compared with 48.2% and 114.3% for NT and LT, respectively). In contrary, LT decreased the variability of SP to the lowest value (18.5%) compared with ST and NT data (98.1 and 146.2%, respectively). Becerril and Wilcox (1994) reported that Box-Cox power transformation decreased coefficient of variation from 105% (untransformed data) to 76.6% for white coat color percent in Holstein Friesian. Kominakis *et al.* (1998) found that CV% was lowest (6.67%) when using logarithmic transformation (LT) compared with non-transformed (NT) and Box-Cox transformed (BCT) data (31.09% and 14.73%, respectively) for milk yield data in Boutsico dairy sheep.

A Kolmogorov statistic test (D) was employed to test the normality of the data (Table 2). Although ST gave the smallest D value for NSC and CR (D= 0.228 and 0.204, respectively), it did not improve the distribution of the data since it resulted in a positive skewness for NSC (0.652) and negative skewness for CR (-0.248). For SP trait, LT gave the smallest D value (D=0.102) compared with NT and ST (0.295 and 0.332, respectively). LT resulted in negative skewness (-0.399) for SP trait compared with NT and ST (1.362 and 0.787, respectively). Kominakis *et al.*, (1998) in Boutsico dairy sheep reported that Box-Cox transformation (BC) gave smallest skewness (-0.0042) value compared with non-transformed (NT) and logarithmic (LT) data (0.458 and -0.324, respectively) for milk yield trait. The same authors added that the transformed data by BC showed smallest D value (0.015) compared with 0.0299 and 0.0389 for LT and NT data, respectively indicating that BC transformed data reasonably followed the normal distribution. Jenko *et al.*, (2015) reported that square root transformation decreased the skewness of distribution for both lifetime milk production (LMP) and length of productive life (PL) from 0.81 for original data to 0.14 for square root transformed LMP data and from 0.60 to 0.02 for PL.

Table 2. Test of normality for non-transformed (NT), Logarithmic (LT) and Square root transformed (ST) data of fertility traits in Friesian heifers.

Statistic ¹	NT	LT	ST
No. of service per conception (NSC)			
Mean	2.07	0.226	1.36
SD	1.449	0.271	0.461
CV%	69.8	119.9	33.8
SK	1.070	0.856	0.652
Ku	-0.352	-1.169	-0.218
D	0.313**	0.339**	0.228**
Conception rate (CR%)			
Mean	0.700	0.028	1.224
SD	0.338	0.032	0.215
CV%	48.2	114.3	17.6
SK	0.358	-0.652	-0.248
Ku	-1.667	-1.460	-1.169
D	0.354**	0.339**	0.204**
Service period (SP)			
Mean	43.93	1.864	4.786
SD	64.23	0.345	4.694
CV%	146.2	18.5	98.1
SK	1.362	-0.399	0.787
Ku	0.564	-0.959	-0.872
D	0.295**	0.102**	0.332**

1: SD= standard deviation, CV%= coefficient of variability percent, SK= skewness, Ku= kurtosis, D= Kolmogorov test., ** P < 0.01.

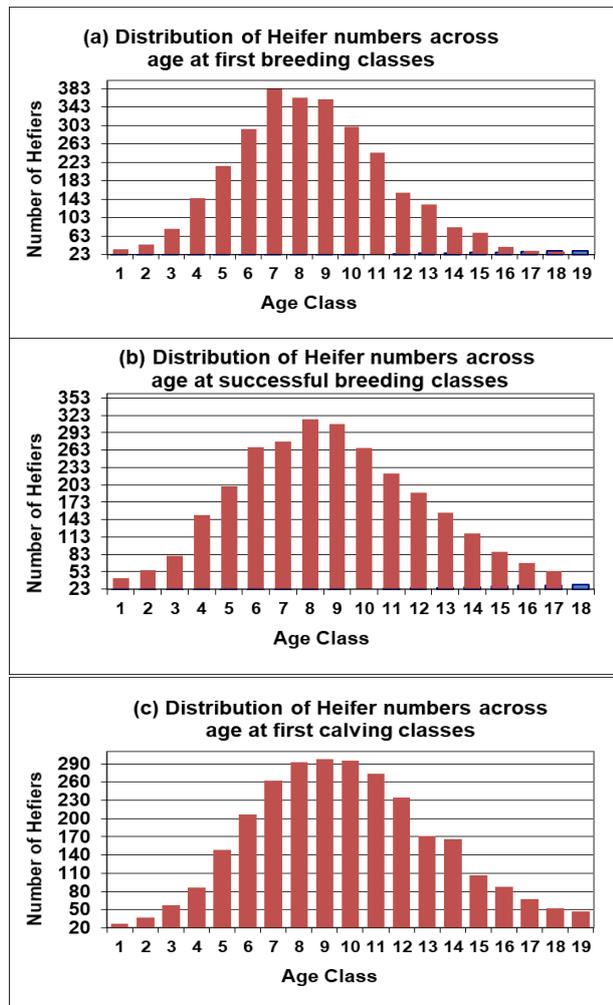


Figure 1. Distribution of heifer numbers in different age classes for (a) AFB, (b) ASB and (c) AFC.

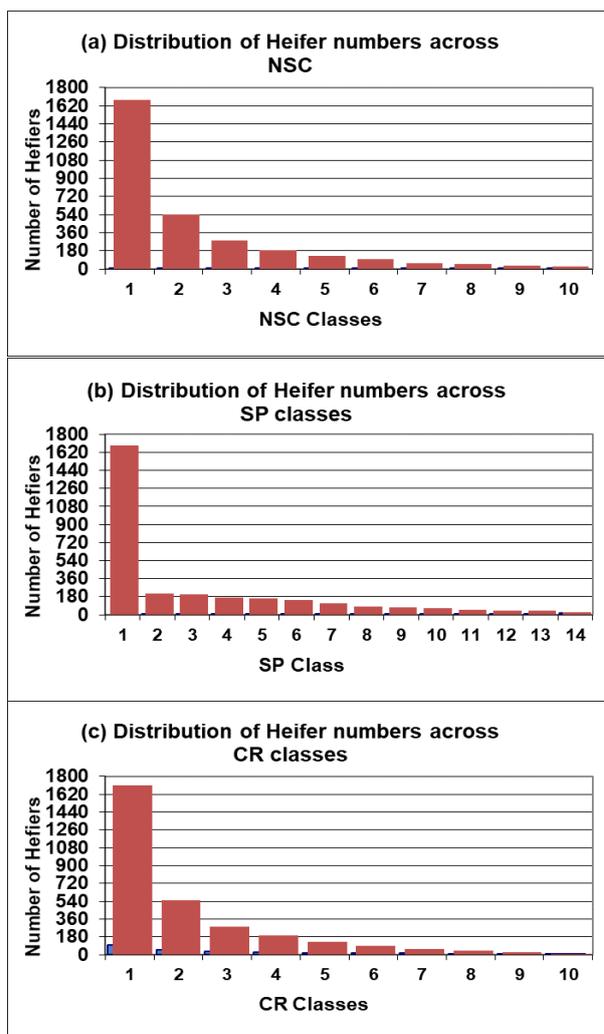


Figure 2. Distribution of heifer numbers in (a) NSC, (b) SP and (c) CR classes.

Factors affecting fertility traits:

Table 3 presents the SAS Type III mean squares and P values. On the factors listed in Table 3, farm had the largest mean square (P<0.0001) for NSC and it was the second most important main effect (P<0.006) for SP, however it was non significant (P=0.33) for CR. EIKarada farm was the best for NSC, CR and SP (1.86, 71.99% and 39.9 d, respectively) than Saka farm (2.3, 67.94 and 48.2 d, respectively). The variation of NSC and SP from one farm to another could be attributed to differences in skills of heat detection. Therefore, an intensive program of heat detection and practices of insemination may significantly reduce the NSC and shorten SP. Age of heifer at breeding (ASB) had the largest MS (P<0.0001), in terms of magnitude of impact on both CR and SP (Table 3), however it was the second important factor for NSC (P<0.0001). Year of first breeding had a significant (P<0.002) effect on NSC (P<0.0001) CR (P<0.002) and SP (P<0.0001) as shown in table (3). The year of first breeding effect are the result of the interaction of a set of environmental, technical and administrative management practices makes its interpretation difficult, however, it is importance source of variation that must be considered in the statistical analysis in order to get clear interpretation of results (Amino *et al.*, 2006). Month of first breeding had a

significant effect on NSC ($P < 0.01$) and SP ($P < 0.004$), however it was non significant on CR (table 3).

Effect of age at first breeding (AFB) on NSC, SP and CR were shown in Figure (3).

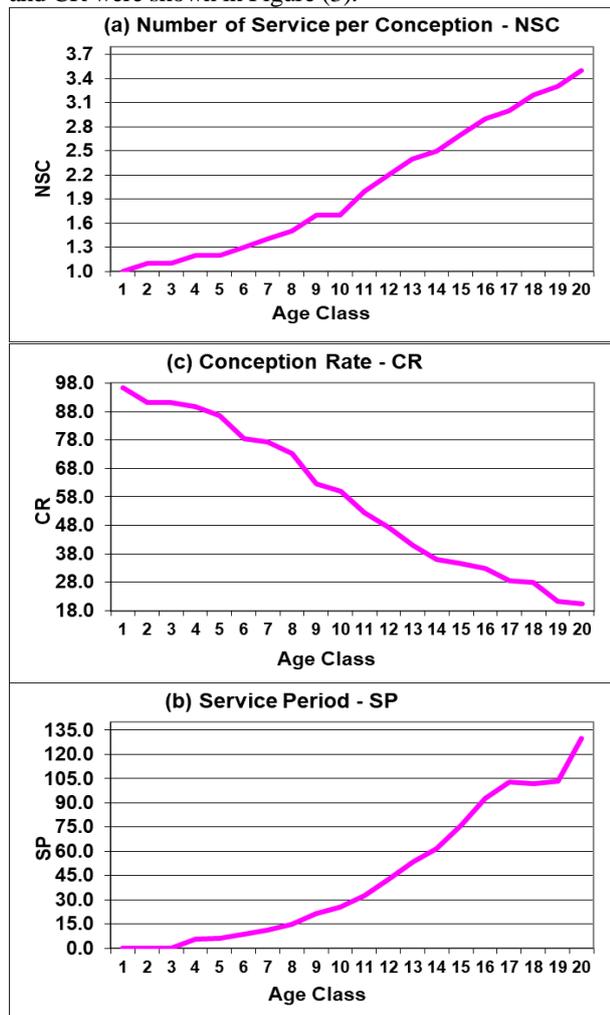


Figure 3. Distribution of heifers across AFB for (a) NSC, (b) SP and (c) CR.

NSC and SP increased as AFB increased, however CR decreased by increasing AFB. Increasing AFB by one month was associated with increased NSC and SP by 0.132 service and 6.8d (i.e., a unfavorable positive effect of increasing AFB on NSC and SP), however CR decreased by 3.99% (unfavorable negative effect of increasing AFB on CR). Youngest heifer at first breeding (13 mo.) needed only one service to be pregnant with zero SP and CR was near to 100%. In contrast, oldest heifer (31 mo.) in AFB needed 3.5 services to be pregnant, SP reached 129.9d and CR decreased to 20.4%. Heifers with low AFC have expressed good fertility in order to conceive and calve at a young age (Eastham *et al.*, 2018). Kuhn *et al.* (2006) reported that breeding heifers at 15-16 mo. of age maximized CR (56.8%), however CR was lower not only for breedings at <14 mo. of age (53.3%) but also for breedings at >26 mo. (48.1%). The most pronounced effect of age was a 5 to 10% lower CR for heifers bred at 26-27 mo. of age (Kuhn *et al.*, 2006). Nilforooshan and Adriss (2004) concluded that due to negative effects of age at first calving on productive life, the reduction of age at first calving to 24 mo. of age could be an effective management

practice. Perhaps these lower means at older ages are not age effects per se but rather resulted from breedings to late-maturing heifers, heifers that were subfertile for other reasons, or that the older heifers may have become overconditioned (Kuhn *et al.*, 2006). Losinger and Heinrichs (1996) demonstrated production to be lower when age at first calving is greater than 27 mo., and concluded that time to breed heifers should be based on body weight rather than age. A combined feeding and breeding management program is necessary for optimal results (Gardner *et al.*, 1988).

Table 3. Linear model mean squares (MS) and P-values for heifer fertility traits .

Source ¹	d.f.	MS	P>F
Number of service per conception (NSC)			
F	1	66.3	<0.0001
AFB	18	58.8	<0.0001
F*Y1B	34	4.5	<0.0001
Y1B	34	3.5	<0.0001
M1B	11	3.2	0.01
F*Y1B*M1B	258	1.7	0.01
Y1B*M1B	364	1.5	n.s.
Conception rat (CR)			
AFB	18	5.1	<0.0001
F*Y1B	34	0.38	0.0006
Y1B	34	0.35	0.002
M1B	11	0.24	0.23
F*Y1B*M1B	258	0.23	0.03
Y1B*M1B	364	0.21	0.15
F	1	0.18	0.33
Service period (SP)			
AFB	18	162068.8	<0.0001
F	1	17630.0	0.006
Y1B	34	9198.3	<0.0001
F*Y1B	34	7655.9	<0.0001
M1B	11	5916.3	0.004
F*Y1B*M1B	258	3533.4	<0.0001
Y1B*M1B	364	3110.4	0.0002

1: F = farm, AFB= age at first breeding, F*Y1B= farm by year of first breeding interaction, Y1B= year of first breeding, M1B= month of first breeding

Month effects (Table 4) were somewhat sporadic in that there was not a clear, definitive pattern across months. April and May were the best months for NSC (1.37-1.41), CR (72.0-72.8%) and SP (33.6-35.0 d), however August and September were the worst months for the same traits (2.3-2.4, 63.2-65.2% and 55.1-55.9 d, respectively). In contrary, Andersen-Ranberg *et al.* (2005) reported that months May, Jun, July and August (Summer months) showed high values for non-return to 56-d rate in heifers. Ron *et al.* (1984) reported highest heifer CR in February (67%) but CR in July was 65.4%.

To further assist the interpretation of month effects, four 3-mo seasons were defined: 22 Dec. to 21 May (Winter season), 22 May to 21 Jun (Spring season), 22 Jun to 21 Sep. (Summer season) and 22 Sep to 21 Dec. (Autumn season). The spring season had the best means for NSC (1.41), CR (71.2%) and SP (37.8 d), however summer season had the worst means for the same traits (2.8, 66.2% and 50.1 d, respectively (Table 4). The summer season clearly has adverse effects on heifer fertility traits. Kuhn *et al.* (2006) in United states reported that April had the highest CR and August the lowest. The same authors added that the spring season

had the highest CR whereas the summer season had the lowest CR. Donovan et al. (2003) using Florida herd found that CR was 23% lower for heifers during the summer months than during winter.

Table 4 . Effect of farm and month of birth on heifer fertility traits.

Trait	No.	NSC (No.)	CR (%)	SP (d)
Farm				
Saka	1495	2.30±0.06 ^a	67.94±1.76 ^a	48.2±1.25 ^a
ElKarada	1583	1.86±0.05 ^b	71.99±1.81 ^b	39.9±1.01 ^b
Month of First Breeding				
Jan.	307	2.10±0.120 ^{bc}	70.04±3.99 ^{ab}	45.6±2.60 ^{ab}
Feb.	313	2.05±0.116 ^{bc}	72.81±4.12 ^a	44.5±2.52 ^{ab}
Mar.	343	2.07±0.112 ^{bc}	70.52±3.81 ^{ab}	44.2±2.39 ^{ab}
Apr.	288	1.37±0.115 ^c	71.55±4.20 ^{ab}	38.4±2.26 ^b
May	258	1.41±0.122 ^c	72.81±4.46 ^{ab}	33.6±2.09 ^b
Jun	218	2.10±0.142 ^{bc}	70.39±4.77 ^{ab}	44.0±2.98 ^{ab}
July	196	2.13±0.152 ^{bc}	66.97±4.78 ^{abc}	45.0±3.21 ^{ab}
Aug.	203	2.30±0.161 ^{ab}	65.16±4.57 ^{bc}	55.1±3.86 ^a
Sep.	211	2.39±0.165 ^a	63.18±4.35 ^c	55.9±3.85 ^a
Oct.	203	1.97±0.138 ^c	72.37±5.08 ^a	40.7±2.86 ^b
Nov.	258	1.98±0.123 ^c	72.53±4.52 ^a	39.5±2.46 ^b
Dec.	280	2.05±0.123 ^{bc}	70.17±4.19 ^{ab}	45.1±2.70 ^{ab}
Season of First Breeding				
Winter	975	2.24±0.067 ^b	70.72±2.2 ^a	45.9±1.48 ^{ab}
Spring	797	1.41±0.070 ^b	71.23±2.52 ^a	37.8±1.34 ^c
Summer	617	2.80±0.09012 ^a	66.23±2.66 ^b	50.1±2.02 ^a
Autumn	707	2.03±0.0765 ^b	71.21±2.68 ^a	42.9±1.62 ^{bc}

^{a, b, c} subclass means followed by different subscripts are significantly differed (P<0.05).

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شكل توزيع صفات الخصوبة في العجلات

أناس عبد السلام أبو العنين بدر
معهد بحوث الإنتاج الحيواني وزارة الزراعة الدقي مصر

تم تجميع بيانات التناسل لعجلات الفريزيان المصرية من سنة 1979 وحتى سنة 2013 وذلك لدراسة شكل توزيع صفات الخصوبة (العمر عند أول تلقيح، العمر عند التلقيح المخصبة، العمر عند أول ولادة، عدد التلقيحات اللازمة للإخصاب، نسبة الخصوبة ومدة التلقيح)، وكذلك لفحص تأثير العوامل البيئية على تلك الصفات. الهدف الثاني من تلك الدراسة هو مقارنة الأعمار المختلفة للعمر عند أول تلقيح وتأثيرها على صفات الخصوبة في العجلات. كان شكل التوزيع لصفات العمر عند أول تلقيح، العمر عند التلقيح المخصبة والعمر عند أول ولادة متماثل وأقرب ما يكون للتوزيع الطبيعي (الشكل الناقوسي) بينما كان التوزيع غير متماثل (شكل حرف L) في صفات عدد التلقيحات اللازمة للإخصاب، نسبة الخصوبة وفترة التلقيح. تحويل البيانات باستخدام الجذر التربيعي كان أفضل في تخفيض معامل الاختلاف في صفات عدد التلقيحات اللازمة للإخصاب ونسبة الخصوبة بينما كان التحويل باستخدام اللوغاريم أفضل في صفة فترة التلقيح. تحتاج العجلات الصغيرة في العمر عن أول تلقيح تلقيح واحدة للإخصاب محققة نسبة 100% خصوبة وأقصر فترة للتلقيح.