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ASSESSMENT OF ARTIFICIAL INSEMINATION AND INFLUENTIAL VARIABLES OF FERTILITY IN CATTLE USING NEGATIVE BINOMIAL REGRESSION

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ABSTRACT

Fertility in cattle is an important trait for the profitability of dairy farms. Some factors affecting fertilization in small and medium-sized dairy farms in Adıyaman and Şanlıurfa provinces located in the Southeastern Anatolia region of Turkey were discussed in this study. The reproductive yield, which is the dependent variable, and the breed of cattle that affect the fertility, and disease were included in the model as categorical variables, while the duration of experience for artificial insemination specialists in the profession and artificial insemination practice were included in the model as continuous variables. The distribution of fertility data was Poisson, however the comparison of mean and variance ($\mu<\sigma^2$) indicated an overdispersion in the data ($p<0.05$). Therefore, the parameters of the criteria affecting the fertility were estimated using NBR model. The fertility rate was low (55%) in study area and the breed (16%) was an important impact on the fertility. The results revealed that the effects of Holstein and Simmental cattle breeds on fertility was higher compared to the local breeds (46.8% and 50.6%, respectively). The fertility decreased 13.6% in cattle with disease symptoms. The race, disease, working time of the specialist and professional experience in the practice had significant effects on fertility in artificial insemination. In addition, the results revealed that the NBR model can be evaluated effectively and easily with the Stata statistical program in case of overdispersion of categorical data such as conception level.

Keywords: Fertility, Overdispersion, Count data, Poisson and Negative binomial regression

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INTRODUCTION

Milk production depends directly on fertility; therefore, fertility and milk yield are the two important parameters affecting the profitability in dairy cattle farms (Ensminger, 1980; Jorjani, 2006; Liu *et al*., 2008; Naya *et al*., 2017; Toledo-Alvarado *et al*., 2017; Craig *et al*., 2018). Success in modern dairy cattle farms is can be achieved by maintaining the fertility at a high level. A cow raised in a farm is expected to give the first birth at about 2-year-old and continues to give birth once in a year (Kaygısız *et al*., 2008; Titterington *et al*., 2015; Van Eetvelde *et al*., 2020). Many studies have been carried out on the milk yield and fertility criteria (Albarrán-Portillo and Pollott, 2013; Kaygısız *et al*., 2015; Berry *et al*., 2016). However, studies investigating the relationship between fertility and milk yield are very rare (Kumuk *et al*., 1999; Yıldız *et al*., 2008; Gürses *et al*., 2014).

In artificial insemination, the conception yield of dairy cattle depends on environmental factors, genetic and physiological characteristics such as body condition, disease, ration and nutrition, farmer's carelessness, age, breed, milk yield, etc. (Varışlı and Akyol, 2018; Ealy and Seekford, 2019; Madureira *et al*., 2020). In addition, the experience of the specialist performing artificial insemination is also an important factor for the conception yield.

Artificial insemination and fertility data including dependent and independent variables can be continuous random, as well as discrete, categorical or count structure. Since the count data in a type of "*how many of the cows who have come to rut in an enterprise have conceived as a result of artificial insemination*" is a discrete variable it can be analyzed using the Poisson approach. In the Poisson approach, the relationship between the mean and variance of variables is important for an accurate decision in model selection. The parameters of the Poisson regression are usually estimated by the maximum likelihood method which only provide consistent results under the assumption of equidispersion (E (μ) = E (σ^2)). However, overdispersion of the dependent variable is often considered as E (μ) < E (σ^2). Inconsistencies caused by overdispersion can be eliminated by calculating robust standard errors. Creating a probability distribution that displays more dispersion than the Poisson distribution is an alternative approach to overcome overdispersion. In this case, negative binomial distribution (NBR) is obtained by assuming that the Poisson parameter has a gamma-distribution (Böhning, 1998; Joe and Zhu, 2005). Since the dependent variable has a categorical characteristic, Poisson and negative binomial regression models were used for evaluation and interpretation in this study. Model parameters obtained were used to estimate the influence ratios of the factors affecting the fertility of the cows.

Some tests were applied to determine the overdispersion encountered in the data sets. An application using alternative NBR model was carried out to overcome the overdispersion problem. In addition, progeny loss (or fertility problem) in the study area is a serious problem, therefore, the model parameters were evaluated using appropriate regression methods. Since fertility-related problems cause losses in milk yield and fertility, the factors affecting fertility were examined in the study.

MATERIALS AND METHODS

Material: The data used in the study were obtained from small and medium-sized dairy farms located in Adiyaman and Sanliurfa provinces of Turkey. The data consisted of the records kept in 2020 by veterinarians engaged in artificial insemination. Simmental, Holstein, and local breeds are dominated in the region. The information on dependent and independent variables used in the models is summarized in Table 1.

Table 1. Dependent and independent variables.

Variables	Parameter	Explanation	Data of the variable
Dependent (Y)	Number of Conception	Total number of pregnant cattle per season in the randomly selected farms	1960
Independent **(X_1)**	Number of artificial inseminations	Total number of artificial inseminations performed by physicians in a season (discrete variable)	3563
(X_2)	Working time	Working time (month) of the veterinarian performing artificial insemination (continuous variable)	40 - 256 months
(X_3)	Cattle disease	Diseases affecting fertility of cows during artificial insemination (categorical variable)	Not sick=0 (Reference) sick=1
(X_4)	Breeds of cows	Breeds of cows inseminated artificially (categorical variable)	Local breed = 1 (Reference) Holstein = 2 Simmental = 3

The number of pregnant cattle by artificial insemination was considered as a dependent variable (Y_i). Other variables (number of artificial inseminations, working time of physicians, the status of disease, and the type of breed) affecting the fertility were included as independent variables (X_i) in the model.

METHOD

Poisson distribution: Events that occur in a given time period and an area, show the Poisson distribution. The most obvious feature of the Poisson distribution is that its mean and variance are equal to each other. The probability density function for the Poisson Maximum Likelihood method is given in Equation 1:

$$f(y_i|x_i) = \frac{e^{-\mu_i}\mu_i^{y_i}}{y_i!} \qquad y_i = 0,1,2,.... \quad (1)$$

The mean parameter is expressed as an exponential mean function as shown in Equation 2.

$$E(y_i|x_i) = \mu_i = \exp(x_i'.\beta) \quad (2)$$

The logarithm of the conditional mean gives the parameters linearly. Therefore, the conditional mean is also defined as a log-linear function as expressed in Equation 3 (Berk and MacDonald, 2008; Cameron and Trivedi, 2013).

$$ln\ (y_i|x_i) = \mu_i = x_i'.\beta \quad (3)$$

Poisson regression model: Poisson regression analysis explains the relationship between count dependent variables and independent variables. The dependent variable takes categorical values (such as Y = 0,1,2,…, n), while there is no limitation for the independent variables. The connection function connecting the linear structure of explanatory variables to the expected value of the response variable is obtained by logarithmic transformation (Tempelman and Gianola, 1996; Çelik and Durmuş, 2020). The disadvantage of the Poisson regression model is being an exponential model, which may cause some difficulties and confusions in the interpretation of the coefficient. However, the observation values are not subjected to any transformation, and they can be interpreted with their original units.

The logarithm of Poisson distribution mean (μ), is a natural function of independent variables, thus, the related function is given under the Poisson distribution assumption as follows:

$$\log(\mu) = b_0 + b_1x_1 + \cdots + b_mx_m \quad (4)$$

Where; μ, is an exponential function of independent variables, and μ can be written as:

$$\mu = \exp(b_0 + b_1x_1 + \cdots + b_mx_m) \quad (5)$$

In the Equation, the Poisson mean and independent variables are associated with the log function (Yeşilova *et al*., 2006).

Negative binomial regression (NBR) model: Poisson regression can only make accurate estimates under the assumption of equidispersion. In practice, the state of overdispersion ($\sigma^2 > \mu$) is encountered more frequently than the equidispersion ($\sigma^2 = \mu$) (Berk and MacDonald, 2008; Hilbe, 2014; Beaujean and Morgan, 2016). In the case of overdispersion, either the Poisson regression model is estimated by calculating robust standard errors, or NBR. The binomial regression is a special case of the Poisson regression model and makes more consistent parameter estimates under overdispersion (Mert, 2016). Negative binomial regression is given in Equation 6:

$$\Pr(Y = y_i) = \frac{\Gamma(y_i+v^{-1})}{y_i!\Gamma(v^{-1})}\left(\frac{v^{-1}}{v^{-1}+\mu_i}\right)^{v^{-1}}\left(\frac{\mu_i}{v^{-1}+\mu_i}\right)^{y_i}, \alpha > 0,\ y = 0, 1, \ldots \quad (6)$$

In the equation, v is the ratio of index to dispersion parameter. The probability function of the dependent variable in the Poisson regression is expressed as:

$$y \sim \text{poisson}(y|\ \mu) = \exp(x'.\beta) \quad (7)$$

Where; x is the vector of independent variables and μ shows the expected value and variance. In overdispersion, the distribution of dependent variable to obtain more consistent estimators is written as:

$$y \sim \text{poisson}(y_i|\ \mu\ ) \quad (8)$$

Here, the distribution of dependent variable (Y) is $v \sim \text{Gamma}(1, \alpha)$, thus it has a negative binomial. Since E(v)=1 and Var(v)=α, the E(Y)=μ and Var(Y)=$\mu(1+\mu v)$ for the dependent variable. The equal dispersion is achieved when $\alpha = 0$, and the expected value of the dependent variable will be equal to its variance. However, the variance will be greater than the mean (E(Y)< Var(Y)) due to the overdispersion (if α>0). Here, α can be defined as an overdispersion parameter and can be used as a test criterion in determining whether the distribution has overdispersion or equidispersion (Mert, 2016).

Model fit criteria: The goodness of fit of the regression line fitted to a data set is considered as a measure of how well the predicted regression line fits the data in linear regression models (Gujarati and Porter, 2009). Akaike Information Criterion (AIC) proposed by Akaike (1974) and Bayesian Information Criterion (BIC) developed by Schwarz (1978) were used to fit the estimated models.

Steps analysis in Stata software: Data were analyzed using Stata statistical software (Stata, 2018). Initially, estimates of the parameters for the Poisson regression analysis under the assumption of equal distribution of variance were tested. The results were obtained by defining and executing the following commands in Stata:

poisson Y X_1 X_2 i.X_3 i.X_4, nolog

The X_3 (disease) and X_4 (breed) were qualitative variables, therefore, the "i." was added as suffix to X_3 and X_4 to derive dummy variables. In calculation of the maximum likelihood value; the term "nolog" was added at the end of the command line to avoid seeing the results of iterative steps. Similarly, the parameters of NBR were estimated using the following command line.

nbreg Y X_1 X_2 i.X_3 i.X_4, nolog

RESULTS AND DISCUSSION

The histogram chart related to the distribution of conception data is given in Figure 1. Due to the variation between the observation values, the data show an extreme right skewed distribution. Normal distribution assumption required for the linear regression model cannot be achieved (p<0.005) even with logarithmic transformation. Gbur *et al*. (2012) also reported that log transformation could not eliminate the skewness of such data.

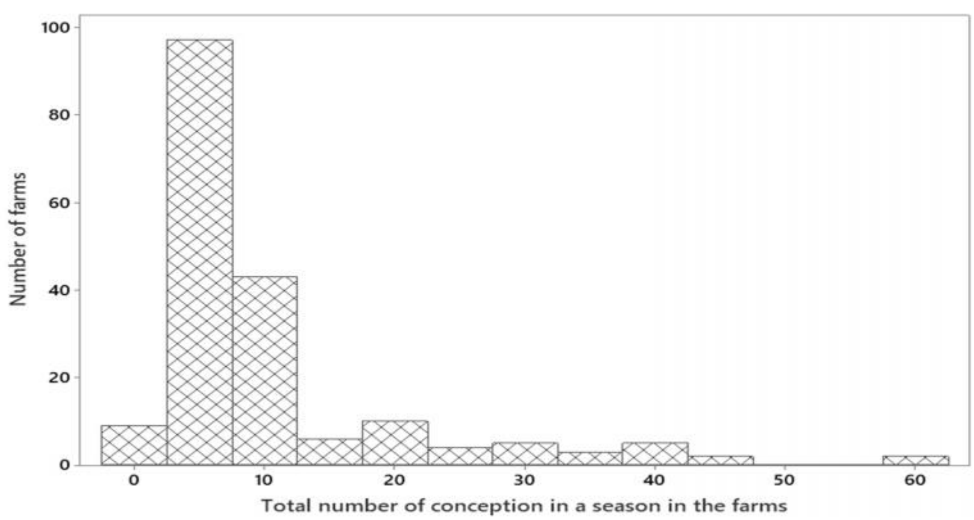

Figure 1. Histogram for the number of conception (Y) in 186 dairy farms

Descriptive statistics for dependent and independent variables for 186 dairy farms located in Sanliurfa and Adiyaman provinces are given in Table 2.

Table 2. Descriptive statistics for dependent and independent variables.

Variable	Breeds of Cows	Farm number	Mean	Std. Error	Std. Dev.	Variance	Total Breed	Percent
Number of Conception (Y)	1	88	5.273	0.22	2.027	4.11	464	0.237
	2	58	14.45	1.67	12.72	161.80	838	0.428
	3	40	16.45	2.07	13.11	171.87	658	0.336
Total	Cattle	186	10.54	0.78	10.66	113.55	1960	1.000
Artificial insemination (X_1)	Not sick. 0	94	14.86	1.45	14.04	197.12	1397	0.392
	Sick. 1	92	23.54	2.37	22.75	517.56	2166	0.608
Total		186	19.16	1.42	19.3	372.61	3563	1.000
Working time (X_2)		69	87.04	5.08	42.17	1778.63		

A total of 3563 artificial inseminations were carried out by 69 veterinarians in 2020 and the number of conceptions was determined as 1960 (55%). The conception rate in the study area is within the risk limit according to the criteria reported by Ata (2013). The conception rates reach to 75% in the western provinces where cultural breeds are common. Unfortunately, the conception rate is much lower in eastern and southeastern provinces of Turkey (Yıldız *et al*., 2008). Low conception rate in the region can be attributed to the local and hybrid breeds of cattle raised in small family farms (Aydemir and Pıçak, 2007). In addition, malnutrition due to the degraded pastures, insufficient veterinary control and lack of careful monitoring during estrous cycling periods have been reported as the other causes of low conception rates in the region. The ratio of local breeds among the conceived breeds of cattle was 23.7%, Simmental breed was 33.6% and Holstein breed was 33.6% (Table 2). The results indicated that 60.8% of the 3563 cattle that were artificially inseminated showed disease symptoms. Metabolic diseases that negatively affect fertilization are oviduct obstructions, abnormal ova, ovarian adhesions, endometritis, and some infectious such as Urea plasma, Mycoplasma, Haemophilus, Brucellosis (Özkoca, 1986; Zobel *et al*., 2011; Nasimento-Rocha *et al*., 2017).

The fertility (Y) variable (that is the number of conception) shows the fitness to the Poisson distribution (Figure 1). However, the mean and variance values are expected to be equal ($\mu = \sigma^2$) in the Poisson distribution. The mean fertility was ($\mu = 10.54 < \sigma^2 = 113.55$) was smaller than the variance (Table 2). This might cause the problem of overdispersion. overdispersion status was checked by obtaining Alpha dispersion test criteria with the NBR model.

Although categorical data based on counting such as the number of artificial inseminations shows Poisson distribution, overdispersion was encountered in the applications. Therefore, alternative regression model such as negative binomial, which gives more consistent parameter estimates under overdispersion should be used. The parameters defined as log functions must be interpreted after linearization when the estimated regression model is not linear. Parameter estimates and goodness of fit statistics for Poisson and NBR models are given in Table 3.

Table 3. Parameter estimates and goodness of fit statistics for Poisson and Negative binomial regression models.

Variables	Poisson Regression			Negative Binomial Regression		
	Coef.	Std. Err.	P	Coef.	Std. Err.	P
X_1	.0218	.0011	<0.001	.0226	.0013	<0.001
X_2	.0034	.0004	<0.001	.0036	.0005	<0.001
X_3 (1)	-.1515	.0514	0.003	-.1346	.0561	0.017
X_4						
(2)	.4310	.0681	<0.001	.4095	.0725	<0.001
(3)	.4343	.0710	<0.001	.3844	.0819	<0.001
Constant	1.239	.0604	<0.001	1.2084	.0676	<0.001
/LNALPHA				-4.5906	.6732	
ALPHA				.01014	.0068	

LR test of alpha=0: chibar^2(01) = 3.07 *Prob >= chibar^2 = 0.040*

The NBR command (***nbreg Y X_1 X_2 i.X_3 i.X_4, nolog***) was written on the command line to determine if the overdispersion provides the test result for the alpha dispersion parameter (α) (Mert, 2016). The null hypothesis of overdispersion parameter of equal to zero was tested against the greater than zero as an alternative hypothesis. The likelihood ratio test was used to test the hypothesis. Test statistic of likelihood ratio test conforms to the existence of chi-square distribution with 1 degree of freedom. The likelihood ratio test statistic value and significant level were 3.07 and 0.04 respectively. Said the results indicated that the assumption of equidispersion in the Poisson distribution could not be achieved and there is overdispersion in the data.

The "fitstat" and "estat ic" commands must be entered to obtain goodness of fit statistics after estimating regression models. Some of the fit statistics are summarized in Table 4. The AIC, BIC and Log Likelihood (LL) values were examined to compare the fit of the model. The model with the smallest AIC and BIC and the largest LL value was preferred.

The comparison of results for the goodness of fit criteria for NBR analysis and the Poisson regression revealed that the NBR model provided a better fit to the data. The fit criteria of NBR (AIC, BIC and LL) were smaller than the fit criteria of the Poisson model, which indicated that the negative binomial produced a better fit to the data (Table 4). In addition, the likelihood ratio (LR chi-square) value of the NBR model was 370.49. The model appears statistically significant (P<0.001). The LL value in the NBR model was higher than the LL in Poisson regression model (PR= -433.53, NBR= -431.99). This supports that the NBR model is better. Therefore, interpretations were performed based on the results of negative binomial model.

Table 4. Some fit statistics for the Poisson and Negative binomial regressions.

Fit Criteria	Poisson Regression	P	Negative Binomial Regression	P
LR chi-square (df:5)	1253.26	<0.001	370.49	<0.001
AIC	879.07		**877.90**	
BIC	900.50		**898.40**	
Pseudo R^2	0.59		0.30	
Mcfadden's R^2	0.59		0.30	
Log Likelihood (LL)	-433.53		**-431.99**	

Pseudo R^2 is used in count data and is not equal or close to 1, which indicates a high level of explanation. Low value of pseudo R^2 does not mean that the model is insignificant or insufficient (Mert, 2016). The Pseudo R^2 value in the Poisson regression was 0.59 and 0.30 in the NBR.

The data on the predicted model fits obtained by typing "estat gof" on the command line are given in Table 5. P values in Deviance and Pearson statistics, were 0.9837>0.10 and 0.989>0.10, respectively. the results indicated that the H_0 hypothesis, which states that the model is fit, can be accepted. That is:

H_0: The Model fits the data.
H_1: The Model does not fit the data.

Table 5. Goodness of fit statistics for the prediction model.

Goodness of fit statistics	DF	Test statistics	P	Interpretation
Deviance	180	141.8426	0.9837	Model fits
Pearson chi-square	180	139.3837	0.9890	Model fits

The interpretation for the coefficients of the parameters given in Table 3 is somewhat difficult, therefore, the Incident Rate Ratio (IRR), which is an exponential form of the Poisson regression coefficient ($e^{coefficient}$) was calculated. On the comment line of Stata software, the commend of "poisson, irr" and "nbreg, irr" were written and executed to obtain IRR values for both models (Table 6).

Table 6. IRR statistics for Poisson and Negative binomial regression models.

Variables	Poisson Regression			Negative Binomial Regression		
	IRR	St. Err.	P	IRR	St. Err.	P
X_1	1.0220	0.00110	<0.001	1.0220	0.0013	<0.001
X_2	1.0034	0.00047	<0.001	1.0036	0.0005	<0.001
X_3(1)	0.8593	0.04420	0.003	0.8740	0.0491	0.017
X_4						
X_4(2)	1.5390	0.10480	<0.001	1.5060	0.1092	<0.001
X_4(3)	1.5440	0.10970	<0.001	1.4680	0.1203	<0.001
Constant	3.4540	0.20860	<0.001	3.3480	0.2265	<0.001

IRR: Incident rate ratios; St. Err.: Standard error

The IRR will be higher than 1 (IRR > 1), when the coefficients of Poisson and NBR models are positive. In contrast, the IRR will be less than 1 (IRR <1), when the coefficients are negative. The effect of independent variable on the dependent variable will be positive when the IRR > 1. The effect of independent variable on the incident number ratio (dependent variable) will be negative, when the IRR < 1 (Mert, 2016). The IRR value for all coefficients of both regression models was significant ($p<0.01$), except the coefficient of the $X_3(1)$ parameter in the NBR model that was significant 5% level ($P=0.017$).

Parameters belonged to NBR are obtained from the exponential function, and the effectiveness of the coefficients for parameters were interpreted using IRR. One-unit increase in the number of artificial insemination results in a 1.022 increase (2.2%) in the conception yield (fertility) of cattle (Table 5). The skills are improved with the experience of a practitioner in field. The results showed that the contribution of veterinarians who practiced more artificial inseminations to fertility was greater. The results are in accordance with the findings of Müller *et al*. (2020). One-month increase in the working time of a veterinarian in the profession resulted in a 0.36% increase in the fertility of cattle. The result showed that the contribution of veterinarians who perform many artificial inseminations to fertility (2.2%) is higher compared to the contribution of veterinarians who spend longer working time (0.36%). Müller *et al*. (2020) stated that experienced experts who have gained experience by doing more artificial insemination are needed reduce the fertility losses and increase yield.

The disease was a dummy variable, and the condition of having symptoms of any disease was defined as "$X_3(1)$". Cattle without symptoms of disease were accepted as the reference value. Accordingly, the IRR coefficient for the disease in the NMR model was 0.874, which indicates that cattle carrying disease will cause 13.6% (1-0.874) more fertility loss than healthy cattle. The results reported by Wathes *et al*. (2020) who investigated the effects of viral disease on fertility of dairy cattle in a large-scale environment are in agreement with our findings

Negative value of (-0.1346) predicted parameter "$X_3(1)$" for the disease variable indicates a decreasing impact on the fertility (Table 3).

Genetic factor is one of the most important factors affecting the fertility of dairy cattle. The differences in fertility between breeds were examined. Local dairy breeds of cattle "$X_4(1)$" was used as the reference value (Table 3). The fertility of Simmental, defined as "$X_4(2)$", and Holstein, defined as "$X_4(3)$" was 50.6 and 46.8% higher than the local breeds of cattle (Table 5).

All the cows and heifers in a herd may not conceive in the first insemination (Ata, 2013; Kim and Jeong, 2019). The conception rate in the first insemination should not fall below 50%, and this rate should increase over 70% in the 2nd and 3rd inseminations, respectively. The fertility in the study area was quite low (55%). The mean pregnancy rate in Şanlıurfa province reported by Kutlu and Varışlı (2012) is coincide with our findings. The results clearly revealed that genetic factor is the most influential factor on fertility. The findings reported in the previous studies carried out in the region (Gürses and Bayraktar, 2012; Kutlu and Varışlı, 2012; Kaygısız *et al*., 2017; Varışlı and Akyol, 2018) support the findings of our study. The results of NBR model revealed that the effects of breed factor on fertility was higher (16%) than all the other factors, and followed by the disease experienced (13.6%), duration of experience in artificial insemination (2.2%) and the duration of veterinarians working time in profession (0.34%). Estrous cycling time is a very important explanatory factors, however, we could not include the estrous cycling time in the model because the veterinarians did not record any information about it.

Conclusion: In the study, the mean value of the dependent variable was much lower than the mean value of the variance (10.54<113.55). Negative binomial regression model was preferred in parameter estimates based on the model fit criteria (AIC = 877.9 and BIC = 898.4). The fertility of the region was low (55%) and the breed factor (16%) was determined as the most influential factor on fertility. The fertility of Holstein and Simmental cattle was 46.8 50.6% higher than the fertility of local breeds. The results finally revealed that the disease causes a 13.6% decrease in fertility of cattle. The experience of expert veterinarians in artificial insemination has a positive effect (2.2%) on reproductive fertility.

The increase in fertility of cattle can be achieved by increasing the populations of cultural breeds. In addition, awareness of the producers on the appropriate treatment of cattle against diseases should be increased. Furthermore, the hand skills and practices of the veterinarians who perform artificial insemination have to be increased to increase the success in artificial insemination.

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