An Observational Analysis of Secondary Sex ratio, Stillbirth and Birth Weight in Iranian Buffaloes (*Bubalus bubalis*)

N. Ghavi Hossein-Zadeh[1*], M. Madad[1], A. A. Shadparvar[1], and D. Kianzad[2]

ABSTRACT

Calving records of Iranian buffaloes from April 1991 to June 2010 comprising 1,151 herds with 34,911 calving events were used to evaluate reported secondary sex ratio, stillbirth, and calf birth weight. Logistic regression models were applied to analyze stillbirth and calf sex at birth, and statistical analysis of calf birth weight was performed using a linear mixed model. Overall, the ratio of males to females was 53:47. It was observed that there were no significant effects of herd, calving year, season of calving, dam parity or interactions between these effects on the odds of male or female rates in Iranian buffaloes. Greater odds of calf stillbirth existed for calves born from primiparous buffaloes than from multiparous ones (Odds Ratio (OR)= 1.83; P< 0.0001). The greatest odds of stillbirth was for spring season (OR= 2.47; P< 0.0001), and male births had greater odds of stillbirth than female ones in Iranian buffaloes (OR= 1.21; P< 0.01). In general, male calves were heavier than the female calves at birth (P< 0.01) and the birth weights of calves from cows of parity 4 and beyond were significantly more than the weights of calves from cows of other parities (P< 0.01). Fall-born calves had significantly greater body weight at birth than calves born in other seasons (P< 0.01). It seems that providing good management practices for primiparous and multiparous buffaloes to minimize stress before parturition can reduce stillbirth incidence.

Keywords: Birth weight, Iranian buffalo, Sex ratio, Stillbirth.

INTRODUCTION

There is documented evidence that water buffalo production has been practiced in Iran since 2500 B.C. Some archeological evidence suggests that water buffaloes have been domesticated in Iran and migrated to southern Europe through this region. Although the ancestry of Iranian buffaloes is not clearly known, it has been proposed that the main progenitors of these animals are Indian buffaloes such as Murrah and so, because of the phenotypic similarity. According to climate conditions, Iranian buffaloes can be classified into three main groups: (i) Azari ecotype (Western and Eastern Azarbaijan); (ii) North ecotype (Guilan and Mazendaran) and (iii) Khuzestan ecotype (Khuzestan). Iranian water buffaloes have also some similarity to Iraqi buffaloes (Tavakolian, 2000). Both groups might be originated from the same ancestor. Furthermore, Iranian buffaloes in northwest of the country (West Azarbaijan), have close resemblance to Mediterranean water buffaloes. Thus, it is thought that they have descended from the same ancestor. There are about 480,000 water buffaloes in Iran. Most of these animals are kept in the south and northwest. All of the Iranian buffaloes are riverine (Naserian and Saremi, 2007).

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[1] Department of Animal Science, Faculty of Agricultural Science, University of Guilan, Rasht, Islamic Republic of Iran.
* Corresponding author, e-mail: nhosseinzadeh@guilan.ac.ir
[2] Animal Breeding Center of Iran, P. O. Box 31585-963, Karaj, Islamic Republic of Iran.

Optimum fertility of breeder buffaloes is the key to dairy economics. Sound reproductive rhythm in each buffalo is essential for ensuring regularity of calving with a narrow dry period to have profitable dairy farming. Stillbirth has been recognized as the most important factor compromising the future reproductive life of the animal.

Animals that had stillbirth had significantly increased risk of culling/death throughout the lactation (Bicalho *et al.*, 2007). Also, economic losses from stillbirth include not just the lost calf, the reduced survival, and increased days open, and increase in days to first breeding but also the decreased milk yield (Bicalho *et al.*, 2008; Maizon *et al.*, 2004). Management of reproductive health for optimization of reproductive performance warrants critical evaluation of the baseline information regarding its various determinants. On the other hand, calf birth weight information is used as an indicator trait for calving ease in animal selection to minimize the risk of dystocia.

Secondary sex ratio is the sex ratio at birth. Most traits can be manipulated effectively in breeding programs, but gender is a special case for which animal breeders simply have to accept the probability that 50% of each bovine conception will result in a male or female calf. But, hypotheses relating fitness to skewed sex ratios have been developed for various organisms and types of social organization. The most notable is the Trivers–Willard hypothesis (Trivers and Willard, 1973) which suggested that females of polygynous species could increase their fitness by skewing the sex ratio of their offspring.

Although factors affecting the stillbirth, secondary sex ratio and calf birth weight have been studied among various breeds of cattle, little is known about the causative factors in buffaloes. Therefore, the objective of this study was to determine factors affecting the secondary sex ratio, stillbirth and calf birth weight in Iranian buffaloes from 1991 to 2010.

MATERIALS AND METHODS

Dataset

Calving records from April 1991 to June 2010 comprising 34,911 calving events from 1,151 buffalo herds of Iran were included in the data set. Information for individual calving events, including herd identification, animal identification, calving date, parity, multiple births, calf sex, calf birth weight and stillbirth were included in the data set. Twin births accounted for 53 of the total observations (0.15%) and were discarded from the analysis. Calf sex was coded as 1 for males and 2 for females. Also, stillbirth was coded as 0 for calves born alive and 1 for calves born dead. Stillbirth was defined as calf death at birth or 24 hours after birth. Months of birth were grouped into four seasons: January to March (winter), April to June (spring), July to September (summer), and October to December (fall). Also, calving years were grouped into four classes: 1991-1995, 1996-2000, 2001-2005 and 2006-2010.

The buffalo farming system in Iran is based on smallholders (99 percent); most of the herds have an average of five animals; a few herds have between 20 and 50 buffaloes and some of them have 300 buffaloes. Smallholders manage their animals according to the opportunities offered by the environment: on pasture, stubble, shrubs and grass. Most of them obtain their feeding by grazing along water sources: streams, rivers, ponds, lakes, integrated with the following products: citrus peels and pulp, sugar cane wastage, etc. In Khuzestan, buffaloes are raised outdoors throughout the year but in the north-west they are housed in the fall and winter. Buffalo farming in Iran can be considered to be at a good level since the owned or rented properties are of a large size and the land available for buffalo farming is also extensive. Buffalo farming has been a traditional activity for many decades (Kianzad, 2000).

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Statistical Analysis

Logistic regression models were used to analyze stillbirth and calf sex at birth in Iranian buffaloes using the maximum likelihood method of the LOGISTIC procedure of SAS 0.9 (SAS Institute, 2002). Model specification was based on the backward elimination method and the fit of all statistical models was evaluated by using the Hosmer and Lemeshow goodness-of-fit test of SAS (Hosmer and Lemeshow, 2000) by including the "lackfit" option in the model statement. Variables (main effects or interaction terms) which were significant by the Wald statistic at $P < 0.05$ were included in the model. The general equation of logistic regression model was defined as follows:

$$\text{Logit}(\pi) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n$$

Where, π was the probability of stillbirth or calf sex at birth; α was the intercept parameter; β_1 to β_n were the logistic regression coefficients (parameter estimates) for the explanatory effects (X_1 to X_n) included in the statistical model. Statistical analysis of calf birth weight was performed using the MIXED procedure of SAS (SAS Institute, 2002), with herd as a random effect. Differences among least-squares means were tested using Tukey's adjustment method.

The final model used to analyze stillbirth included the fixed class effects of herd, calving year, calving season, parity of dam, calf sex and the interaction effects of calving year by calving season, calving year by parity and calving season by parity. The final model used to analyze calf birth weight included the fixed class effects of calving year, calving season, parity, calf sex and the interaction effects of calving year by calving season, calving year by parity, calving year by calf sex and calving season by calf sex. The initial model of analysis for secondary sex ratio included the fixed class effects of herd, calving year, parity, calving season and their interaction effects, but all of these effects were found non-significant and were excluded from the final model of analysis for calf sex at birth.

RESULTS AND DISCUSSION

Table 1 shows the number and percentage of male and female births by parity of dam, year of calving and season of calving in Iranian buffaloes. Overall, the ratio of males to females was 53.0:47.0. In general, it was observed that there were no significant effects of herd, calving year, season of calving, dam parity or interactions between these effects on the odds of male or female rates. Several factors have been proposed to influence sex ratio, including vaginal and

Table 1. Reported secondary sex ratios for Iranian buffaloes by parity of dam, calving year and calving season.

Effect	Class	Male births		Female births		Total	
		N	%	N	%	N	%
Parity of dam	1	2800	54.1	2379	45.9	5179	100
	2	2780	53.8	2386	46.2	5166	100
	3	2624	53.1	2314	46.9	4938	100
	≥4	10286	52.4	9342	47.6	19628	100
Calving year	1991-1995	132	45.7	157	54.3	289	100
	1996-2000	4042	52.5	3650	47.5	7692	100
	2001-2005	8421	53.7	7248	46.3	15669	100
	2006-2010	5895	52.3	5366	47.7	11261	100
Calving season	Spring	3259	54.1	2766	45.9	6025	100
	Summer	6830	52.6	6151	47.4	12981	100
	Fall	6666	52.6	6013	47.4	12679	100
	Winter	1735	53.8	1491	46.2	3226	100

uterine pH, and maturity of the oocyte and time of artificial insemination (AI) (Pursley *et al.*, 1998). Contrary to the current results, Naqvi and Shami (1999) reported that male sex ratio was higher in spring as compared to summer in Nili-Ravi buffaloes, but similar to the results of this study, they reported a non-significant effect of parity on the sex ratio. Visscher *et al.* (2004) observed non-significant effects of year and maternal lactational status on fetal sex ratio in the African buffalo and fetal sex ratio was 50.8:49.2 in favor of males. As shown in Table 1, the ratio of males to females varied from 52.4:47.6 to 54.1:45.9, 45.7:54.3 to 53.7:46.3 and 52.6:47.4 to 54.1:45.9 across the parities, calving years and seasons of calving, respectively. According to Trivers–Willard hypothesis (Trivers and Willard, 1973), the benefit of a skewed sex ratio would occur when a female could maximize her fitness through her offspring, in accordance with her present condition and the external constraints of the environment. Mothers in good condition, therefore, are expected to produce sons while those in poorer condition are expected to produce daughters.

The overall incidences of stillbirth by parity of dam, calving year and season of calving are shown in Table 2. Also, estimated odds ratios, and parameter estimates for the effects of calving year, parity of dam, calving season and calf sex on the stillbirth of Iranian buffaloes are reported in Table 3. The overall incidence of stillbirth was 12.8% and varied from 10.6% to 15.2% across the parities. The effect of herd was significant on the stillbirth (P< 0.0001). Greater odds of calf stillbirth existed for calves born from primiparous buffaloes than from multiparous ones (Odds Ratio (OR)= 1.83; P <0.0001). Therefore, stillbirth rate was the highest for first calving buffaloes, partly because of a disproportion between the size of the calf and the pelvic area, which causes a difficult calving and increases stillbirth parturition incidence (Steinbock *et al.*, 2003; Hansen *et al.*, 2004). The overall incidence of stillbirth ranged from 0 to 24.5% over the years and the odds of stillbirth increased from 1991 to 2010 (OR= 1; P<0.0001). Also, the overall incidence of stillbirth ranged from 10.9 to 14.4% across the seasons and the greatest odds of stillbirth was for spring season (OR= 2.47; P< 0.0001). In general, male births had greater odds of stillbirth than female ones (OR= 1.21; P< 0.01). This effect could be probably due to greater body size of male calves than female ones at birth. The calving year by parity interaction effect was significant for the model of analysis for stillbirth and the odds of stillbirth was the greatest for the combination of calving year 2006-2010 and primiparous buffaloes (P< 0.0001). Also, the calving season by parity interaction effect was significant for the model of analysis for stillbirth and the odds of stillbirth was the greatest for the

Table 2. Reported stillbirths for Iranian buffaloes by parity of dam, calving year and calving season.

Effect	Class	Alive births		Dead births		Total	
		N	%	N	%	N	%
Parity of dam	1	3087	84.8	555	15.2	3642	100
	2	2808	89.4	334	10.6	3142	100
	3	2675	89.2	324	10.8	2999	100
	≥4	10560	86.9	1587	13.1	12147	100
Calving year	1991-1995	148	100	0	0	148	100
	1996-2000	4756	99.6	19	0.4	4775	100
	2001-2005	8309	90.6	860	9.4	9169	100
	2006-2010	5917	75.5	1921	24.5	7838	100
Calving season	Spring	3790	86.4	599	13.6	4389	100
	Summer	6646	89.1	816	10.9	7462	100
	Fall	6605	85.6	1113	14.4	7718	100
	Winter	2089	88.5	272	11.5	2361	100

Table 3. Estimated odds ratios, and their 95% Confidence Intervals (CI) and parameter estimates for the effects of calving year, calving season, parity of dam, and calf sex on the stillbirth of Iranian buffaloes from 1991 to 2010.

Effect	Class	Estimate±SE	Odds ratio	95% CI	Type 3 *P*-value
Intercept	1	11.19±39.98	-	-	0.78
Calving year	1	-3.34±1.17	0.002	0.001-0.05	<0.0001
	2	-1.62±0.42	0.01	0.007-0.02	
	3	2.16±0.41	0.53	0.44-0.63	
	4	-	1		
Calving season	Spring	0.36±0.09	2.47	1.84-3.32	<0.0001
	Summer	0.04±0.07	1.80	1.36-2.37	
	Fall	0.16±0.07	2.02	1.55-2.64	
	Winter	-	1	-	
Parity of dam	1	0.55±0.08	1.83	1.50-2.24	<0.0001
	2	-0.33±0.09	0.76	0.60-0.96	
	3	-0.17±0.08	0.89	0.72-1.12	
	≥4	-	1	-	
Calf sex	1	0.09±0.04	1.21	1.05-1.38	0.008
	2	-	1	-	

combination of spring season and primiparous buffaloes (P< 0.0001). In addition, the odds of stillbirth was the greatest for the combination of the effects of calving year 2006-2010 and spring season (P< 0.0001). Herd managers should apply the most appropriate calving procedures to assure that suitable timing and calving assistance techniques are used when providing assistance during parturition. Furthermore, providing a good environment for primiparous and multiparous buffaloes to minimize stress before parturition can reduce stillbirth incidence.

Least-squares means and their standard errors for calf birth weight in Iranian buffaloes are given in Table 4. The average birth weight of calves was 32.68 kg. In general, male calves were heavier than the female calves at birth (P< 0.01) and the birth weights of calves from cows of parity 4 and beyond were significantly more than the weights of calves from cows of other parities (P< 0.01). Fall-born calves had significantly greater body weights at birth than calves born in other seasons (P< 0.01). The results of the present study indicated that the weights of calves born in calving years 2001-2005 and 2006-2010 were significantly greater than the birth weights

of calves born in other years (P< 0.01). There was a significant parity by year interaction effect on calf birth weight and calves born in calving year 2006-2010 and from buffaloes in their parity 4 and beyond had greater birth weights than other combinations (P< 0.01). There was a significant season by year interaction effect on calf birth weight and calves born in year 2006-2010 and fall season had greater birth weight than other calves (P< 0.01). Also, buffaloes calved in calving year 2006-2010 and in their fourth and beyond parities had calves with greater birth weight (P< 0.01). In addition, there were significant interaction effects of calving year by calf sex and calving season by calf sex on birth weight, and male calves born in calving year 2006-2010 or fall season had greater birth weight than other calves (P< 0.05). Consistent with our results, Abdelaziz *et al.* (2010) reported that calf birth weight increased from the first parity (27.68 kg) up to the sixth (36.03 kg) in the Egyptian buffaloes. Also, Sethi (2003) reported average birth weight of 27.9 kg for Toda buffaloes in India which was lower than the average birth weight of Iranian buffaloes. Usmani *et al.* (1987) reported that calf birth weight averaged 38.2 kg and ranged from 17 to 48 kg in Nili-Ravi

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Table 4. Least-squares means and their standard errors for calf birth weight in Iranian buffaloes [a].

Effect	Class	N	Calf birth weight
Calving year	1991-1995	148	31.79 ± 0.47^b
	1996-2000	4756	31.87 ± 0.08^b
	2001-2005	8309	32.91 ± 0.06^a
	2006-2010	5917	33.16 ± 0.08^a
Calving season	Spring	3790	31.70 ± 0.09^d
	Summer	6646	32.76 ± 0.07^b
	Fall	6605	33.31 ± 0.07^a
	Winter	2089	32.57 ± 0.12^c
Calf sex	Male	10165	33.40 ± 0.06^a
	Female	8965	31.95 ± 0.06^b
Parity	1	3087	31.26 ± 0.11^d
	2	2808	32.50 ± 0.11^c
	3	2675	32.70 ± 0.11^b
	≥ 4	10560	32.92 ± 0.05^a

[a] Least-squares means within a column that do not have a common superscript (a–d) are significantly different (P< 0.05).

buffaloes and similar to the current results, they observed that bull calves were heavier at birth than were heifer calves (39.0 vs. 37.5 kg).

CONCLUSIONS

The overall incidence of stillbirth is the highest for first calving buffaloes. The odds of stillbirth increased from 1991 to 2010 and buffaloes calved in fall had the greatest rates of stillbirth compared with other seasons. Also, male births had greater odds of stillbirth than female ones in Iranian buffaloes. In general, male calves were heavier than the female calves at birth and the birth weights of calves from cows of parity 4 and beyond were significantly more than the weights of calves from cows of other parities. Fall-born calves had significantly greater body weight at birth than calves born in other seasons. It seems that providing good management practices for primiparous and multiparous buffaloes to minimize stress before parturition can reduce stillbirth incidence. In general, the results of the present study indicated that the increase in the rate of male births in buffaloes was concurrent with the increase in calf birth weight and stillbirth over the

recent years. Greater body weights of male calves at birth could be a risk factor for dystocia occurrence in buffaloes; therefore, providing assistance at calving for buffaloes carrying male calves may reduce complications associated with dystocia and may reduce economic losses by reducing the incidence of neonatal calf mortality or stillbirth.

REFERENCES

1. Abdelaziz, M. A., Shalaby, N. A. and Al-Hur, F. S. 2010. Comparison between Genetic Parameters for Birth Weight of Egyptian Buffaloes Estimated by Random Regression and Multi-trait Models. *Proceedings of the 9th World Congress on Genetics Applied to Livestock Production*, Leipzig, Germany, http://www.kongressband.de/wcgalp2010/assets/pdf/0175.pdf

2. Bicalho, R. C., Galvão, K. N., Cheong, S. H., Gilbert, R. O., Warnick, L. D. and Guard, C. L. 2007. Effect of Stillbirths on Dam Survival and Reproduction Performance in Holstein Dairy Cows. *J. Dairy Sci.*, **90**: 2797-2803.

3. Bicalho, R. C., Galvão, K. N., Warnick, L. D. and Guard, C. L. 2008. Stillbirth Parturition Reduces Milk Production in Holstein Cows. *Prev. Vet. Med.*, **84**: 112-120.

4. Hansen, M., Misztal, I., Lund, M. S., Pedersen, J. and Christensen, L. G. 2004. Undesired Phenotypic and Genetic Trend for Stillbirth in Danish Holsteins. *J. Dairy Sci.*, **87**: 1477–1486.

5. Hosmer, D. and Lemeshow, S. 2000. *Applied Logistic Regression.* 2nd Edition, Wiley-Interscience, John Wiley and Sons, New York, NY, Pp.392.

6. Kianzad, D. 2000. A Case Study on Buffalo Recording and Breeding in Iran. *ICAR Tech. Ser.*, **4**: 37-44.

7. Maizon, D. O., Oltenacu, P. A., Gröhn, Y. T., Strawderman, R. L. and Emanuelson, U. 2004. Effects of Diseases on Reproductive Performance in Swedish Red and White Dairy Cattle. *Prev. Vet. Med.*, **66**: 113-126.

8. Naqvi, A. N. and Shami, S. A. 1999. Secondary Sex Ratio in Nili-Ravi Buffalo. *Pak. J. Biol. Sci.*, **2(3)**: 1030-1033.

9. Naserian, A. A. and Saremi, B. 2007. Water Buffalo Industry in Iran. *Ital. J. Anim. Sci.*, **6 (Suppl 2)**: 1404-1405.

10. Pursley, J. R., Silcox, R. W. and Wiltbank, M. C. 1998. Effect of Time of Artificial Insemination on Pregnancy Rates, Calving Rates, Pregnancy Loss, and Gender Ratio after Synchronization of Ovulation in Lactating Dairy Cows. *J. Dairy Sci.*, **81**: 2139-2144.

11. SAS Institute. 2002. *User's Guide: Statistics, Version 9.1 Edition.* SAS Inst., Inc., Cary, NC.

12. Sethi, R. K. 2003. Improving Riverine and Swamp Buffaloes through Breeding. *Proc. of the Fourth Asian Buffalo Congress,* 25 to 28 Feb., New Delhi, India, PP. 51-60.

13. Steinbock, L., Nä sholm, A., Berglund, B., Johansson, K., and Philipsson, J. 2003. Genetic Effects on Stillbirth and Calving Difficulty in Swedish Holsteins at First and Second Calving. *J. Dairy Sci.*, **86**: 2228–2235.

14. Tavakolian, J. 2000. *An Introduction to Genetic Resources of Native Farm Animals.* Anim. Sci. Res. Inst., Karaj. Iran, Pp.451

15. Trivers, R. L. and Willard, D. 1973. Natural Selection of Parental Ability to Vary the Sex Ratio of Offspring. *Sci.,* **179**: 90 –92.

16. Usmani, R. H., Lewis, G. S., and Naz, N. A. 1987. Factors Affecting Length of Gestation and Birth Seight of Nili-Ravi Buffaloes. *Anim. Reprod. Sci.,* **14(3)**: 195-203.

17. Visscher, D. R., van Aarde, R. J., and Whyte, I. 2004. Environmental and Maternal Correlates of Foetal Sex Ratios in the African Buffalo (*Syncerus caffer*) and Savanna Elephant (*Loxodonta africana*). *J. Zool., Lond.,* **264**: 111–116.

تجزیه مشاهداتی نسبت جنسیت ثانویه، مرده‌زایی و وزن تولد گوساله در گاومیش-های ایران

ن. قوی حسین زاده، م. مدد، ع. ا. شادپرور و د. کیانزاد

چکیده

رکوردهای زایش گاومیش‌های ایران از آوریل ۱۹۹۱ تا ژوئن ۲۰۱۰ مشتمل بر ۱۱۵۱ گله و ۳۴۹۱۱ رکورد زایش برای ارزیابی نسبت جنسیت ثانویه، مرده‌زایی و وزن تولد گوساله مورد استفاده قرار گرفت. مدل‌سازی تابعیت لجستیک برای تجزیه مرده‌زایی و جنسیت گوساله در زمان تولد و تجزیۀ آماری وزن تولد گوساله از طریق یک مدل خطی مختلط صورت گرفت. به طور کلی، نسبت نرها به ماده‌ها در گاومیش‌های ایران ۵۳ به ۴۷ بود. مشاهده شد که آثار گله، سال زایش، فصل زایش، شکم زایش مادر یا آثار متقابل بین این اثرات بر احتمال نرخ ماده‌زایی یا نرزایی گاومیش‌های ایران معنی‌دار

نبودند. احتمال زیادتر مردهزایی برای گوسالههای متولد شده از مادران شکم اول زایش نسبت به مادران چندشکم زایش مشاهده شد (احتمال خطر (OR)=1.83؛ $P<0.0001$). بیشترین احتمال مردهزایی در فصل بهار بوده ($OR=2.47$؛ $P<0.0001$) و تولدهای نر احتمال مردهزایی بیشتری نسبت به تولدهای ماده در گاومیشهای ایران داشتند ($OR=1.21$؛ $P<0.01$). به طور کلی، گوسالههای نر در هنگام تولد وزن بیشتری نسبت به گوسالههای ماده داشته ($P<0.01$)، و وزن تولد گوسالههای مربوط به مادران شکم زایش ۴ و بالاتر بهطور معنی داری بیش از وزن گوسالههای مربوط به مادران شکمهای زایش پایینتر بود ($P<0.01$). گوسالههای متولد شده در فصل پاییز وزن تولد بالاتری در مقایسه با سایر فصول داشتند ($P<0.01$). به نظر میرسد که فراهم کردن روشهای مدیریتی مناسب برای گاومیشهای شکم اول و بالاتر جهت به حداقل رساندن تنش قبل از زایش میتواند سبب کاهش وقوع مردهزایی شود.