



Effect of variable interservice interval on conception in crossbred cows

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ABSTRACT

Reducing number of services per conception holds the key for profitable dairying. The widely considered estrous cycle length of 21 d has been refuted, a change that can seriously implicate fertility. The present investigation evaluated association amongst interservice interval (ISI, the period intervening two successive inseminations) and services per conception alongwith certain cow related variables (age, lactation number, days in milk to AI, days in milk at conception, age at conception and 305 d lactation yield). Data on 768 ISIs (range: 17 to 24 d) from 1,479 AIs (600 in normal; 879 in repeat breeders) in 193 lactating Jersey crossbred cows conceiving to different number of inseminations, was utilized. The ISI data in form of average, immediately prior to conception and pattern (regular – if ISI between AIs was of same duration; irregular – if ISI between AIs was of different durations) was considered for analysis. Nearly 84% of ISI ranged from 19 to 22 d, merely 22.6% being of 21 d (overall average of 20.3 ± 0.44 d). Correlation matrix revealed a solitary, significant and negative relation of ISI, both average and prior to conception, with services per conception. The ISI of ≥ 21 d culminated consistently into more number of pregnant cows as well as lesser services per conception. The forward selection regression model predicted reduction in ISI by 0.17 d with each additional insemination. Irregular pattern of ISI was also unfavourable for conception. The probable mechanisms for variable ISI in light of conception have been exhaustively elaborated, which could pave potential future studies aiming reduced reproductive wastage.

Keywords: Conception, Crossbred cows, Interservice interval

Reproductive performance determines the profitability from dairy cows (Le Blanc 2007). Number of services per conception is indicative of fertility (Cavestamy and Galina 2001). Conception failure generates an interservice interval (ISI), which is extremely helpful in monitoring and management of fertility as also an improvement of estrus detection or its prediction. The ISI ranges from 18 to 24 d (Forde *et al.* 2011) which, however, is widely considered to be of 21 d in multiparous and 20 d in nulliparous cows (Sartori *et al.* 2004). However, a recent study abroad indicated increase in ISI by one day in multiparous cows (22 d) and nulliparous heifers (21 d) (Remnant *et al.* 2015). Alteration of ISI by one day can affect conception (Bleach *et al.* 2004) and could therefore be a potential reason for reduced fertility worldwide over last few decades. The present investigation revisited ISI and its relation to conception and some other variables in crossbred cows.

MATERIALS AND METHODS

The herd data was collected from Jersey-upgraded cows

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(upto 75% exotic inheritance) raised in the University Dairy Farm of CSK Himachal Pradesh Agricultural University, Palampur, situated at a latitude 3206' N longitude 7603' E. The information was collected for 16 calendar years (2000 to 2015). The total number of cattle heads in the farm during the period of study ranged from 136 to 175.

Feeding and management: The feeding practice, on total dry matter basis, comprised of concentrates (one – third) and seasonally available roughages (two – third) fed as per the reproductive and lactation demands of cows (BIS: 2052). The cows were managed in loose housing system and had round the clock access to clean drinking water. Milking was done manually twice a day and lactation yield was recorded.

Breeding and reproduction indices: The cows in spontaneous estrus were inseminated as per AM – PM rule using 0.25 ml of frozen thawed certified semen. Pregnancy diagnosis was performed 90 d after A.I. A voluntary waiting period / days in milk postpartum (DIM) of atleast 40 d was the benchmark for first postpartum insemination. The cows with excessively prolonged DIM (exceeding normal lactation length) were excluded from data as outliers.

The information was collected for 1,479 AIs that generated 768 ISIs in 193 lactating cows. Out of 1,479 AIs, 600 were in normal cows (conceiving to ≤ 3 AIs; 369 cows conceived to first postpartum AI), while the remaining were

in repeat breeders (conceiving with > 3 AIs). Accordingly, the first service conception rate in the investigated herd was 51.8%. The range of modal ISI (days) considered in the present study was 17 to 24 d that was based on the work of Forde *et al.* (2011) as well as follicular dynamics investigations undertaken for 86 interovulatory intervals in 59 cows belonging to the same herd (Sood 2017). The ISI of 34 to 48 days (multiple of 17 to 24 day normal ISI), probably due to a missed or unobserved estrus after AI, and ISI shorter than 17 d, considered to be mid-cyclic estrus (Sood *et al.* 2009b) were also excluded. The selected cows were strictly ensured to have normal estrus duration (no repeated AI in same estrus) and not treated for any disease during the period of study. The cows enrolled in different fertility trials/ controlled breeding programs during the period of interest were also excluded.

It is pertinent to mention that all the cows included in the study eventually conceived, though to different number of inseminations. Accordingly, no ISI was available in the cows conceiving to first AI. The cows repeating once and conceiving to second AI generated one ISI, while the cows inseminated seven times produced six ISIs for analysis. During the course of inseminations, the cows getting conceived to any of the insemination (s) were designated as pregnant, if not, these were said to be non-pregnant. The average ISI (produced from cows receiving ≥ 3 AIs) and ISI immediately preceding conception (in cows receiving ≥ 2 AIs) were evaluated for their relation to S/C and other cow related factors comprising of lactation number (LN), days in milk to first postpartum AI (DIM), days in milk at conception (DIMC), age at conception (AC) and 305 d lactation yield (LY). The data pertaining to pregnant and non-pregnant cows was also analysed in relation to varying ISI intervals. The cows exhibiting more than two consecutive estrous cycles also yielded a pattern of ISIs. If the duration of ISI between different AIs in the same cow was similar, the ISI pattern in that cow was considered as regular; if not, the pattern was considered to be irregular. The ISI pattern was analysed for its relation to services per conception (S/C). The average (range) – age, LN, DIM and LY of the cows was 5.69 ± 0.08 years (1.7 to 12.6 years), 3.08 ± 0.06 (1 to 9), 105.43 ± 1.50 d (40 to 284 d) and 2254.00 ± 21.70 L (320 to 4395 L), respectively.

Statistics: The average values have been presented as Mean $\pm$ SEM. Pearson's correlation coefficient was determined between average ISI and S/C, LN, DIM, DIMC, AC and LY. As the average ISI exhibited a significant relation with S/C, the ISI prior to conception was also tested with all the aforesaid variables for strengthening of analysis and interpretation. Another analysis comprising of a forward selection regression model was designed to predict ISI in relation to service number. Different variables were added to the model sequentially and coefficient of determination (r^2), standard errors of the coefficient estimate variables with high r^2 value were retained in the model and were finally selected when the 95% confidence interval was greater than twice the standard error. Difference for

significance between two means was tested by Student's t-test, three or more means by Kruskal –Wallis H Test and difference in proportions by chi-square analysis. A difference of $P < 0.05$ (at least) was considered to be significant and $P = 0.10$ as a tendency for difference. The entire analysis was done using statistical package (SAS 9.3).

RESULTS AND DISCUSSION

Delayed return to normal ovarian cyclicity, attenuated estrus expression and increased services per conception are some ramifications of continuous genetic upgradation of cattle for increased milk yield (Walsh *et al.* 2011). Of late, a change in the ISI duration and its relation to different variables has been found in the cows raised abroad (Remnant *et al.* 2015). Lack of such studies in Indian cattle prompted us to undertake the present investigation.

Distribution of varying ISI is presented in Fig. 1 (Panel A). Considering the ISI range of 17 to 24 d, nearly 84% ISI ranged from 19 to 22 d. The overall average ISI was 20.3 ± 0.44 d, which was slightly less than the more widely accepted estrous cycle length of 21 d (Olds and Seath 1951) and even more short than estrous cycles longer than 21 d (Bleach *et al.* 2004, Sartori *et al.* 2004, Wolfenson *et al.* 2004, Remnant *et al.* 2015). Variation in estrous cycle length can be due to increased milk yield (Walsh *et al.* 2011) and breed differences (Britt 1995).

The correlation matrix of ISI with S/C and other variables is presented in Table 1. The ISI, both average (presented in a horizontal frame) and before conception (presented in a vertical frame), were negatively and significantly correlated to S/C, but not with the other variables. Another interaction worth mentioning was a negative correlation between DIM and S/C, suggesting that a shorter postpartum DIM does not favour conception, which otherwise improves with increase in days postpartum. This can be due to (i) a diminishing influence of negative energy balance with increase in postpartum interval, (ii) proportion of cows returning to normal cyclicity is higher in late lactation and (iii) more estrous cycles before AI are associated with higher conception rate (Tenhagen *et al.* 2003). A significant and positive correlation of LY with S/C (implying increase in S/C with increase in milk yield) also testifies the aforesaid explanation corroborating to available literature (Campos *et al.* 1994). Stepwise regression analysis (Table 2) reaffirmed a solitary and colossal interaction between ISI and S/C. Further application of forward selection model demonstrated a weak association of ISI with DIM and DIMC, both of which were eventually eliminated from the model due to no improvement in r^2 value. The step 1 of the forward regression predicted a reduction in ISI by 0.17 d for each additional service. A similar analysis in a recent study indicated a significant association between ISI and S/C (found in present study), DIM (weak association in present study) and LY (Remnant *et al.* 2015). Absence of a significant interaction between ISI and LY in present study may be due to absence of variation in LY between the cows (average 2254.00 ± 21.70 L; median 2152 L).

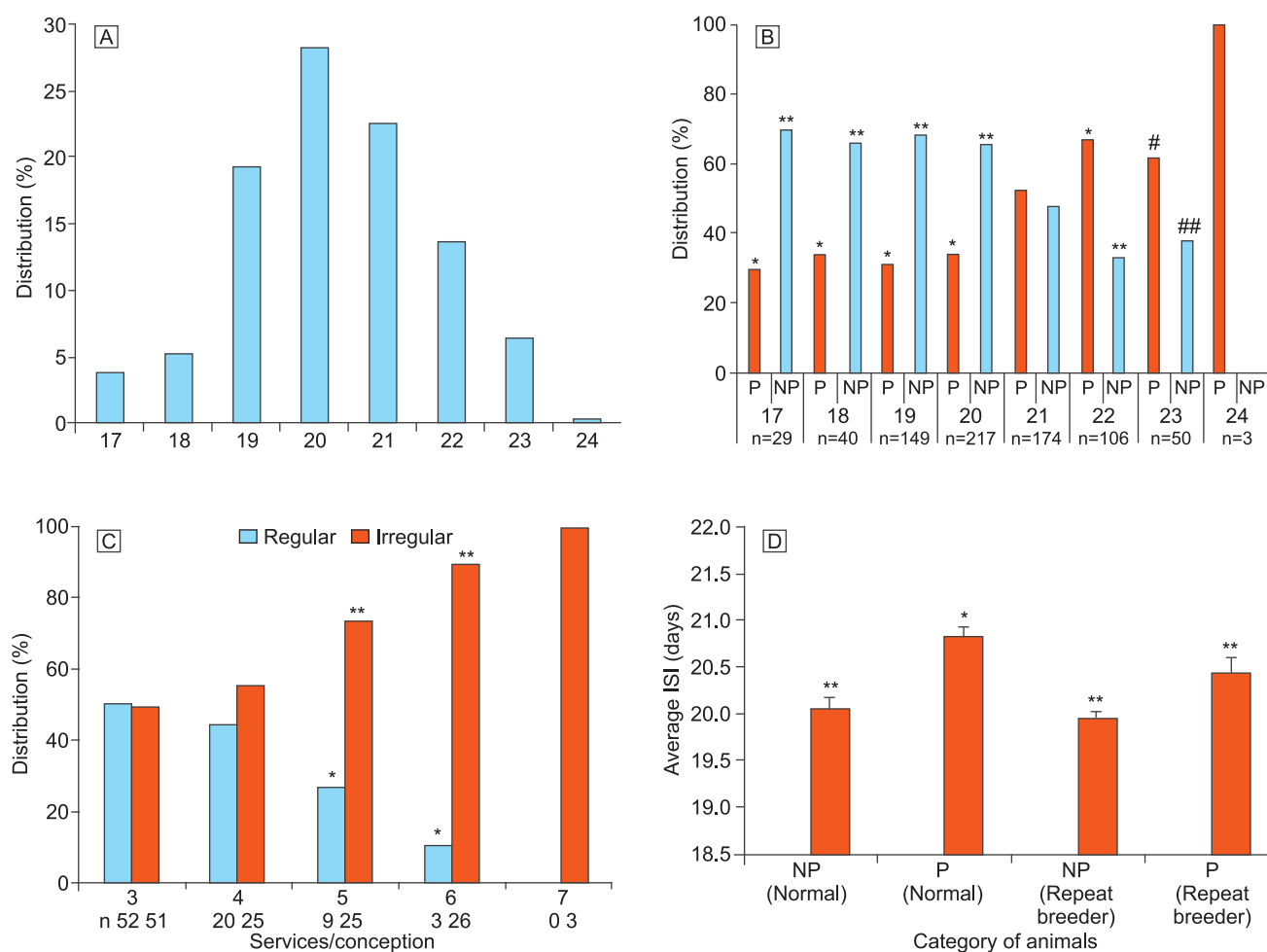


Fig. 1. Histograms showing distribution percentage of – interservice intervals (ISI) of different durations (Panel A); pregnant (P) and non pregnant (NP) cows exhibiting different interservice intervals (ISI) (Panel B); regular and irregular interservice intervals in relation to variable number of services per conception (Panel C) and average interservice intervals in pregnant (P) and non pregnant (NP) cows under normal and repeat breeding categories (Panel D). n is the number of cows in each category.

Single vs. paired asterisk between adjacent bars within same category denote a significant difference (* vs. ** as $P < 0.05$ at least and # versus ## as $P = 0.10$).

The proportion of pregnant and non-pregnant cows in relation to different ISIs is presented in Fig. 1 (Panel B). It is evident that the 17 to 20 d ISI displayed a greater proportion of non – pregnant than pregnant cows, whereas a subsequent increase in ISI of ≥ 21 d culminated consistently in higher proportion of pregnant than non – pregnant cows. A longer ISI interval and its impact on pregnancy can be attributed to number of follicular waves within an estrous cycle. Cows with 3-wave compared to 2-wave pattern of follicular growth had a longer estrous cycle length ($P < 0.01$) as well as higher pregnancy rate (81.0 vs. 63%), respectively. A reduced pregnancy rate in 2-wave cycles was believed to be due to older follicles shedding oocytes of reduced fertilization and development competence (Townson *et al.* 2002). Shorter interestrus intervals were more common during 2-wave cycles in a subset of Jersey crossbred cows belonging to the herd where the present study was conducted (Sood 2017) as well as in Holstein Friesian cows (Sood *et al.* 2009a).

The pattern of ISI (regular vs irregular) also had a

significant impact on S/C (Fig. 1, Panel C). The proportion of cows having regular or irregular ISI were almost similar in cows taking three S/C. However, the irregular ISI superseded the regular pattern in cows taking ≥ 4 S/C. It is inferred that inconsistent ISI is detrimental for conception or conversely more number of services are required in cows with irregular ISI. In confirmation, nearly one-third of the cows exhibiting at least one atypical cycle (irregular) had significantly poor fertility than the other cows with regular cyclicity (Remnant *et al.* 2015). Agreeing to the findings that the number of follicular waves dictate the length of estrous cycles as mentioned *vide supra*, the irregular ISI pattern may be due to switching of 2- and 3-follicular pattern between consecutive estrous cycles within same cows. The proportion of cows having inconsistent wave pattern ranged from 30 to 46% (Jaiswal *et al.* 2009, Sichtar *et al.* 2010) justifying a substantial variation between cycles within a cow than between the cows or herds (Remnant *et al.* 2015). On the contrary, we found a much lower inconsistent wave pattern of nearly 8% in Jersey crossbred cows (Sood 2017).

Table 1. Pearson's correlation coefficients between interservice interval (average in horizontal direction and before conception in vertical direction) and other variables in Jersey crossbred cows

Trait	ISI (BC)	S/C	LN	DIM	DIMC	AC	305 LY
ISI (Average)		-0.26**	-0.02	0.04	0.07	-0.08	-0.05
S/C	-0.15*		0.07	-0.16**	0.50**	0.33**	0.18**
LN	0.04			0.01	0.05	0.75**	0.09
DIM	-0.001				0.77**	-0.05	-0.08
DIMC	-0.04					0.16**	0.04
AC	-0.04						0.17**
305 LY	-0.02						

*and ** indicate a significant association between two variables across the corresponding row and column, respectively, at $P < 0.05$ and $P < 0.01$. (ISI, interservice interval; S/C, services per conception; LN, lactation number; DIM, postpartum days in milk at first AI; DIMC, days in milk at conception; AC, age at conception; 305MY, 305 day lactation yield).

Table 2. Stepwise multiple regression analytical model for predicting interservice interval in Jersey crossbred cows

Variable	Parameter estimate	Standard error	Pr>F	r ²
<i>Step 1</i>				
Intercept	21.28	0.210	< 0.0010	49.41
Service/conception	-0.17	0.060	0.0040	
<i>Step 2</i>				
Intercept	20.97	0.300	< 0.0010	51.41
Service/conception	-0.25	0.080	0.0019	
DIM	0.03	0.002	0.1372	
<i>Step 3</i>				
Intercept	25.31	0.380	< 0.0010	57.92
Service/conception	-4.36	0.290	< 0.0010	
DIM	0.21	0.014	< 0.0010	
DIMC	0.20	0.015	< 0.0010	

Hence, the unexplained variation in ISI within a cow from different studies indicates that the cycle length and therefore potentially the follicular wave number may be less consistent than thought. The findings of present study therefore suggest an inherent variability in cycle length for individual cows, which warrants further studies to evaluate the underlying physiological mechanisms. The other possible reason for ISI variation within same cows is embryonic mortality. Upto 80% of embryonic loss is sustained between day 8 and 16 after AI, which is the period prior to maternal recognition of pregnancy thereby not altering the length of estrous cycle (Sreenan and Diskin 1986). However, embryonic losses after day 16 delay the onset of subsequent estrus or extend the ISI in present context (Diskin *et al.* 2011). The latter phenomenon is also possible in present study. There was a gradual increase in the average ISI subsequent to conception failure for cows conceiving to 3 to 5 AIs; the respective ISI values in chronology being 20.07 ± 0.11 and 20.61 ± 0.12 d for 3 AIs ($P < 0.001$), 19.56 ± 0.18 , 20.16 ± 0.18 and 20.60 ± 0.25 d for cows consuming 4 AIs (first and third average differs; $P < 0.01$) and 19.44 ± 0.23 , 19.76 ± 0.22 , 20.32 ± 0.25 and 20.24 ± 0.26 d for 5 AIs (first differs from third and fourth value; $P < 0.05$), respectively. Whether the increasing ISI interval with

increase in number of S/C was a gradual adjustment, say immunological, for the developing embryo, needs to be ascertained. In contrast, the ISI pattern was slightly asymmetrical in cows conceiving to 6 AIs, even though the difference between some of the values was significant (19.17 ± 0.19 , 20.14 ± 0.26 , 20.52 ± 0.3 , 20.41 ± 0.3 and 20.45 ± 0.33 d; first average differs from third and fourth; $P < 0.05$). Such a trend was, however, missing for the cows conceiving to 7 AIs and the ISI between different AIs ranged from 20.00 ± 0.99 to 21.33 ± 0.66 d. Maternal recognition of pregnancy occurs between day 16 to 19 of estrous cycle (Sreenan and Diskin 1986), presumably for estrous cycles lasting for 21 d or more. This may be untrue and may occur much earlier in cows with a shorter ISI of 17 days or so. Hence, more conception failures in cows having shorter ISI (Fig. 1, Panel B) might be due to less available time for embryo at a relatively earlier development stage to signal its presence. The underlying endocrine mechanism may be an uterine exposure to progesterone for a shorter duration that allows premature secretion of $\text{PGF}_{2\alpha}$ (Cooper *et al.* 1991) to act as an embryotoxin (Breuel *et al.* 1993). The latter mechanism may be parallel to a much reduced fertility during first postpartum estrous cycles that are happen to be short (Perea *et al.* 1998).

The average ISI immediately preceding to conception (pregnant) or conception failure (non-pregnant) was compared between normal vs. repeat breeder cows (Figure 1, Panel D). Considering the average ISI prior to conception in normal pregnant cows, as benchmark, it was longer ($P < 0.05$ at least) than non-pregnant—normal and repeat breeder cows as well as pregnant repeat breeder cows. The overall average ISI between normal vs. repeat breeder cows was 20.60 ± 0.07 vs. 20.08 ± 0.7 d, respectively ($P < 0.01$), a difference of 0.52 d (or 12.48 h). The ISI preceding fifth AI or later has been predicted to be short by approximately 19.2 h than the interval between first and second insemination (Remnant *et al.* 2015). Two studies substantiate a shorter ISI in the repeat breeder cows. The time taken from exogenous $\text{PGF}_{2\alpha}$ administration to estrus onset (corroborates to proestrus) was 8.2 h ($P < 0.001$) and 19.8 h ($P < 0.05$) short in repeat breeder Israeli Holstein (Sood *et al.* 2015) and Jersey crossbred (Sood *et al.* 2016)

cows than their normal contemporaries. Extrapolating the findings of induced estrus to spontaneous estrus suggests a possibility of precocious estrus onset (or short proestrus) after endogenous release of $\text{PGF}_2\alpha$ in repeat breeders. A short proestrus may not only reduce ISI, but is also not good for conception (Xu *et al.* 1996, Bridges *et al.* 2010)

In conclusion, the present study brings to the fore that a blanket consideration of widely accepted ISI of 21 d becomes questionable. The variable ISI pattern characterised by a shorter duration and irregular pattern needs to be mitigated for fertility enhancement.

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