Interventions in mating strategies of broiler breeder during peak and post peak phase influence hatching traits

Muhammad Shabir Shaheen,[†,1] Shahid Mehmood,[†] Athar Mahmud,[†] Amjad Riaz,[‡] and Sohail Ahmad[†]

[†]*Department of Poultry Production, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan; and* [‡]*Department of Theriogenology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan*

ABSTRACT The study evaluated the effect of housing system, insemination frequency, and sperm concentration on hatching traits of commercial broiler breeder. Experiment was set up as $2 \times 4 \times 4$ factorial arrangement under completely randomized design. A total of 960 broiler breeder females (Ross-308) were divided evenly (480) into two groups for Artificial Insemination in cages (**AIC**) and on deep litter floor (**AIF**) with 41 and 48 males were allocated for aforesaid flocks, respectively. Females birds of both flocks (AIC and AIF) were further divided into 4 treatment groups to apply 4 various insemination frequencies at 4, 6, 8, and 10th days. These treated groups were further divided into 4 subgroups to apply each of insemination frequencies with 4 different sperm concentrations per insemination dose 100, 125, 150, and 175×10^6 sperms during peak phase of production which were replaced with 200, 225, 250, and 275×10^6 sperms in post peak phase. According to the results, significantly higher egg production, fertility, hatchability and number of chicks were documented when AI was conducted in cages as compared to deep litter floor. Although, the best reproductive performance was observed on 4 and 6th day insemination frequencies on all subjected sperm concentrations during peak; however, these parameters were found better on only 4th day during post peak. Sperms concentrations of 150, 175×10^6 during peak and 250 and 275×10^6 during post peak brought forth the best reproductive performance on all insemination frequencies. Although, embryonic mortality was significantly higher, when AI was conducted in floored flocks particularly when repeated after 4th day; however, various sperm concentrations found inert. In conclusion, AI found advantageous in caged flock as compared to floored. The consortium of different insemination frequencies and sperms concentrations are required for sustainable reproductive traits with progression of breeder age.

Key words: artificial insemination, housing systems, insemination frequencies, sperm concentration, hatching traits

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INTRODUCTION

Continuous revolutionary and scientific approaches in health care management along with effective breeding programs in commercial poultry enabled it to fetch a lion share in economy of many nations (Dhama et al., 2007). In addition to breeding goals, the housing systems are being corroborated as the most cogent factor in overall performance of a parent flock (**PS**) in progressive poultry sector (Campbell et al., 2019). Despite of some advantages and disadvantages, two housing systems i.e., floor and cage are being used globally (Valkonen et al., 2008) in commercial poultry. Although, deep litter system is common and cheaper housing system (Anonymous, 2016a), yet its dampness made it inappropriate bedding material which may negatively affect overall welfare and performance of birds (De Jong et al., 2014; Petek et al., 2014). However, floor rearing system as compared to cages, to some extent satisfies the natural behavior of the bird with lesser capital cost in consort with natural mating (**NM**) and artificial insemination (**AI**). Perhaps, it can be blemished for a bit higher ratio of dirty eggs than in cages. These dirty eggs can contaminate other clean eggs at farm and may lead to more exploder and dead in shell (**DIS**) in hatchery (De Reu et al., 2009). Cages not only maintain quality of eggs, especially the physicochemical properties (Gianenas et al., 2009), but also prevents the nutrient wastage by restricting movement of birds thus better production with less feed consumption has been noticed (Hetland et al., 2004). However, installation of cage system

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[1]Corresponding author: shabir.shaheen@uvas.edu.pk

escalates the capital cost (Valkonen et al., 2008) as well as the welfare requirements of birds are also being compromised (Matthews and Sumner, 2015).

It is suggested that reproduction technique is another important factor affecting production performance (Khazaei Koohpar et al., 2010). Where, artificial insemination (AI) has emerged as an evolutionary biotechnological valuable procedure where males are used more efficiently which might not be possible through natural mating (NM). As cockerel semen is highly concentrated and carries about $3-7 \times 100^9$ sperms/mL depending upon age, fleshing and health status (Gordon, 2005; Khazaei Koohpar et al., 2010). With AI, one male can serve up to 100 hens as compared to only 10 in case of natural mating (Villaverde-Morcillo et al., 2015; Mohan et al., 2017a). Now a days, AI is being extensively proceeded with freshly collected semen due to ease of collection and insemination at farm level (Kharayat et al., 2016; Santiago-Moreno et al., 2016) and exhibited far better results as compared to NM (Dhama et al., 2007). Actually, the worth of AI practice depends upon mainly on quality and quantity of spermatozoa along with insemination frequency (Tabatabaei, 2010; Beulah, 2017; Soliman and El-Sabrout, 2020).

An appropriate quantity of sperms at regular intervals is essential to maintain high level of fertility throughout the flock (Santiago-Moreno et al., 2016). Tabatabaei (2010) and Mohan et al. (2018) recorded 88% and 89% fertility in broiler PS when they inseminated 100×10^6 and 80×10^6 sperms, respectively. However, Beulah (2017) observed 94% fertility on 89×10^6 sperms/insemination during peak phase of breeder. Although, females need more semen of better quality after shorter intervals yet the deterioration of semen quality is quite common with the progression of its age (Douard et al., 2003; Slanina et al., 2015). Requirement of sperm concentration per insemination largely depends upon the age and body weight of a hen (Talebi et al., 2009). Conversely, production of sperms per ejaculation is influenced by body weight rather age of males (Prieto et al., 2011). The requirement of sperms/insemination increases with progression of PS can be attributed to some extent that more sperms are decayed in sperm host glands (proctodium) in older hens than younger ones. Therefore, higher concentration of spermatozoa/ insemination can ensure a greater safe margin for fertility (Mohan et al., 2017b). Some scientists are convinced that the reduction in egg production (short clutch) in post peak can be associated with poor fertility as ovulation induces stimulation for transportation of sperms from storage gland (Tumova and Gous, 2012).

AI usually resulted in better fertility; however, handling stress and minor negligence from the operator can lead to some adverse impact on productive and reproductive performance of a flock. To minimize the handling stress owing to AI, certain techniques are under way like to increase the insemination interval (Froman et al., 2011) and this study can be the part of this endeavor. Single AI frequency along with one sperm concentration has been attempted in most of studies conducted so far. Thus, combination of different AI frequencies along with various sperm concentrations need to be tried to get some more efficient and bird friendly AI protocol in caged and floored flock. Similarly, the utilization of semen can be used even more professionally by précising the quantity of sperms/insemination according to requirement of hen with progression of age. Keeping in view the above-mentioned facts the following study has been planned with the objective to investigate the effect of different AI frequencies and semen concentration rates on productive and reproductive traits of broiler breeder during peak and post peak phase in cage and floor production systems during peak and post peak phases as well as collectively across the production cycle.

MATERIALS AND METHODS

The present study was conducted at a commercial broiler breeder farm (N = 30.912, E = 73.354) with collaboration of Pakistan Poultry Association and University of Veterinary and Animal Sciences (**UVAS**) Lahore, Pakistan to investigate the effect of various interventions in AI in two housing systems on productive and reproductive performance of broiler breeder during peak (29−45 wk) and post peak phase of production cycle (46−62 wk). All the birds were reared under experimental animal care procedures approved by the Ethical Review Committee (vide letter No. DR/1053) of University of Veterinary and Animal Sciences, Lahore, Pakistan.

A total of 980 pullets along with 97 males of 18 wk old (Ross 308) PS were picked up randomly from a commercial breeder flock having uniform body weight (±7.5%). These birds were divided into two groups i.e., AI in caged flock (AIC) and AI in floored flock (AIF). During brooding and rearing, PS was vaccinated according to schedule of breeding company and flock was graded accordingly to achieve flock uniformity ~ 90 % before light stimulation which was induced after 147 days. Prior to light stimulation, 5 lux light intensity for 8 h was provided (15 to 147 days) that was gradually replaced with 60 lux for 15 h on achieving targets like body weight, fleshing, abdominal fat and intra pubic space (Anonymous, 2016b). After one week of light stimulation the birds were divided into two flocks having similar number of females (480) along with 41 males for AIC and 48 males for AIF.

A total of 480 females (pullets) of AIC were placed in 96 colony cages (0.2×0.4 m) having 5 pullets each (8 females/m^2) while 41 males (8.5% of females) were kept in individual cages (5 males /m^2). Similarly, 480 females of AIF for AI on floor, were placed in 16 pens having 30 females each (5 females/m^2) on deep litter floor production system with 48 males (10%) in 8 replicates having 6 males each (3 males /m^2). Pullets of both flocks (AIC and AIF) were further divided into 4 groups (480/4=120 females) to apply 4 various AI frequencies i.e., 4, 6, 8, 10th day. However, all these 4 groups were possessing 120 females. After this arrangement, all these aforesaid four different frequencies of AI (4, 6, 8, and 10 days) were applied on both PS (AIF and AIC) from 29 to 62 wk of age.

Each group assigned for an insemination frequency was further fragmented into 4 subgroups (120/4=30 females) to apply 4 different semen concentrations (100, 125, 150, and 175 × 106 sperms/insemination) during peak which were replaced with boosted concentration regimen (200, 225, 250, and 275 × 10^6 sperm/insemination) during post peak phase. 30 females of these subgroups were kept in 6 replicates/pens (30/6=5/pen). During 2nd trial, data of post peak of experimental flocks were collected while all procedures and parameters were same as practiced in the first trial (peak phase) except sperm concentration regimen was changed. Although, semen volume/insemination was adjusted after every 4 wk to assert the required sperm concentration/concentration by using ONGO machine (working on CASA principle) at experimental site yet these results were further substantiated with computerized assisted semen analyzer (**CASA**) machine at Department of Theriogenology, UVAS, Lahore, Pakistan.

On the subject of feed and feeding, recommendations of PS guide book (Anonymous, 2016c) were followed. Peak feed (mesh) was given to replicates on attaining 70% egg production and same quantity of feed (g) was maintained unless decline in production was started.

According to feeding program, 01 g feed/female was increased with increase of 01% in production to achieve peak feed i.e., 170 and 165g/ hen for AIF and AIC flocks respectively. About 12 to 18% feed was reduced from the offered peak feed according to situation of body and egg weight (Anonymous, 2016b). Male feed was increased at 3g/male/week to maximum 165 and 170 g for a male of AIC and AIF, respectively. However, body weight and egg weight were also considered as indicators for the adjustment of feed quantity offered to each replicate.

Production was started after 3 wk of light stimulation (168 days), AI was started in all replicates of both flocks on 27th wk of age. Eggs were collected 5 times a day, dirty eggs were washed with luke warm water having Dimethyl Diethyl Benzalkonium chloride (1:10) and clean (apparently) were fumigated with Paraformaldehyde powder at 1g/10m^3 area. However, badly soiled, double yolk (jumbo) and cracked eggs were discarded and only settable eggs (SE) were stored at 17°C and 60% RH. All SE were marked properly and stored for 5 days prior to shifting in a commercial hatchery (N = 30.5437, E = 73.3953) where these eggs were stored further for 1−2 days according hatchery schedule. The stored eggs were subjected to prewarming before setting in setter machine. Candling was conducted on 12th day of incubation to estimate the fertility %. Dead in shell (DIS) were examined by breaking the unhatched fertile eggs for embryo-diagnosis after completion of each hatch.

The semen (S) was collected through abdominal message method developed by Burrows and Quinn (1937). Semen of 3−4 males were pooled into a glass funnel of 2 mL with wider brim and this fresh semen was diluted with commercial diluent (1:10). Then the exact required semen volume carrying concerned number of sperms was inseminated to hen's vagina within few minutes (5−8 min) with micropipette.

Parameters Evaluated

Productive Performance Daily laid eggs by hens of each replicate of a treatment were noted while weekly production was calculated by dividing the weekly aggregate of all eggs of a replicate with its Hen Housed (**HH** of a replicate; 6) and answer was divided by 100 to get answer in ratio (%). Average production % of each treatment during a phase was calculated by dividing the aggregate of all weekly production % with 17 (weeks of a phase).

Settable Eggs

All laid eggs by hens of a replicate were noted and unsettable eggs i.e., cracked, jumbo, badly soiled, misshapen were subtracted to find out the settable eggs (SE) /replicate which were added for a week to calculate the SE/week and this aggregate of all SE of 6 replicates of each treatment were divided with HH (480) of a treatment to get total SE/HH/week. Weekly SE of 17 wk of a phase were aggregated to get the total SE/treatment in production phase. Every SE produced by either treatment was kept in store for 5 days prior to shifting in hatchery. Consignment of SE of a treatment and subtreatments were different owing to different in production with progression of age.

Fertility

The unfertile eggs were sorted out through candling on 12th day of incubation which were subtracted from SE and remaining eggs (supposed to be fertile) were divided with SE and multiplied by 100 to calculate apparent fertility. To calculate average fertility % of a treatment, the aggregate of fertility % of all hatches was divided with number of hatches in a phase. Each fertile egg of a treatment has to stay in egg store for 5 days then being shifted to hatchery.

Hatchability

On completion of each hatch, the hatched eggs (HE) were subtracted from total SE and then HE was divided by SE and multiplied by 100 to get answer in ratio. Aggregate of hatched egg % of all hatches was divided by 17 and 34 to get average hatchability of a phase and of whole experiment respectively.

Embryonic Mortality (EM)

Unhatched fertile eggs were subtracted from hatched eggs on the completion each hatch and ratio were calculated. After completion of a hatch breakout analysis of unhatched eggs was carried out to evaluate embryonic mortality (EM) hereafter which can be termed as dead in shell (DIS). Where, exploder eggs after candling in setter, dead pips, quitters, gravely breathed chicks in shell were considered in DIS.

Number of Chicks

All the chicks (salable) of each hatch were added to find out the total chicks of a treatment at the end of each phase, total produced chicks of a treatment during the phase were divided by HH. The chicks produced by HH of each treatment during both phases (peak and post peak) were added to get cumulative chicks/HH/ treatment.

Statistical Analysis

Effect of different housing system, insemination frequency, and sperm concentration in broiler breeder were evaluated on hatching traits during peak and post peak phase. All the collected data were set up as completely randomized design under $2 \times 4 \times 4$ factorial arrangement of treatments and analyzed through factorial ANOVA using PROC GLM in SAS software (SAS, 2002-2003; version 9.1). Housing system, insemination frequency, and sperm concentration were considered as main effects and their interaction were tested, too. Data for weekly production, fertility, and hatchability percentage were analyzed through repeated measures ANOVA. Significant treatment means were compared by Duncan's New Multiple Range test (Duncan, 1955) considering probability at $P \leq 0.05$. Following mathematical model was applied:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha \times \beta)_{ij} + (\alpha \times \gamma)_{ik} + (\beta \times \gamma)_{jk} + \epsilon_{ijkl}$$

Where,

Y_{ijkl} = observation of dependent variable recorded on i^{th}, j^{th}, and k^{th} treatment groups
μ = population mean
α_i = effect of i^{th} housing system (i = 1, 2)
β_j = effect of j^{th} insemination frequency (j = 1, 2, 3, 4)
γ_k = effect of k^{th} sperm concentration (k = 1, 2, 3, 4)
ϵ_{ijkl} = residual effect associated with i^{th}, j^{th}, and k^{th} treatment groups NID $\sim 0, \sigma^2$

RESULTS AND DISCUSSION

Productive Performance

Production performance is the most imperative factor for any PS to gauge its economic worth. In this experiment, the sway of different interventions (4 insemination frequencies and 4 sperm concentrations) in AI under two housing systems were examined on production performance of PS. According to results of both trials, housing systems noticeably interfered the production performance, as the hens in cages (AIC) significantly ($P \leq 0.05$) laid higher number of eggs during post peak phase (Tables 3,4 and 5, Figure 1). However, this difference was recorded nonsignificant ($P > 0.05$) during peak phase among both flocks subjected to AI but kept in two different housing systems (Tables 1, 2, and 5; Figure 1). Moreover, the birds which were being inseminated after every 10 and 8th day showed better performance ($P \leq 0.05$) than of 4th and 6th day during both phases respectively (Tables 1−5; Figure 2). When the influence of two housing systems on AI frequencies was evaluated, significantly ($P \leq 0.05$) better production was recorded on all applied AI frequencies in AIC as compared to AIF especially during post peak phase (Table 5; Figure 2). Different sperm concentrations could not exert any significant impact on production performance neither in peak nor in

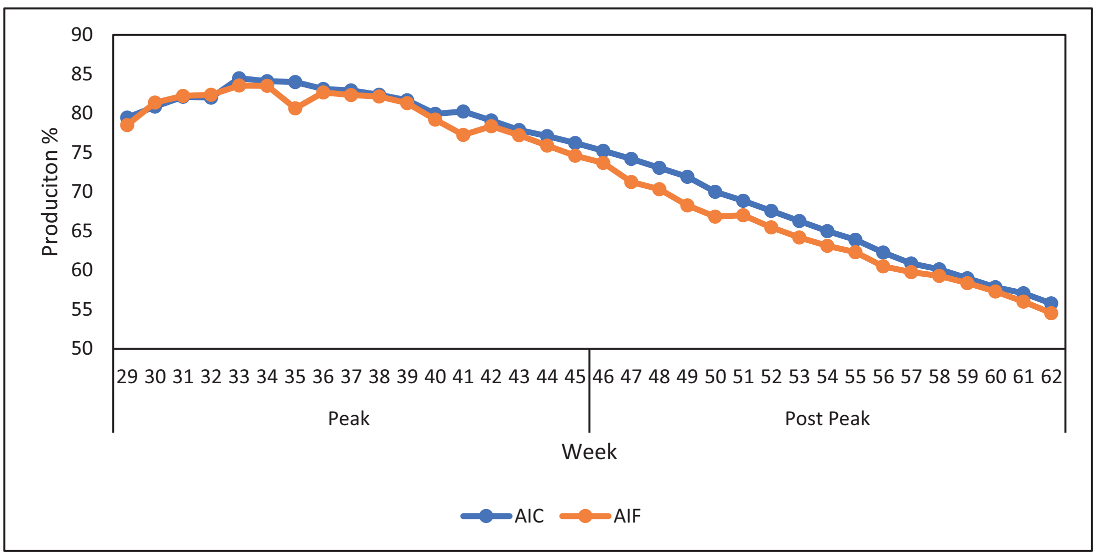

Figure 1. Trend of weekly production % affected by different housing systems; Abbreviations: AIC, artificial insemination in cages; AIF, artificial insemination in floor.

Table 1. Effect of housing systems, AI frequencies and sperm concentrations during peak phase on production and hatching traits.

Treatment		Production %	Settable eggs %	Fertility %	Hatchability %	Chick / HH	Embryonic mortality %
Housing systems	**AIC**	77.10 ± 0.16	88.05 ± 0.16[a]	91.43 ± 0.32[a]	86.83 ± 0.75[a]	74.95 ± 0.25[a]	4.61 ± 0.55
	AIF	77.31 ± 0.18	87.19 ± 0.18[b]	90.59 ± 0.52[b]	85.29 ± 0.72[b]	73.61 ± 0.17 [b]	4.89 ± 0.49
***P*-value**		0.063	0.038	0.021	0.0128	0.044	0.074
Insemination frequency	**4**	75.34 ± 0.11[c]	86.90 ± 0.22[b]	91.96 ± 0.32[a]	86.96 ± 0.62[a]	74.94 ± 0.11[a]	4.89 ± 0.33
	6	77.59 ± 0.15[b]	87.20 ± 0.21[b]	91.15 ± 0.32[ab]	86.06 ± 0.69[ab]	74.59 ± 0.55[ab]	4.89 ± 0.35
	8	77.99 ± 0.14[ab]	89.30 ± 0.22 [a]	89.39 ± 0.45[b]	84.39 ± 0.67[b]	73.75 ± 0.44[b]	4.83 ± 0.29
	10	78.22 ± 0.14[a]	89.84 ± 0.22 [a]	88.16 ± 0.39[c]	83.56 ± 0.66[c]	73.22 ± 0.14[b]	4.54 ± 0.38
***P*-value**		<0.0001	0.011	<0.0001	<0.0001	<0.0001	0.063
Sperm concentration (10^6)	**100**	77.13 ± 0.20	88.74 ± 0.22	87.87 ± 0.65[c]	83.26 ± 0.70[c]	73.63 ± 0.20 [b]	4.63 ± 0.18
	125	77.65 ± 0.24	87.96 ± 0.24	88.78 ± 0.65[b]	84.10 ± 0.65[b]	73.86 ± 0.24 [b]	4.68 ± 0.18
	150	76.91 ± 0.18	89.12 ± 0.19	90.71 ± 0.65[a]	86.04 ± 0.65[a]	75.91 ± 0.18[ab]	4.67 ± 0.18
	175	77.45 ± 0.29	88.51 ± 0.16	90.94 ± 0.66[a]	86.21 ± 0.66[a]	76.45 ± 0.29[a]	4.73 ± 0.18
***P*-value**		0.236	0.091	<0.0001	<0.0001	0.003	0.1123

Superscripts on different means within column differ significantly at $P \leq 0.05$.
Abbreviations: AIC, artificial Insemination in cages; AIF, artificial Insemination in floored flock; HH, hen housed.

Table 2. Interactive effects of mating strategies, AI frequencies and sperm concentrations during peak phase on production and hatching traits.

Treatment			Production%	Settable eggs	Fertility%	Hatchability %	Chick/H.H	EM%
HS × IF	AIC	4	76.43 ± 0.16[bc]	87.83 ± 0.36[bc]	92.14 ± 0.39[a]	87.49 ± 0.46[a]	74.92 ± 0.25[a]	4.67 ± 0.07
		6	77.13 ± 0.19 [b]	87.04 ± 0.24[bc]	91.88 ± 0.36[ab]	87.21 ± 0.44[ab]	74.29 ± 0.95[ab]	4.67 ± 0.03
		8	77.83 ± 0.20[ab]	88.21 ± 0.23[ab]	89.67 ± 0.56[b]	85.14 ± 0.64[b]	73.16 ± 0.95[b]	4.57 ± 0.04
		10	78.16 ± 0.20[a]	89.78 ± 0.20[a]	87.19 ± 0.42[c]	83.24 ± 0.55[c]	71.49 ± 0.96[c]	4.96 ± 0.05
	AIF	4	75.25 ± 0.16[c]	86.51 ± 0.16[c]	91.64 ± 0.39[ab]	87.05 ± 0.37[ab]	73.13 ± 0.75[b]	4.59 ± 0.03
		6	77.23 ± 0.19[b]	87.17 ± 0.19[bc]	91.12 ± 0.36[b]	86.60 ± 0.46[ab]	73.04 ± 0.94[b]	4.56 ± 0.04
		8	77.77 ± 0.20[ab]	88.09 ± 0.22[ab]	88.58 ± 0.57[bc]	84.00 ± 0.65[b]	72.83 ± 0.95[c]	4.50 ± 0.05
		10	77.88 ± 0.20[ab]	89.41 ± 0.20[a]	84.85 ± 0.41[c]	80.24 ± 0.56[d]	70.82 ± 0.96[d]	4.61 ± 0.06
	P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.071
HS × SC	AIC	100	77.33 ± 0.26	88.63 ± 0.27	89.35 ± 0.97[bc]	84.82 ± 0.80[c]	72.54 ± 0.42[c]	4.54 ± 0.23
		125	77.53 ± 0.33	88.94 ± 0.33	89.92 ± 0.88[bc]	85.34 ± 0.93[c]	72.13 ± 0.52[b]	4.50 ± 0.25
		150	76.52 ± 0.25	88.11 ± 0.25	92.34 ± 0.89[b]	87.87 ± 0.94[ab]	72.71 ± 0.39[ab]	4.49 ± 0.25
		175	77.34 ± 0.38	88.95 ± 0.39	92.53 ± 0.90[ab]	88.07 ± 0.94[ab]	73.43 ± 0.53[a]	4.49 ± 0.24
	AIF	100	77.24 ± 0.30	87.84 ± 0.30	90.20 ± 0.95[c]	85.59 ± 1.00[bc]	72.15 ± 0.40[c]	4.66 ± 0.26
		125	77.76 ± 0.35	88.38 ± 0.36	90.97 ± 0.89[bc]	86.18 ± 0.94[b]	72.75 ± 0.50[ab]	4.79 ± 0.28
		150	76.70 ± 0.35	87.29 ± 0.28	93.57 ± 0.87[ab]	88.90 ± 0.93[a]	73.17 ± 0.38[a]	4.60 ± 0.28
		175	77.56 ± 0.44	88.17 ± 0.45	92.54 ± 0.91[a]	88.10 ± 0.97[ab]	72.91 ± 0.52[ab]	4.44 ± 0.29
	P-value		0.2368	0.111	<0.0001	<0.0001	<0.0001	0.0907
IF × SC	4	100	75.58 ± 0.21	88.15 ± 0.22	91.16 ± 0.11[bc]	86.53 ± 0.32[c]	73.81 ± 0.33[bc]	4.67 ± 0.09
		125	75.53 ± 0.20	88.10 ± 0.22	91.85 ± 0.15[b]	87.34 ± 0.36[bc]	73.95 ± 0.38[b]	4.52 ± 0.10
		150	75.40 ± 0.26	87.96 ± 0.27	94.63 ± 0.15[a]	90.22 ± 0.38[a]	75.87 ± 0.43[ab]	4.55 ± 0.10
		175	74.85 ± 0.12	87.90 ± 0.12	94.95 ± 0.15[a]	90.40 ± 0.20[a]	76.07 ± 0.18[a]	4.50 ± 0.06
	6	100	76.39 ± 0.24	87.91 ± 0.24	87.52 ± 0.22[bc]	82.99 ± 0.50[cd]	72.28 ± 0.33[c]	4.53 ± 0.06
		125	75.10 ± 0.18	88.72 ± 0.18	90.93 ± 0.15[bc]	86.47 ± 0.32[b]	74.33 ± 0.21[b]	4.48 ± 0.08
		150	76.76 ± 0.29	88.85 ± 0.29	94.15 ± 0.28[b]	89.62 ± 0.35[b]	75.27 ± 0.41[b]	4.53 ± 0.09
		175	76.12 ± 0.18	87.54 ± 0.19	94.45 ± 0.15[b]	89.90 ± 0.27[b]	75.15 ± 0.53[b]	4.56 ± 0.09
	8	100	75.52 ± 0.20	88.13 ± 0.21	87.31 ± 0.17[cd]	83.00 ± 0.33[cd]	69.91 ± 0.34[d]	4.31 ± 0.08
		125	76.22 ± 0.12	88.84 ± 0.12	90.91 ± 0.18[c]	84.54 ± 0.33[c]	70.17 ± 0.22[c]	4.34 ± 0.10
		150	76.88 ± 0.25	87.48 ± 0.26	90.95 ± 0.16[bc]	86.18 ± 0.41[bc]	72.89 ± 0.41[c]	4.77 ± 0.10
		175	75.63 ± 0.24	87.76 ± 0.24	91.31 ± 0.17[bc]	86.54 ± 0.31[bc]	73.47 ± 0.55[bc]	4.75 ± 0.10
	10	100	76.04 ± 0.20	87.66 ± 0.21	84.91 ± 0.18[d]	80.20 ± 0.45[d]	68.76 ± 0.35[d]	4.71 ± 0.10
		125	75.74 ± 0.21	88.38 ± 0.12	85.05 ± 0.17[d]	80.70 ± 0.45[d]	69.83 ± 0.23[d]	4.35 ± 0.11
		150	77.04 ± 0.25	88.01 ± 0.26	85.95 ± 0.14[cd]	81.46 ± 0.42[d]	69.74 ± 0.42[d]	4.49 ± 0.10
		175	78.70 ± 0.21	88.33 ± 0.22	87.61 ± 0.08[c]	83.19+0.39[cd]	70.19 ± 0.50[cd]	4.45 ± 0.10
	P-value		0.4590	0.3660	<0.0001	<0.0001	<0.0001	0.0901

Superscripts on different means within column differ significantly at $P \leq 0.05$; Abbreviations: AIC, artificial Insemination in cages; AIF, artificial insemination in floored flock; EM, embryonic mortality; HH, hen housed; HS, housing systems; IF, insemination frequency; SC, sperm concentration (10^6).

post peak phase ($P > 0.05$) (Tables 1 and 3; Figure 3). Similarly, interaction of different sperm concentrations with two housing systems and four AI frequencies were also found nonsignificant in both phases (Tables 2 and 4).

It is imperative from the results that AIC seemed better than AIF in average as well as in weekly production performance (Figure 1) and this fact could be attributed that cage production system would be conducive for production as compared to floor (Hetland et al., 2004). These findings are also in line with the results of some other researchers (Khan and Khan, 2018; Yan Li et al., 2018) who concluded better production in cages than floor. Contrarily to Roll et al. (2009) who found no difference in production performance

Table 3. Effect of housing systems, AI frequencies and sperm concentrations during post peak phase on production and hatching traits.

Treatment		Production%	Settable eggs	Fertility %	Hatchability%	Chick/HH	EM%
Housing systems	AIC	58.83 ± 0.12^{a}	67.55 ± 0.12^{a}	89.92 ± 0.39^{a}	84.60 ± 0.52^{a}	56.56 ± 0.25^{a}	5.32 ± 0.67^{b}
	AIF	57.59 ± 0.44^{b}	66.84 ± 0.14^{b}	88.17 ± 0.46^{b}	82.15 ± 0.52^{b}	54.68 ± 0.27^{b}	5.95 ± 0.55^{a}
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Insemination frequency	4	56.61 ± 0.17^{c}	65.74 ± 0.15^{c}	91.96 ± 0.08^{a}	86.18 ± 0.22^{a}	55.36 ± 0.35^{a}	5.79 ± 0.49^{a}
	6	57.51 ± 0.14^{b}	65.93 ± 0.17^{b}	89.46 ± 0.11^{b}	84.06 ± 0.27^{b}	54.16 ± 0.15^{b}	5.40 ± 0.53^{b}
	8	58.54 ± 0.13^{ab}	67.05 ± 0.13^{ab}	86.61 ± 0.12^{c}	81.15 ± 0.32^{c}	51.98 ± 0.13^{c}	5.45 ± 0.55^{b}
	10	60.15 ± 0.16^{a}	67.77 ± 0.16^{a}	83.31 ± 0.16^{d}	77.06 ± 0.39^{d}	45.09 ± 0.21^{d}	5.05 ± 0.55^{c}
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sperm concentration (10^6)	200	57.94 ± 0.18	66.14 ± 0.19	89.20 ± 0.61^{d}	83.30 ± 0.69^{c}	54.21 ± 0.35^{b}	5.88 ± 0.20
	225	58.01 ± 0.20	67.04 ± 0.21	89.38 ± 0.61^{c}	83.81 ± 0.57^{b}	54.30 ± 0.38^{b}	5.59 ± 0.20
	250	57.06 ± 0.14	66.00 ± 0.15	90.22 ± 0.60^{b}	85.27 ± 0.37^{ab}	54.63 ± 0.35^{ab}	5.82 ± 0.20
	275	57.64 ± 0.21	65.95 ± 0.21	90.97 ± 0.59^{a}	86.03 ± 0.48^{a}	55.44 ± 0.38^{a}	5.65 ± 0.20
P-value		0.236	0.910	<0.0001	<0.0001	<0.0001	0.9801

Superscripts on different means within column differ significantly at $P \leq 0.05$.
Abbreviations: AIC, artificial insemination in cages; AIF, artificial insemination in floored flock; HH, hen housed; EM, embryonic mortality.

Table 4. Interaction effect of housing system, sperm concentration & insemination frequency during post peak phase on production and hatching traits.

Treatment			Production%	Settable eggs	Fertility%	Hatchability%	Chick/H.H	Embryonic mortality %
HS × IF	AIC	4	57.13 ± 0.20^{c}	65.33 ± 0.20^{c}	91.83 ± 0.13^{a}	86.14 ± 0.19^{a}	56.34 ± 0.21^{a}	5.71 ± 0.04^{b}
		6	57.96 ± 0.17^{bc}	66.00 ± 0.17^{b}	90.40 ± 0.15^{ab}	84.65 ± 0.19^{ab}	55.99 ± 0.12^{ab}	5.67 ± 0.05^{a}
		8	59.15 ± 0.17^{ab}	67.21 ± 0.20^{a}	87.88 ± 0.14^{c}	82.89 ± 0.15^{b}	55.02 ± 0.13^{b}	5.57 ± 0.04^{c}
		10	60.89 ± 0.20^{a}	67.73 ± 0.16^{a}	84.96 ± 0.15^{d}	80.01 ± 0.22^{c}	51.56 ± 0.14^{c}	5.48 ± 0.05^{c}
	AIF	4	56.97 ± 0.18^{c}	65.15 ± 0.19^{c}	91.08 ± 0.15^{a}	85.20 ± 0.19^{ab}	54.94 ± 0.05^{ab}	6.07 ± 0.03a
		6	58.07 ± 0.18^{b}	66.28 ± 0.18^{b}	89.80 ± 0.15^{b}	83.52 ± 0.15^{b}	53.08 ± 0.22^{bc}	5.94 ± 0.05^{ab}
		8	59.72 ± 0.15^{ab}	66.37 ± 0.20^{ab}	87.34 ± 0.19^{c}	81.66 ± 0.21^{bc}	52.98 ± 0.14^{bc}	6.08 ± 0.05^{a}
		10	59.91 ± 0.23^{ab}	67.44 ± 0.23^{ab}	83.65 ± 0.16^{d}	77.83 ± 0.19^{d}	49.0s5 $\pm 0.15^{d}$	5.79 ± 0.05^{b}
	P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HS × SC	AIC	200	57.93 ± 0.27	66.15 ± 0.27	89.82 ± 0.80^{c}	84.49 ± 0.56^{c}	53.59 ± 0.40^{c}	5.39 ± 0.26
		225	58.26 ± 0.29	66.49 ± 0.29	90.37 ± 0.78^{b}	85.01 ± 0.53^{bc}	53.99 ± 0.35^{b}	5.20 ± 0.25
		250	57.44 ± 0.13	65.65 ± 0.13	90.58 ± 0.79^{ab}	85.59 ± 0.59^{ab}	54.25 ± 0.39^{ab}	5.27 ± 0.26
		275	58.50 ± 0.17	66.07 ± 0.17	91.91 ± 0.79^{a}	86.67 ± 0.52^{a}	55.29 ± 0.42^{a}	5.25 ± 0.26
	AIF	200	56.94 ± 0.19	66.94 ± 0.19	88.44 ± 0.94^{cd}	83.11 ± 0.45^{c}	52.68 ± 0.56^{d}	5.29 ± 0.26
		225	57.77 ± 0.28	65.99 ± 0.29	89.99 ± 0.95^{c}	84.84 ± 0.66^{bc}	53.11 ± 0.57^{bc}	5.41 ± 0.26
		250	58.67 ± 0.22	66.88 ± 0.23	$90.62 \pm 0.93\ ^{b}$	85.11 ± 0.51^{b}	53.66 ± 0.50^{b}	5.36 ± 0.30
		275	58.39 ± 0.33	66.60 ± 0.33	90.79 ± 0.91^{ab}	85.35 ± 0.51^{b}	54.31 ± 0.53^{ab}	5.89 ± 0.25
	P-value		0.1800	0.1953	<0.0001	<0.0001	<0.0001	0.0861
IF × SC	4	200	56.55 ± 0.7	66.74 ± 0.17	90.20 ± 0.12^{b}	84.71 ± 0.18^{b}	55.08 ± 0.09^{b}	5.49 ± 0.04
		225	56.59 ± 0.29	67.79 ± 0.13	90.71 ± 0.11^{ab}	85.53 ± 0.10^{ab}	55.83 ± 0.22^{b}	5.27 ± 0.04
		250	56.25 ± 0.35	66.44 ± 0.36	91.01 ± 0.17^{a}	86.12 ± 0.17^{a}	57.05 ± 0.24^{a}	5.15 ± 0.05
		275	56.81 ± 0.30	67.01 ± 0.51	91.47 ± 0.18^{a}	86.47 ± 0.18^{a}	57.19 ± 0.25^{a}	5.00 ± 0.06
	6	200	57.17 ± 0.32	67.37 ± 0.33	88.19 ± 0.17^{b}	83.00 ± 0.21^{bc}	54.19 ± 0.20^{c}	5.24 ± 0.05
		225	57.91 ± 0.27	66.95 ± 0.26	89.15 ± 0.19^{b}	84.11 ± 0.16^{bc}	55.18 ± 0.36^{bc}	5.28 ± 0.06
		250	57.10 ± 0.20	67.30 ± 0.20	90.18 ± 0.15^{ab}	85.07 ± 0.15^{b}	56.55 ± 0.12^{b}	5.14 ± 0.04
		275	57.87 ± 0.26	67.09 ± 0.27	90.69 ± 0.19^{ab}	85.52 ± 0.12^{b}	56.99 ± 0.13^{a}	5.16 ± 0.06
	8	200	57.51 ± 0.26	66.72 ± 0.26	84.86 ± 0.25^{cd}	79.00 ± 0.30^{c}	53.17 ± 0.34^{c}	5.10 ± 0.07
		225	58.06 ± 0.16	66.58 ± 0.16	85.68 ± 0.20^{bc}	80.43 ± 0.22^{bc}	53.70 ± 0.34^{c}	5.21 ± 0.07
		250	57.25 ± 0.14	67.46 ± 0.14	87.41 ± 0.13^{bc}	82.12 ± 0.22^{bc}	54.09 ± 0.19^{bc}	5.13 ± 0.07
		275	58.22 ± 0.23	67.44 ± 0.24	87.99 ± 0.17^{bc}	82.93 ± 0.15^{bc}	54.42 ± 0.18^{bc}	5.06 ± 0.07
	10	200	59.51 ± 0.34	67.74 ± 0.35	80.90 ± 0.33^{d}	75.30 ± 0.23^{d}	48.62 ± 0.59^{d}	5.28 ± 0.10
		225	60.20 ± 0.20	67.44 ± 0.21	82.06 ± 0.38^{c}	76.56 ± 0.19^{d}	49.27 ± 0.47^{c}	5.34 ± 0.13
		250	59.63 ± 0.18	67.86 ± 0.19	86.48 ± 0.29^{cd}	81.09 ± 0.20^{c}	51.65 ± 0.38^{c}	5.23 ± 0.09
		275	59.87 ± 0.28	67.06 ± 0.29	86.78 ± 0.27^{cd}	80.55 ± 0.18^{cd}	50.91 ± 0.17^{c}	5.57 ± 0.05
	P-value		0.2400	0.2637	<0.0001	<0.0001	<0.0001	0.0610

Superscripts on different means within column differ significantly at $P \leq 0.05$; Abbreviations: AIC, artificial Insemination in cages; AIF, artificial insemination in floored flock; EM, embryonic mortality; HH, hen housed; HS, housing systems; IF, insemination frequency; SC, sperm concentration (10^6).

under both housing systems. During experiment 1−2% drop in production was noticed after every AI insemination particularly during post peak. Similarly, a bit hasty decline in production might be indicative that older hens endured more AI procedural stress as compared to younger age. These findings were also reinforced by the results of adjacent fragment of this study that the hens which were being frequently inseminated i.e., 4 and 6 days yielded lesser eggs than those which were being subjected to far apart insemination frequencies i.e., 8 and 10 days. In nut shell, AI in cages seemed lesser retro-productive housing systems as compared to floor. But the bird's welfare concern cannot be over ruled in cages (Campbell et al., 2019). Production was not influenced by different sperm concentrations is quite logical as there would be no difference in physical

Table 5. Cumulative effect of housing systems, artificial insemination frequencies and sperm concentrations during peak and post peak phase on production and hatching traits.

Treatment			Production%	Settable Eggs	Fertility %	Hatchability %	Chick/HH	EM%
Housing systems	AIC		67.97 ± 0.70^{a}	155.60 ± 0.34^{a}	90.88 ± 0.51^{a}	86.09 ± 0.52^{a}	131.51 ± 0.28^{a}	4.80 ± 0.29^{b}
	AIF		66.45 ± 0.66^{b}	154.03 ± 0.28^{b}	89.63 ± 0.44^{b}	84.22 ± 0.44^{b}	128.29 ± 0.35^{b}	5.41 ± 0.37^{a}
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Insemination	4		67.08 ± 0.35^{c}	152.64 ± 0.19^{c}	92.96 ± 0.25^{a}	87.89 ± 0.55^{a}	130.30 ± 0.44^{a}	5.09 ± 0.39^{a}
Frequencies	6		67.25 ± 0.29^{b}	153.13 ± 0.22^{b}	91.53 ± 0.25^{ab}	86.54 ± 0.51^{ab}	129.74 ± 0.51^{ab}	4.94 ± 0.35^{ab}
	8		68.66 ± 0.33^{ab}	156.35 ± 0.18^{a}	88.50 ± 0.28^{b}	83.81 ± 0.52^{b}	126.74 ± 0.45^{b}	4.69 ± 0.41^{b}
	10		69.25 ± 0.27^{a}	157.61 ± 0.20^{a}	85.74 ± 0.23^{c}	81.08 ± 0.59^{c}	119.31 ± 0.46^{c}	4.64 ± 0.39^{b}
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.041
Sperm concentrations (10^6)	*100	¥200	67.78 ± 0.11	155.38 ± 0.22	87.80 ± 0.17^{c}	81.98 ± 0.55^{d}	126.22 ± 0.15^{c}	5.31 ± 0.18
	125	225	67.65 ± 0.14	155.15 ± 0.28	89.29 ± 0.14^{b}	83.25 ± 0.49^{c}	128.35 ± 0.19^{b}	5.33 ± 0.19
	150	250	66.91 ± 0.12	155.12 ± 0.24	90.29 ± 0.16^{ab}	85.08 ± 0.50^{b}	131.27 ± 0.15^{a}	5.27 ± 0.19
	175	275	66.85 ± 0.15	154.46 ± 0.24	91.58 ± 0.19^{a}	86.29 ± 0.47^{a}	131.89 ± 0.18^{a}	5.36 ± 0.19
P-value			0.731	0.920	<0.0001	<0.0001	<0.0001	1.000

Superscripts on different means within column differ significantly at $P \leq 0.05$.
Abbreviations: AIC, artificial insemination in cages; AIF, artificial insemination in floored flock; EM, embryonic mortality; HH, hen housed; HS, housing systems; IF, insemination frequency; SC, sperm concentration (10^6).
*Sperm concentration (10^6) during peak phase;
¥Sperm concentration (10^6) during post peak phase.

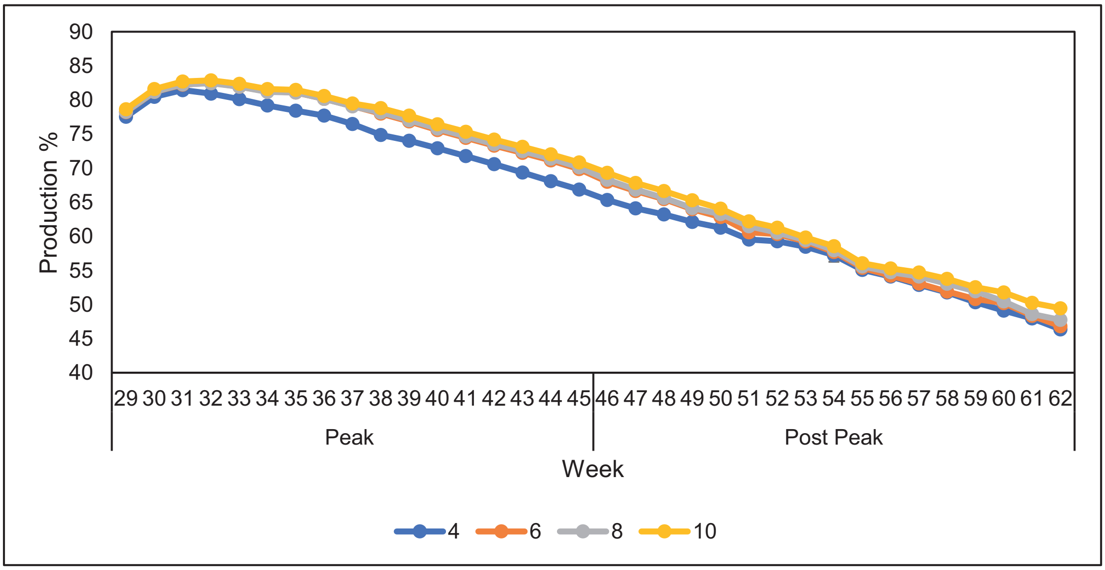

Figure 2. Trend of weekly production % of broiler breeders affected by different insemination frequencies
4, 6, 8, and 10 = Days of insemination frequencies.

and physiological stress being exerted by various sperm concentrations.

Settable Eggs (SE)

Although, eggs are the ultimate product of production of any PS yet the SE/HH are actually the goal of a breeder farmers rather just total eggs. Many contributory factors can be enlisted which establish SE % out of total produced eggs by a hen. When the impact of housing systems on SE was recorded in this study, cages (AIC) found comparatively more facilitative to attain more SE during peak as well as in post peak phase ($P \leq 0.05$) than floor (AIF) (Tables 1–5). However, reciprocal relation of insemination frequencies to SE has been recorded as greater number of SE were achieved on 10 and 8th day insemination frequencies as compared to 4 and 6th day respectively during peak and post peak phase ($P \leq 0.05$) (Tables 1, 3). Interaction of various frequencies with both housing systems proved to be futile ($P > 0.05$) in influencing any differential impact (Tables 2 and 4). Similarly, as it was in egg production %, the applied sperm concentration rates remained inert to play meddling role (Tables 1 and 3).

It is revealed from the results that SE of a treatment basically determined by its production, thus the flock AIC possessed the better SE/HH simply as its production was better as compared to AIF. This sequence of total SE/HH seemed plagiaristic to pattern of production % of subjected treatments. In pursuance of research procedure, some factors other than production % was also traced which was actually production systems and egg quality. That's why the difference in average production% of AIC and AIF was although nominal yet AIC excelled numerically to AIF in SE/HH indicative that eggs were safer in cages.

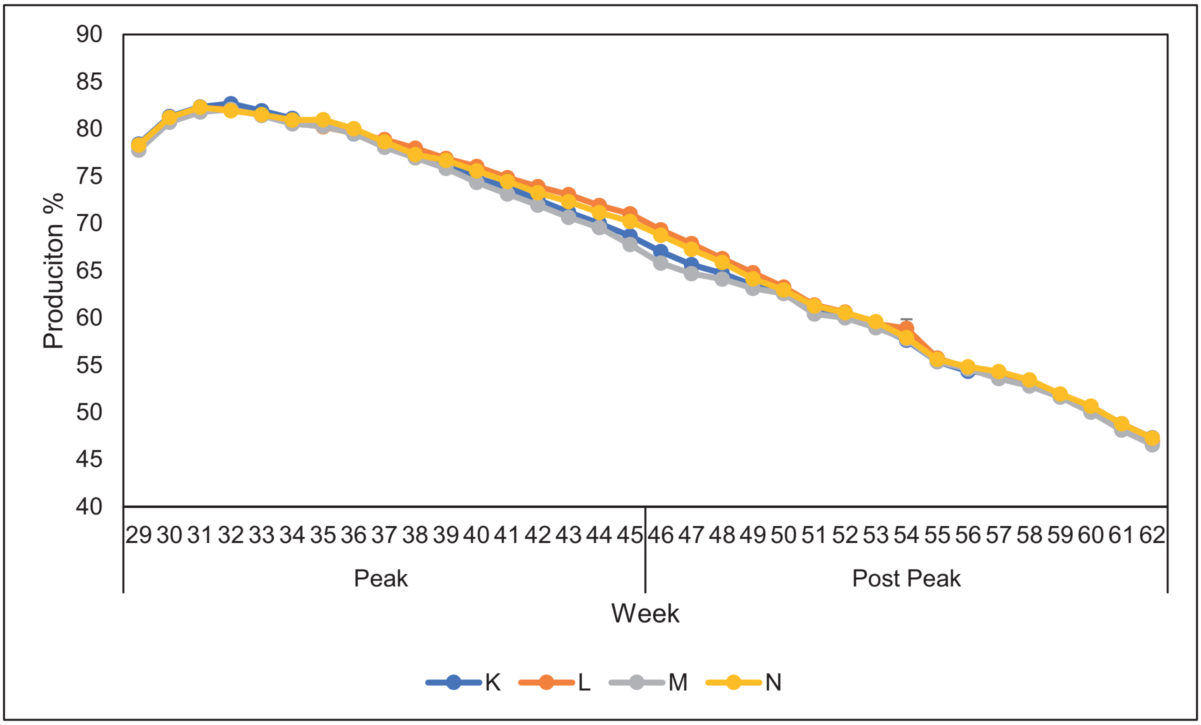

Figure 3. Trend of weekly production % of broiler breeders affected by different sperm concentrations
K = 100, L = 125, M = 150, and175 in million (during peak) K = 200, L = 225, M = 250, and N = 275 in million (during post peak).

Where the ratio of unsettable eggs particularly cracked, toe balled and extremely soiled eggs were lesser in cages as compared to deep litter floor. Though, the similar order of SE among the treatments was observed but this rift of difference widened gradually with progression of age (post peak). However, it could be concluded that number of SE of a flock mainly depends upon its production% followed by housing systems. Resultantly, the flock possessed better production yielded more SE/HH i.e., AIC and AIF, respectively. The factors (discussed earlier) which would have affected the production actually have indirectly influenced the SE. Regarding both flocks subjected to AI (AIC and AIF), The ratio of SE/HH out of total eggs/HH was better in cages is quite explicable as there would be lesser dirty, broken and toe balled eggs as compared to AIF where the environment was more dusty and dirty which might have increased the rejected eggs (Yan Li et al., 2018). These results are also being reinforced by some earlier work like (Gianenas et al., 2009) who found better egg quality in cages while the egg quality reduces the egg breakage.

Fertility

It is quite fathomable that just SE are worthless so as not to be fertile. Fertility of SE is regulated by reproductive competency of breeder along with cautious handling of eggs which further depends upon simply management and housing systems. The influence of housing systems on fertility was probed during both trials i.e., peak and post peak separately, where higher fertility % was manifested by birds being kept in cages (AIC) as compared to birds being kept on deep liter floor (AIF) during peak and this difference in fertility became more obvious during post peak phase ($P \leq 0.05$) as mentioned in data (Tables 1, 3, and 5; Figure 4). Thus, significantly ($P \leq 0.05$) higher average fertility noticed in AIC “affirmed the proficiency of consortium of cages and AI'' (Figures 4, 5, and 6).

AI frequency can be considered among the most vital contributory factors in success of AI. Thus, when 4 various AI frequencies were tested, the best fertility ($P \leq 0.05$) was recorded at 4 and 6th day followed by 8 and 10th day respectively during peak; but the results varied a little during post peak as exclusively 4th day AI frequency germinated the highest no of eggs followed by 6th and 8thday while the least average fertility % was recorded at 10th day interval of AI insemination. Although, AI frequencies seemed pivotal yet number of sperms given at the mentioned intervals did matter a lot to attain a sustainable and satisfactory level of average fertility throughout the production cycle of PS. As, the significant difference in fertility was noticed on various sperm concentrations applied during peak and post peak phases. Albeit, during peak, even moderate sperm concentrations/insemination like 175×10^6 and 150×10^6 generated satisfactory level of fertility yet a bit higher concentration of sperms (275×10^6 and 225×10^6) could brought forth the desirable fertility as found in post peak phase.

As far as interactions of AI frequencies to sperm concentrations are concerned, consortium of 175×10^6 and 150×10^6 the sperm concentration with 4th and 6th day AI frequencies generated significantly ($P < 0.0001$) the best results during peak while in post peak, combination

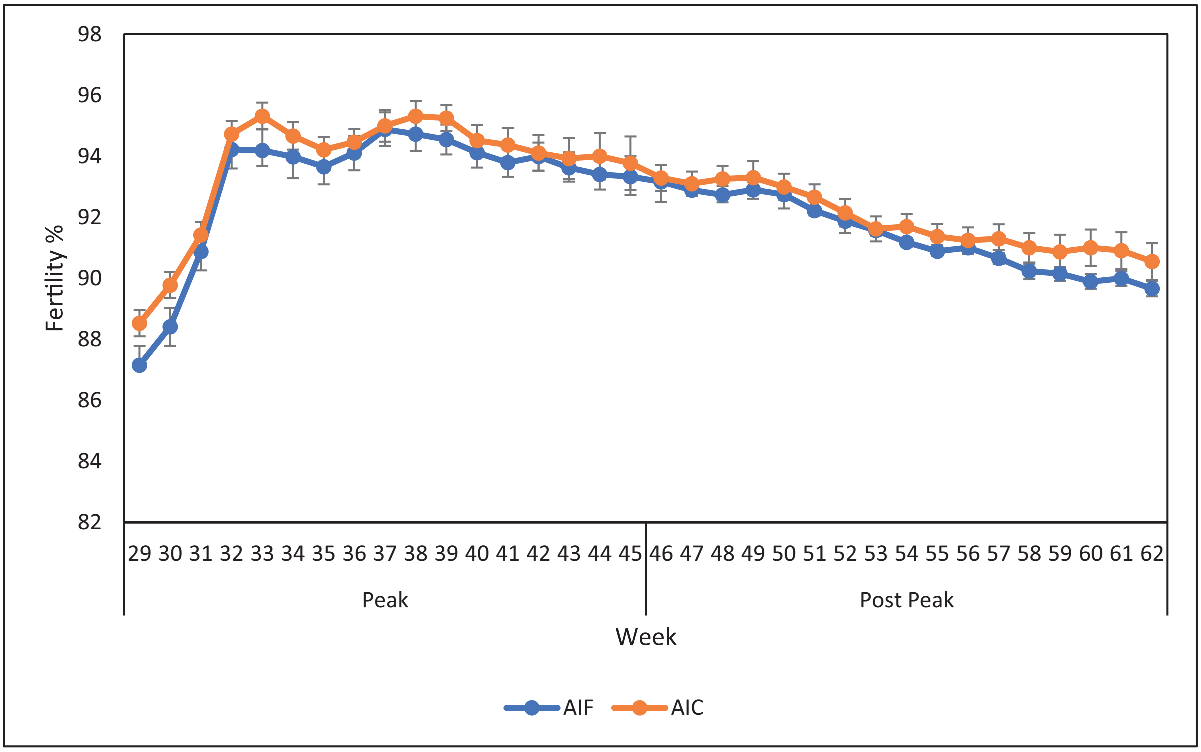

Figure 4. Trend of weekly fertility % of broiler breeders affected by different housing systems
Abbreviations: AIC, artificial insemination in cages; AIF, artificial insemination in floor.

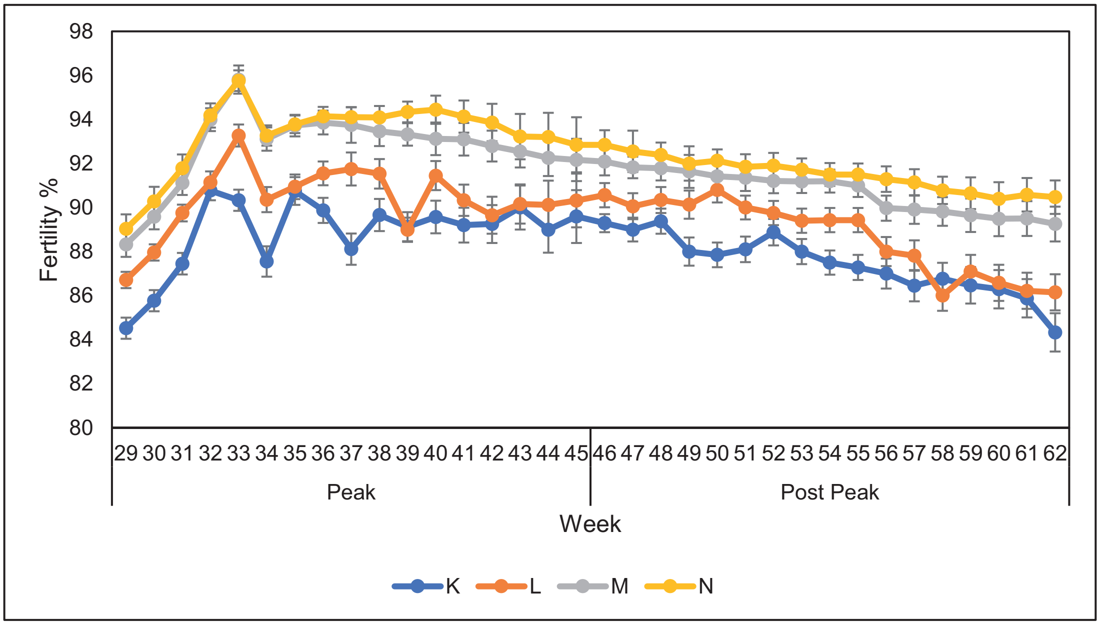

Figure 5. Trend of weekly fertility % of broiler breeders affected by different sperm concentrations
K = 100, L = 125, M = 150, and N = 175 in million (during peak) K = 200, L = 225, M = 250, and N = 275 in million (during post peak).

of a bit higher sperm concentrations i.e., 275×10^6 and 250×10^6 germinated the highest number of SE on the said frequencies. Thus, shortly it is being perceived that the best fertility % was achieved by using 175×10^6, 150×10^6 during peak and 275×10^9 and 225×10^6 sperms/insemination during post peak respectively. Furthermore, fluctuation and inconsistency in weekly fertility % was observed on lower sperm concentrations along with distantly apart AI frequencies (8 and 10 days) with progression of age (Figures 5 and 6). So, the results are reminiscent that fertility % was being influenced more profoundly by AI frequencies in comparison to quantity of spermatozoa/insemination particularly up to 48 to 50 wk while afterword both the frequencies as well as sperm concentration proved to be equally vital for a sustainable fertility (Figures 5 and 6).

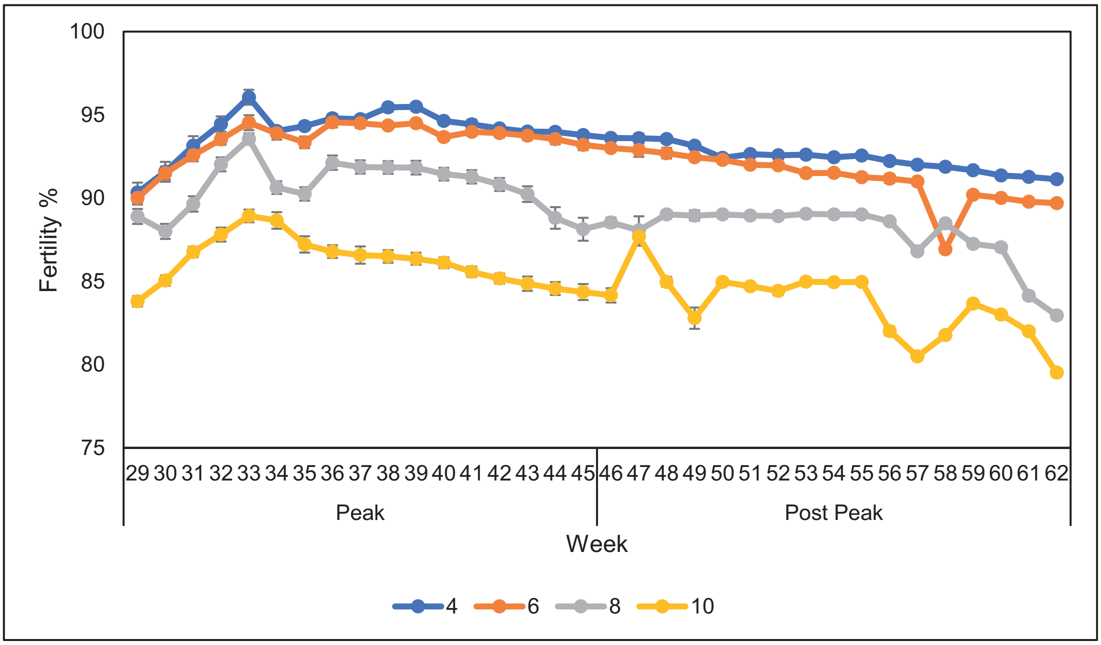

Figure 6. Trend of weekly fertility % of broiler breeders affected by different insemination frequencies
4, 6, 8, and 10 = Days of insemination frequencies.

Predictably, AI led to better results especially when practiced in cages as compared to AI in floored flock might be referred to fact that somehow it would be convenient to maintain uniform body weight and fleshing of males and females in cages as compared to floored flock (AIF) where sizing in body conformation along with cannibalism would not let some of the of males to produce good quality semen. To some extent uneven feed distribution might be blemished for the said variations in AIF males. These findings might be linked with obscuring reproductive potential of experimental breeder birds particularly of older males. AI served well might be by delivering required quantity of spermatozoa per insemination to hens on the appropriate time which would have accomplished the need of hens to lay fertile eggs (Dhama et al., 2007; Khazaei Koohpar et al., 2010) who have suggested that reproduction technique (AI) is an important factor affecting the breeder's reproductive performance. Consequently, AI proved to be a handy and useful technique to cater with the swift declining of male's reproductive incompetency with the progression of age. Albeit, this debility of males can be linked with traumatic (leg deformities, bumble foot), pathological (diseases) yet mainly manage mental (body weight, depleted fleshing) motives can be blemished.

Contrarily to male, with progression of age the female's requirement of no sperms/insemination after shorter intervals (4th day) increases to lay fertile eggs. These findings are in line with the work of Tabatabaei (2010) and Beulah (2017) who revealed that success of AI depends upon the supply of good quality and quantity of sperms to establish satisfactory fertility. However, study of Douard et al. (2003) revealed that sperm holding capacity of reproductive tract usually decreases as hens get older. These findings are in consistent with work of Hofacre (2002) who suggested that decline in fertility is due to lack of mating desire or unsuccessful copulation. Results of another integral segment of this study, directed that 4th and 6th day AI frequencies brought about the satisfactory average fertility % which was closely followed by 8th day while sperm count/insemination did matter to lesser extent during peak phase. These results might be attributed to the good quality semen and prolonged sperm retention capability of the hens in their reproductive tract. These outcomes are similar to findings of Froman et al. (2011) who have even attempted an AI frequency more than a week by using 125×10^6/insemination regardless to age to age of breeder.

During post peak phase, fertility on frequencies of 6 and 8th days even with higher sperm concentrations could not compete with 4th day on all available concentration rates. Hence, these results can be referred to the fact that semen quality and quantity drops in males when its requirement rises in females with progression of age. The aforesaid discussion can be further illustrated, when only 4th day frequency worked efficiently with relatively higher sperm concentration i.e., 250 and 275×10^6 especially after 52 to 53 wk indicated that older hen needs more sperms with frequent intervals of insemination. Another reason for the need of this extensive insemination in older PS was pointed out by Tumova and Gous (2012) who associated this phenomenon with clutch size of hens. As ovulation induces stimulation for transportation of sperms from storage gland (proctodium) to the site of fertilization (infundibulum). The clutch size becomes shorter from 6 to 3.52/week (average) (Anonymous, 2016b) with progression of age of hens. Furthermore, irrational increase in sperm count per insemination might be not so effective rather an appropriate number of spermatozoa after suitable intervals according to age of hen is required (Beulah, 2017;

Mohan et al., 2018). Thus, it can be concluded that insemination frequency seems of greater concern rather sperms count in early life of PS but sperm count especially viable and progressive motile/insemination become equally or more important for fertility in later half of life which would have not been produced by older males. In addition to milking intervals, age, body weight and fleshing of males appeared critical for steady supply of good quality semen for optimum reproductive performance (Talebi et al., 2009; Nahak et al., 2015).

Embryonic Mortality (EM) /Dead in Shell (DIS)

Ideally, each fertile egg should produce a healthy chick in commercial poultry. However, this optimum is never been achieved so far. As, the situation is being complicated by numerous factors affecting embryonic livability including lethal chromosomal abnormalities, nutrition in addition to hygiene and storage conditions of fertile eggs. The embryonic mortality (EM) or DIS termed as hatchery loses which is preventable to much extent. Egg hygiene plausibly associated with housing systems (cage and deep litter floor). So, the sway of housing systems on EM was recorded; Although, statistically ($P > 0.05$) lesser EM/DIS was recorded in caged flock as compared to floored during peak yet this difference became significant ($P \leq 0.05$) in post peak (Tables 1, 3). Regarding influence of various AI frequencies, it was revealed that EM/DS was the highest in experimental groups being inseminated after every 4th day followed by 6th, 8th and 10th particularly post peak ($P \leq 0.05$) (Tables 1 and 3). Thus, there was a little difference of EM ratio among both experimental flocks during peak yet it was escalated by AI with progression of age particularly in floored flock. Though, the impact of various applied sperm concentrations on EM was found inert .by assuming that number sperms would have not interfered the EM (Tables 1, 3, and 5).

These results are being harmonized with the findings of Christensen (2001) who blemished to lethal gene along with hygienic conditions for early embryonic mortality (EEM) to contribute in EM in peak phase. According the results, it was noticed that EM ratio was higher during post peak as compared to peak phase. These finding seems logical as reproductive tract would have become septic with progression of age particularly in those hens which were subjected to AI as it was proceeded in environment of shed. These results are in line to some extent with (Fairchild et al., 2002) who suggested that hen age influences embryonic mortality in modern commercial poultry. EM particularly late embryonic mortality (LEM) was significantly higher in flocks being subjected to AI in floor is indicative that AI might have exposed the embryo to multiple infections especially with *E. coli, Salmonella and Mycoplasam gallisepticum (MG)* and fungus which would have led to embryonic mortality (Cox et al., 2002; Buhr et al., 2005; Tomar et al., 2006). Similarly, Amer et al. (2017) have worked on contribution of contamination towards EM and found that multiple bacterial infection played a vital role in death of embryo. *Salmonella, E. coli and MG* can be easily transmitted vertically during AI through transovarian route as per findings of Donoghue and Wishart (2000). Grochowska et al. (2019) narrated that breeder age, storage of egg and hygienic status can be the main cause of hatchery loses (DIS/EM). However, the role of fungus in EM has not been acknowledged so far which would be one of the most lethal subscribers among the causative elements for EM. It is highly suggestive to expose the contribution of fungus and mycotoxins in embryonic death in commercial poultry particularly flocks being subjected to AI. ***Hatchability%***

The ultimate purpose of PS flock is to produce good quality and quantity of chicks which are being hatched from good condition fertile eggs. So, the hatchability is a primary reproductive parameter which affix the economy of a PS. Hatchability is mainly derived by the reproductive capability of birds which can be improved by adopting better mating strategies and housing. When the impact of different housing systems on hatchability of experimental PS were compared, statistically better average hatchability % was attained through AI in caged flock (AIC) as compared to AI in floored flock (AIF) (Tables 1 and 3; Figure 7).

During peak, better ($P \leq 0.05$) average hatchability % was recorded on 4th and 6th day AI frequencies as compared to 8 and 10th day. At 4th day frequency led to the highest hatchability followed by 6th day which followed by 8 and 10th in post peak (Tables 1 and 3; Figure 9).

At all the applied AI frequencies, sperm concentration of 175×10^6 and 150×10^6 gave comparatively ($P \leq 0.05$) better hatchability than 125×10^6 and 100×10^6 respectively. In post peak 200×10^6 and 225×10^6 exhibited almost similar results but significantly ($P \leq 0.05$) lesser to the sperm concentration of 275×10^6 which was found the best sperm concentration among the all other applied sperm concentrations particularly in terminal phase of post peak (55−62 wk) (Figure 8).

From weekly data it was noticed that AI frequencies did matter more profoundly in comparison to quantity of spermatozoa/insemination in hatchability particularly up to 50 wk but afterword both the frequencies and sperm concentrations proved to be equally vital for a sustainable hatchability. Furthermore, frequent fluctuation in hatchability was also observed on long intervals of AI (8 and 10th day) applied with low sperm concentrations (125×10^6 and 100×10^6 in peak, and 200×10^6 and 225×10^6 in post peak) (Figure 8). Conclusively, the trend of hatchability % was in accordance to the fertility pattern i.e., AIC, AIF respectively. As, it mainly depends upon fertility of a flock which is quite fathomable. The context of variations in average hatchability % among treatments could be attributed to the factors which have been affecting the fertility of experimental flocks i.e., aging, physiological and pathological etiologies (Uni et al., 2012; Ogbu and Oguike, 2019).

It was deducted from this study that hatchability of fertile eggs seems reciprocal to age of flock that might be due to deterioration of semen and egg quality which

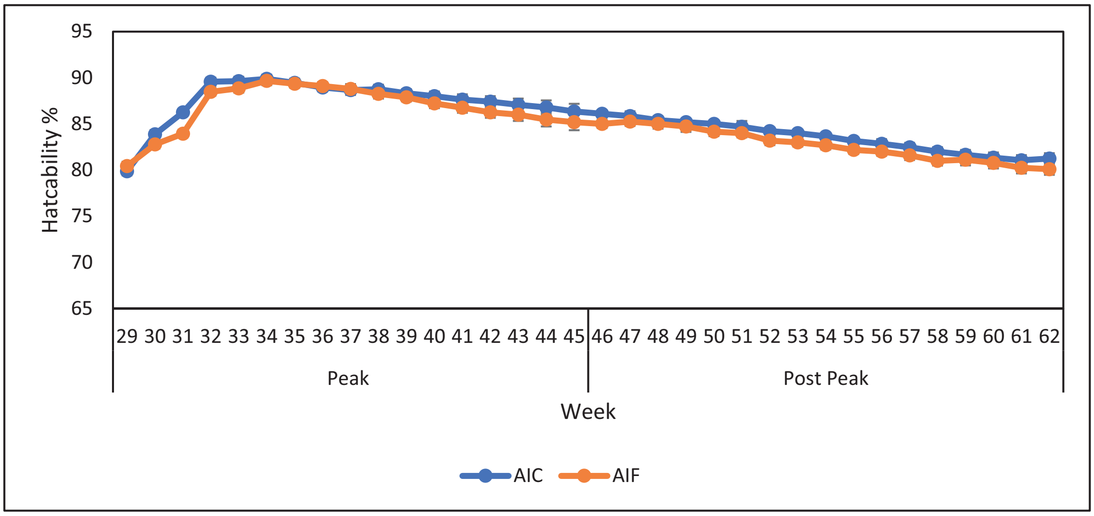

Figure 7. Trend of weekly hatchability % of broiler breeders affected by different housing systems
Abbreviations: AIC, artificial insemination in cages; AIF, artificial insemination in floor.

would have happened with progression of age. Hygienic conditions of eggs were different on floor and cages which might be another major influential factor affecting the hatching % of the fertile eggs. The margin of difference in hatchability by AIC to its competitors might be due to EM/DIS or hatchery loses in caged as compared to floored flocks (AIF). Whereas, dirty litter of floored flocks can be blemished for dirty fertile eggs which would lead to more exploder and DIS in hatchery. This argument is compatible with the study of Blount (2016) and Duru et al. (2017) who have studied that egg quality and hygiene are vital obligations for hatching of fertile eggs. We observed that poor quality fertile eggs (miss-shaped, week shelled, rounded eggs) irrespective to production systems and mating types might have dented the hatchability even hygienically were not bad (Peeble et al., 2000).

Regarding role of AI frequencies, excellent level of hatchability % was accomplished through AI repeated after 4 and 6th day even too good at 8th day in peak (Tables 1 and 2; Figure 9). However, the sustainable hatchability % was attained by repeating AI on 4th day and to satisfactory level on 6th day particularly in terminal phase of post peak. Although, similar work was conducted by Mohan et al. (2017c) where he got some satisfactory result at 7th AI yet age of PS not been discussed. These findings indicated that with progression of age, female required frequent insemination as its reproductive tract would have not able to preserve sperms for longer period (Froman et al., 2011).

The study unveiled another fact that the concentration of sperms per insemination seems not be a decisive contributor as compared to AI frequency in a younger PS (up to 45−48 wk of age). Contrarily, both AI frequency and sperm count /insemination do matter for older PS particularly after 50 wk. On the analysis of weekly data interaction (Figures 8 and 9), following regimen of AI frequency and sperm concentration could be

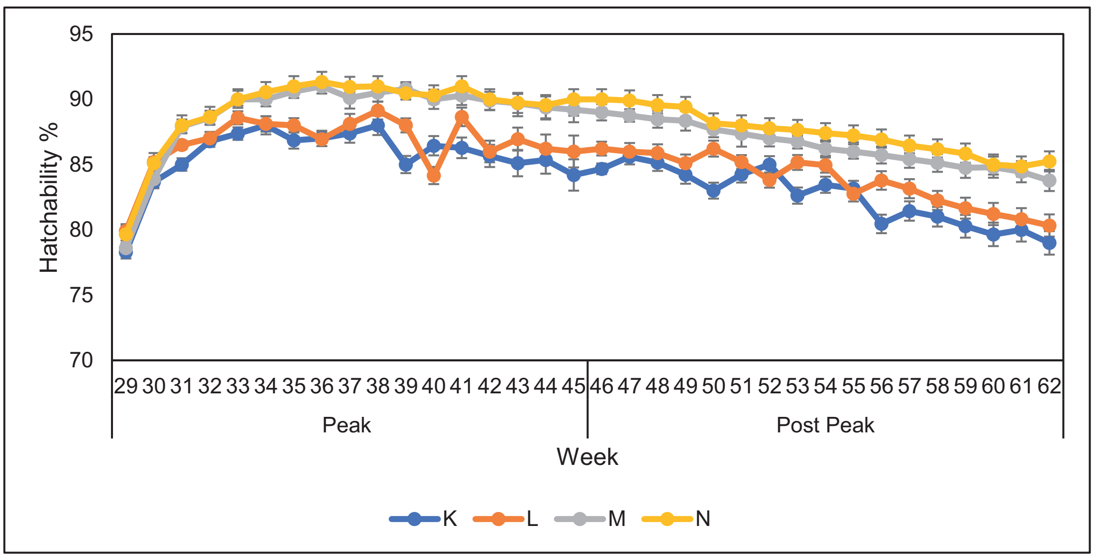

Figure 8. Trend of weekly hatchability % of broiler breeders affected by different sperm concentrations
K = 100, L = 125, M = 150, and N = 175 in million (during peak) K = 200, L = 225, M = 250, and N = 275 in million (during post peak).

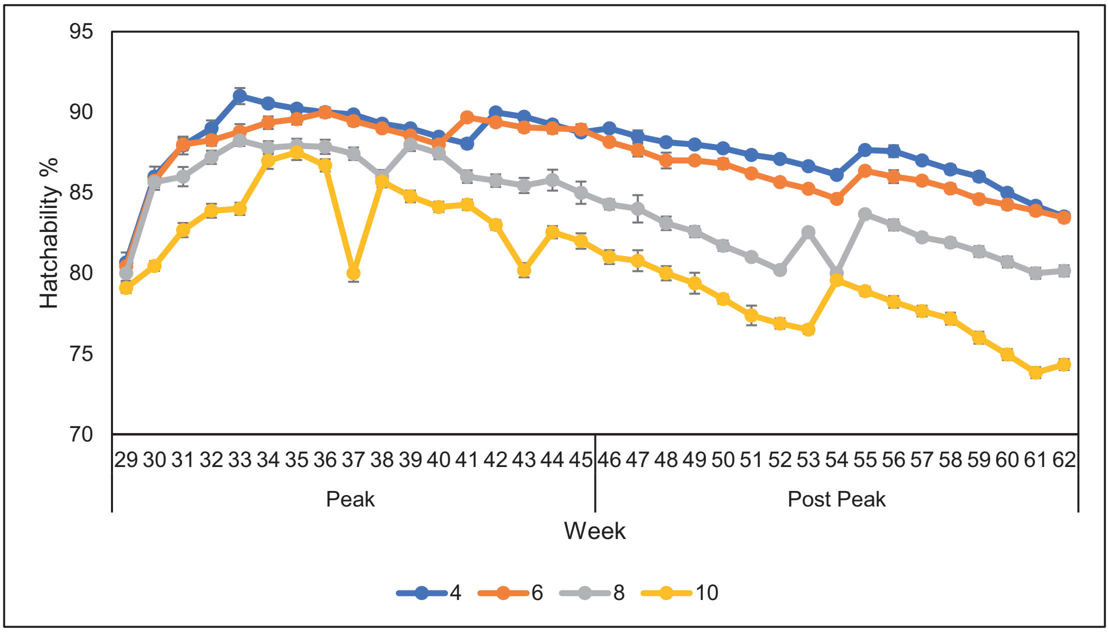

Figure 9. Trend of weekly hatchability % of broiler breeders affected by different insemination frequencies 4, 6, 8 and 10, Days of insemination frequencies.

established. During early peak phase (29 to 40 week) the 6 and 8th day frequencies along with moderate sperm concentrations (150×10^6 and 175×10^6) can produce hatchability close to standard (Anonymous, 2016b) but after that (40 to 50 week). AI should be repeated at 6th day interval with sperm concentration of 175×10^6. During the age of 50 to 55 wk only 4th day frequency proved up to the mark with sperms 250×10^6/concentration and afterward 275×10^6 sperms/insemination in terminal phase (56−64 wk). So retro production impact of AI could be mitigated by implementing the said schedule. This suggested program is in accordance to the fact that need of spermatozoa/insemination is directly proportional to the age of a hen while production sperms/milking reciprocal to the age of its counterpart (Beulah, 2017; Mohan et al., 2018). After peak, sharp decline of fertility and hatchability has been observed which could be associated with improper mounting capacity of males along with management issues (Hocking and Bernard, 2010). Moreover, we found during research that execution of vaginal douching, semen deposition along with hygienic measures are herculean tasks rather AI frequencies and sperm concentrations. Minor procedural negligence can spoil the whole exercise. Thus, through good management male's efficient reproductive activities can orient equally good hatchability even with natural mating.

Number of Chicks

Indeed, day old broiler chick (DOC) is ultimate product of breeder farmer as well as it is an earning tool for broiler farmer. Number of chicks/HH is real gage of production performance which can be influenced by certain intrinsic like health status and genetic potential and extrinsic factor including housing systems. The impact of latter one was explored and results directed that significantly ($P \leq 0.05$) more cumulative chicks/HH were produced by hens of AIC as compared to AIF (Tables 1, 3, and 5). But during peak phase AIC could excelled with minor difference of ($P \leq 0.05$) (Table 1). However, in post peak phase AIC significantly ($P \leq 0.05$) swerved from AIF as it produced higher chicks than AIF (Table 3).

Regarding interaction of frequencies, it was noticed that 4 and 6th day AI frequencies yielded the highest ($P \leq 0.05$) number of chicks than 8 and 10th day, respectively in peak phase (Tables 1 and 2). However, during post peak the highest ($P \leq 0.05$) chicks were achieved by repeating AI after every 4th day followed by 6 and 8 while the least no chicks could be received when AI was repeated on 10th intervals days (Table 3, 4).

Sperm concentrations of 175×10^6 and 150×10^6 on all frequencies were found better ($P \leq 0.05$) than 125×10^6 and 100×10^6 in peak phase (Tables 1 and 2). A 275×10^6 Sperm concentrations on all frequencies were found better ($P \leq 0.05$) than 250×10^6 which was significantly better than 225×106 and 200×10^6 in post peak phase (Tables 3 and 4). The stated findings are quite in accordance with pattern of fertility and hatchability of respective flocks i.e., AIC and AIF respectively. It could be concluded that fertility and hatchability made the decisive role in ultimate product the chicks (Hassan, 2006, 2009) which were attained through AI when conducted in cages as compared to being exercised in floored flock. Higher performance of AIC might be linked with the uniform body weight of experimental birds in cages (Silveira et al., 2014) as compared to floored birds where male's body weight was more ununiformed (Romero-Sanchez et al., 2004).

It is summarized that the best reproductive traits i.e., fertility%, hatchability% and chicks/HH were chronicled in flocks which were experiencing AI in cages as compared to on floor. Moreover, AI generated better average fertility, hatchability when repeated at the frequencies of 4 and 6th day followed by 8th day with sperms concentration of 150 and 175 × 10^6 during peak phase. Although, in early post peak (46−50 wk) the 4th and 6th AI frequency delivered the same results but after 50 wk only 4th day emerged as purposeful AI interval followed by 6th day by using 250 and 275 × 10^6 sperms. However, lesser EM was documented in fertile eggs being produced by caged flock or through natural mating.

CONCLUSIONS

It is concluded from the study that better productive (production %, SE) and reproductive performance (fertility %, EM, hatchability, chicks) were attained through AI in caged flock as compared to floored flock particularly during post peak phase. Similarly, the insemination frequencies proved to be more decisive than the sperm concentrations during peak production but both were found equally vital for reproductive performance in post peak phase. Moreover, requirement of sperms after shorter intervals increases with progression of age of hen.

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DISCLOSURES

No potential conflict of interest was found by the authors.

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