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Turkey hen fertility and egg production after artificial insemination and multiple oviduct eversion during the pre-laying period

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Summary. The onset of egg production (mean 18·3 days after the onset of photostimulation) and the rate of egg production (flock averaged 4·9 eggs per bird per week for the first 8 weeks of egg production) were not affected by 5 days of twice daily oviduct eversion ('venting') in the pre-laying period when compared to unvented controls. After the onset of photostimulation, pre-laying hens were inseminated twice daily on Days 12 to 16 with 3 µl semen containing 15×10^6 spermatozoa, and compared with groups of hens inseminated once daily on Days 15 and 16 with 15 µl semen containing 75×10^6 spermatozoa or 41 µl semen containing 200×10^6 spermatozoa. Fertility remained high for the first 5 weeks of egg production. However, by Week 6 the fertility of the hens receiving frequent low doses of semen dropped significantly below that of the others, which suggests that multiple inseminations with a low semen volume containing relatively low numbers of spermatozoa does not lead to an increase in the efficacy of sperm transport and storage in the oviduct.

Keywords: turkey; artificial insemination; pre-laying hens; photostimulation

Introduction

Artificial insemination (AI) of turkey hens before the onset of egg production generally results in higher hen fertility than in hens inseminated initially after the onset of egg production (McIntyre *et al.*, 1982, 1986). Although the physiological basis of these observations has not been established, McIntyre *et al.* (1982) suggested that there is "... an increased receptiveness of the hen's oviduct to spermatozoa during the time that natural mating frequency is highest".

The efficacy of sperm transport to the sperm-storage tubules of the uterovaginal junction may be related to the volume of semen introduced into the vagina. Considering that the male turkey copulates several times per day during the breeding season (Margolf *et al.*, 1947), one may assume that a small volume of semen is actually ejaculated during copulation. Sefton & Hawes (1971) noted that the chicken ejaculates about 20 µl semen during copulation. This, along with the observation that turkey hens before the onset of egg production are subjected to numerous completed and incompleted attempts at copulation by the male, would suggest that multiple inseminations of a small volume of semen may be efficacious with respect to the transport of spermatozoa to the sperm-storage tubules and subsequently lead to sustained high fertility. Therefore, the aim of this work was to investigate the fertility and duration of fertility of photostimulated hens inseminated before the onset of egg production with different volumes and numbers of spermatozoa. The effect of oviduct eversion (also referred to as 'venting' or 'breaking') on the onset and rate of egg production was also examined.

Materials and Methods

Large White turkey hens were moved to a light-proof environment-controlled house at 30 weeks of age, caged individually, and, after 3 days of acclimatization, photostimulated (lights on from 03:00 to 17:00 h). Hens were divided into four treatment groups, 14 hens/group. On Days 12 through 16 after the onset of photostimulation, Group 1 was inseminated with 15×10^6 spermatozoa (mean 3·1 µl semen) between 08:30 and 10:00 h and again between 13:00 and 14:30 h. Hens in Groups 2 and 3 were similarly subjected to oviduct eversion 8 times over a 5-day period but were not inseminated on Days 12, 13 and 14: on Days 15 and 16, these hens were inseminated at 08:30–10:00 h with 75×10^6 spermatozoa (mean 15·3 µl semen; Group 2) and 200×10^6 spermatozoa (mean 40·9 µl semen; Group 3). To assess the effects of oviduct eversion (everting the cloaca to exteriorize the vagina) on the onset and rate of egg production, hens in Group 4 were not handled.

Semen was collected daily between 08:00 and 08:30 h, diluted with an equal volume of Beltsville Poultry Semen Extender (BPSE; Sexton, 1980), mixed thoroughly, and divided into two equal parts. The semen to be used in the afternoon was stored at 4°C and occasionally agitated. Sperm concentration (Cecil, 1982) and viability (Bilgili & Renden, 1984) was determined immediately before each artificial insemination (AI) period. For AI, the tip of a micropipettor (Gilson Pipetman, Rainin Instru. Co., Inc., Woburn, MA) was placed gently against the everted wall of the vagina and the semen ejected simultaneously with the inversion of the vagina.

Eggs were collected 3 times daily, stored in a cooler at 15°C, and set weekly. Candling fertility was determined after 7 days of incubation. Hatchability could not be determined due to an incubator malfunction. Fertility (percentage) was calculated weekly from the first egg of the flock (Week 1) until the last fertile egg (Week 8).

Four hens from each of Groups 1, 2, and 3 were killed with an injection of pentobarbitone sodium 24 h after the last insemination and the utero-vaginal junctions were fixed in neutral buffered formalin. Sperm-storage tubules from sections of paraffin-wax embedded tissue were quantified by the method of Van Krey *et al.* (1971) with the exception that sperm-storage tubules were classified as 'with' or 'without' spermatozoa.

Statistical analyses were performed using the general linear models procedure of the Statistical Analysis System (SAS, 1982). Percentage data were subjected to arcsine or square root transformation for analyses. Duncan's multiple range test was used to indicate differences ($P < 0·05$) between means.

Results

Results for the first 8 weeks of egg production indicated that oviduct eversion twice daily for 5 consecutive days before the onset of egg production (Groups 1, 2, 3) did not affect the onset of egg production (mean ± s.e. time after the onset of photostimulation 18·3 ± 0·9 days) or rate of egg production (Table 1) when compared to unhandled control hens (Group 4) (flock averaged 4·9 eggs per week).

Table 1. Rate (mean ± s.e.) of egg production (total number of eggs laid by hens per week divided by 7) between hens subjected to oviduct eversion twice daily for 5 consecutive days (Groups 1, 2, and 3) and unhandled hens (Group 4) over an 8-week laying period*

	Weeks in production							
Group	1	2	3	4	5	6	7	8
1	0·40 ± 0·07	0·73 ± 0·06	0·79 ± 0·05	0·81 ± 0·04	0·70 ± 0·05	0·70 ± 0·86	0·69 ± 0·06	0·70 ± 0·06
2	0·40 ± 0·10	0·77 ± 0·06	0·83 ± 0·05	0·81 ± 0·04	0·80 ± 0·04	0·71 ± 0·04	0·73 ± 0·04	0·73 ± 0·03
3	0·55 ± 0·10	0·73 ± 0·05	0·81 ± 0·02	0·79 ± 0·04	0·84 ± 0·02	0·66 ± 0·04	0·70 ± 0·04	0·72 ± 0·03
4	0·34 ± 0·07	0·81 ± 0·03	0·76 ± 0·05	0·75 ± 0·06	0·71 ± 0·07	0·73 ± 0·07	0·62 ± 0·06	0·62 ± 0·08
Means averaged across treatments	$0·39^{d}$	$0·76^{ab}$	$0·79^{a}$	$0·78^{a}$	$0·75^{abc}$	$0·70^{bc}$	$0·67^{c}$	$0·68^{bc}$

*Contrasts between all Groups and Groups 1 + 2 + 3 *vs* 4 were not significant.

^{a,b,c,d}Presented as backtransformed least square means: means with different superscripts are significantly different ($P < 0·05$).

Table 2. Percentage fertility (mean ± s.e.) of eggs from hens inseminated in the morning and afternoon with 15 × 10^6 spermatozoa (mean volume 3·1 µl) on Days 12–16 (Group 1), or in the morning with 75 × 10^6 spermatozoa (mean volume 15·3 µl semen) or 200 × 10^6 spermatozoa (mean volume 40·9 µl semen) (Groups 2 and 3, respectively) on Days 15–16, after the onset of photostimulation

	Weeks in production							
Group	1	2	3	4	5	6	7	8
1	95·0[a] ± 3·3	93·2[a] ± 8·8	98·6[a] ± 1·4	86·1[a] ± 4·9	92·2[a] ± 4·1	64·3[b]* ± 8·5	23·9[c]* ± 5·3	0·0[d]
2	89·6[a] ± 5·6	96·3[a] ± 2·5	98·0[a] ± 2·0	93·3[a] ± 2·7	98·0[a] ± 2·0	85·7[a] ± 5·8	46·7[b] ± 8·5	5·7[c] ± 4·2
3	85·7[a] ± 5·9	95·8[a] ± 2·8	95·0[a] ± 2·5	96·3[a] ± 2·5	88·1[a] ± 4·2	86·0[a] ± 5·2	51·3[b] ± 11·1	2·0[c] ± 2·0

*Means significantly different ($P < 0·05$) from remaining means in column.
[a,b,c,d]Within each row means with different superscripts are statistically different ($P < 0·05$).

Semen volume and sperm number inseminated had little effect on fertility for the first 5 weeks of egg production (Table 2). Fertility averaged about 93, 95 and 92% for Groups 1, 2 and 3, respectively, for the first 5 weeks of egg production. Group 1 fertility dropped significantly in Week 6 to 64% and in Week 7 to 24%. Although fertility for Groups 2 and 3 remained high (about 86%) in Week 6, by Week 7 both groups dropped to 47% and 51% fertility, respectively. All groups were nearly infertile by Week 8 and infertile in Week 9. No significant differences were observed between the percentages (mean ± s.e.) of sperm-storage tubules containing spermatozoa 24 h after the last insemination in Groups 1 (36·2 ± 2·9%), 2 (28·2 ± 4·3%), and 3 (33·5 ± 4·0%).

Discussion

Since the 2–3-week period between photostimulation and the first oviposition corresponds to the period of highest mating activity in the turkey (Margolf *et al.*, 1947; Carte & Leighton, 1969), it was felt that multiple inseminations of low semen volumes may result in more efficient oviducal sperm transport to the sperm-storage tubules at the uterovaginal junction. However, the results do not support this hypothesis. While multiple AI and oviduct eversion of the hen do not delay the onset of egg production or the rate of egg production (at least for the first 8 weeks of egg production), they also do not increase the percentage or duration of fertility. To the contrary, the duration of fertility was greater in hens inseminated once on 2 consecutive days with 15 µl semen containing 75 × 10^6 spermatozoa (Group 2) than in hens inseminated twice daily for 5 consecutive days with 3 µl semen containing 15 × 10^6 spermatozoa (Group 1), even though both groups received a total of 150 × 10^6 spermatozoa. Furthermore, the fertility of hens inseminated once on 2 consecutive days with 41 µl semen containing 200 × 10^6 spermatozoa (for a total of 400 × 10^6 spermatozoa) (Group 3) was essentially identical to the fertility of the Group 2 hens receiving fewer spermatozoa.

The lower fertility during Weeks 6 and 7 and the overall shorter duration of fertility of Group 1 hens when compared to that of hens in Groups 2 and 3 is difficult to explain, particularly since the percentages of sperm-storage tubules containing spermatozoa were not significantly different between the three groups. However, such data are no indication of the total number of spermatozoa within the sperm-storage tubules. For Groups 1 and 2, the sperm concentration used for AI (5 × 10^6 spermatozoa/µl) and the total number of spermatozoa inseminated (150 × 10^6 spermatozoa) were identical. It is unlikely that holding semen for 3–6 h affected fertility since similar short-term (6 h) semen storage procedures using BPSE as the diluent have resulted in high hen fertility (Sexton *et al.*, 1984; Sexton, 1986). In addition, sperm viability (Bilgili & Renden, 1984) scores immediately before the morning (77%) and afternoon inseminations (76%) were nearly identical. It

is possible that short-term storage of turkey semen may not affect the rate of sperm acceptance into the sperm-storage tubules, but might, in some subtle manner, negatively affect the sperm viability after 5 weeks of storage in the tubules. That sperm-storage tubules were not fully differentiated either morphologically or functionally 12 or 13 days after the onset of photostimulation should be discounted since spermatozoa inseminated the first day of photostimulation will enter sperm-storage tubules already present at the utero-vaginal junction (Bakst, 1987) and such hens lay fertile eggs for about 3–4 weeks after the onset of egg production (unpublished data). Possibly extremely low volumes of semen do not allow adequate dispersal and optimal sperm transport of spermatozoa to the utero-vaginal junction. Another consideration when using such low volumes is the loss due to semen retention within the inseminating straw/tip. A submicrolitre quantity of semen retained in such a manner may represent a significant percentage of the total sperm number.

Compton & Van Krey (1979) suggested that only a finite number of spermatozoa are capable of entering the sperm-storage tubule within a given period. The absence of significant differences in fertility between Groups 2 and 3 may be because an insemination dose of 200×10^6 spermatozoa (Group 3) is in excess of the maximum number of spermatozoa which can be accepted by sperm-storage tubules in a given period.

Whether sustained high fertility in the turkey can be achieved after one or two inseminations of 3 µl semen containing 15×10^6 spermatozoa is not known. However, Lake & Ravie (1987) reported fertility levels greater than 90% in eggs laid on Days 2–8 after insemination in chicken hens inseminated with 20 µl of a 10-fold dilution (containing 10×10^6 spermatozoa) or 50 µl of a 46-fold dilution (containing $5{\cdot}45 \times 10^6$ spermatozoa) of semen diluted with chicken seminal plasma. They suggested that the homologous seminal plasma had a stimulatory effect on the spermatozoa at the time of insemination. Although turkey hen fertility ranged betweeen 36 and 75% over a 20-week period following AI of semen diluted 1:4 with synthetic diluents and containing 20×10^6 spermatozoa (Sexton, 1977), the implications of the work of Lake & Ravie (1987) with regard to turkey AI technology are that, given the appropriate diluent, e.g. homologous seminal plasma, extremely low sperm numbers may be capable of maintaining turkey hen fertility at adequately high levels. However, this needs to be investigated.

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