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Comparison of Oviductal Sperm Age on Fertility, Hatchability and Embryonic Death Rates in Iranian Indigenous and Ross-308 Broiler Breeder Chickens

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Abstract: The aim of this study, was to compare oviductal sperm age on fertility, hatchability and embryonic death in Iranian Indigenous and Ross-308 broiler breeder chickens. Twenty four Ross-308 broiler breeder and 24 Indigenous hens were used in this study. For insemination of those hens, 8 Indigenous and 8 Ross-308 broiler breeder roosters were used. Semen was collected from roosters by abdominal massage. Semen of each rooster was diluted in range that each insemination volume (0.1 mL) contains 100 billion sperm. After dilution, semen of each rooster was inseminated to 3 hens with the same strains of it. After single insemination of all hens, eggs were collected daily from the 2nd day after insemination which was the 1st day of fertile eggs. The collection and incubation of eggs continued for 21 days post insemination. Altogether, 3 hatches were taken for this study. The fertility, total hatch, hatch of fertile eggs, death of fertile eggs, early death, mid death and late death percent in each hatch were recorded for both Indigenous and Ross chickens. Maximum fertility for Ross and Indigenous chickens were on 3 days and 2 of fertility, respectively. For consecutive days, the fertility, total hatch and hatch of fertile rates in Indigenous chickens were significantly higher than Ross chickens ($p<0.05$). In both strains, there was a significantly positive correlation between fertility and hatch of fertile eggs rates and negative correlation between fertility and embryonic mortality rates ($p<0.01$). There was no trend toward the embryonic mortality at a younger age with increasing age of the spermatozoa.

Key words: Chicken, embryonic development, sperm seniority, strain

INTRODUCTION

Unlike to many mammalians, female birds have the capacity to store spermatozoa in their oviduct for varying amounts of time depending on the species. The utero-vaginal glands, defined as Sperm Storage Tubules (SST) are primary storage sites of spermatozoa in the bird's oviduct (Gumulka and Kapkowska, 2005). Infundibulum is the second storage region for spermatozoa (Bakst, 1993). Mechanism of sperm storage and slow release assures a succession of fertilized eggs in the absence of repeated copulation or artificial insemination (Brillard and Bakst, 1990). The exact mechanisms of prolonged sperm storage in the SST are unknown, but are thought to include reversible suppression of respiration and motility of spermatozoa as well as stabilization of the plasma membrane and maintenance of the acrosome (Donoghue and Wishart, 2000; Bakst, 1993). Fertility and hatchability performance of eggs depend on genetic, physiological, social and environmental factors (Islam *et al*., 2002). The fertility and hatchability are interrelated heritable traits and varies among breeds, varieties and individuals within breeds and variants (Islam *et al*., 2002). In domestic fowl, sperm numbers, type of hens (broiler or layer types) and age may affect the in vivo storage of spermatozoa and subsequently, the fertility of eggs. Strain effects on oviductal sperm storage were generally much higher than age and dose effects (Brillard, 1992). Sperm concentrations of 50×10^6-100×10^6 were adequate for good fertility in chickens and turkeys (Bratte and Ibe, 1989). Heritability of embryonic viability was moderate (Buss, 1989; Yoo and Wientjes, 1991; Beaumont *et al*., 1997). Early Embryonic Mortality (EEM) occurs during the 1st week of incubation, Middle Embryonic Mortality (MEM) occurs after the 1st week of incubation and before transfer in to hatchery and death in shell or Late Embryonic Mortality (LEM) occurs during the hatching period (Beaumont *et al*., 1997). The factors that affect on hatchability are egg fertility and embryonic mortality (Fairchild *et al*., 2002). Some of causes for embryonic mortality are prolonged egg storage, abnormal egg storage conditions, season of year, nutrition, egg

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size, age of the breeders and incubation problems (Fasenko *et al*., 1992; Meijerhof, 1992; Wilson, 1997; Fairchild *et al*., 2002). The aim of our study, was to evaluate the effect of oviductal sperm age (Sperm storage interval of SST) on fertility, total hatch, hatch of fertile eggs, death of fertile eggs and stages of death in Iranian Indigenous and Ross-308 broiler breeder chickens.

MATERIALS AND METHODS

This study was performed, from November 2007 in Department of Clinical Science, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran.

Twenty four Ross broiler breeder strain 308 and 24 indigenous hens with the same ages (30 weeks) and nearly the same weights (2.8 kg) were used at the beginning of this study. For insemination of this hens, eight Ross broiler breeder strain 308 and eight Indigenous roosters with the same ages (30 weeks) and nearly the same weights (3.2 kg) were used. Females were separated from males throughout the study. All hens and roosters were maintained in enclosed houses and were fed with their own standard breeder diet. All males and females received 16 h light day^{-1} throughout the study. Before beginning of AI, the hens were separated from roosters for 1 month and after this time, eggs collected and incubated for several days, to ensure that the females didn't have any fertile eggs. The roosters were trained to give semen 10 days before the collection began. Semen was collected by the abdominal massage method (Lake, 1957). Modified Ringer's solution was used as a diluent of semen (Martin, 2004). Immediately, after collection, semen of each rooster was evaluated microscopically by hemocytometer for sperm concentration and then each of this samples diluted in a range that every insemination volume (0.1^{cc}) contains the insemination dose of sperm (100×10^6 sperm). Immediately, after collection and dilution, the semen of each Ross and Indigenous rooster was inseminated to three Ross and Indigenous hens, respectively. The semen collection and insemination were made at the afternoon, because during the morning most hens have an egg in the oviduct, thus obstructing the free passage of semen to the ovary (Johnson and Parker, 1970). Day of inseminating was determined as a 0 day of fertilization. After single insemination of all hens, eggs were collected daily from the 2nd day post insemination, which was the 1st day of fertile eggs. Therefore, on throughout this study, 1 day represent 1st day of fertility, not 1st day after artificial insemination. The eggs were labeled in order to determine the strains (Ross or Indigenous) and days of fertility and were stored at a temperature of 15-18°C, with 70-80% relative humidity. The collected eggs over the period of 6 days were set for incubation. After 18 days of incubation, the eggs were candled and the eggs that didn't contain live embryos were removed and broken out for macroscopic examination of the early death (1-7 days), mid death (8-18 days) and infertile germinal disc. On 18 day of incubation, the eggs were transferred to hatchery. The eggs that failed to hatch were broken out in order to determine the late death (19-21 days). The collection and incubation of eggs continued for 21 days after single insemination, the time that there was no fertile egg. Altogether, sum of 1930 eggs of three hatches were taken in this study. The fertility, total hatch, hatch of fertile eggs, death of fertile eggs, early death, mid death and late death percent in each hatch were recorded for both Indigenous and Ross chickens.

Statistics: Statistical analysis was performed using the SPSS version 16. The percentages were reported as Mean±SEM. For each indigenous and ross chickens, differences in mean percentages of the variables for consecutive days were analyzed by one-way repeated analysis of variance and subsequent Duncan's multiple comparison test (post-hoc). Differences in mean percentages of the variables between strains were determined by Paired-Samples t-test. The correlations between fertility and embryonic mortality rates and also, fertility and hatchability of fertile eggs rates in both strains were analyzed by Pearson's bivariate correlation test (Petrie and Watson, 2006).

RESULTS AND DISCUSSION

Comparison of oviductal sperm age on fertility, total hatch, hatch of fertile eggs, death of fertile eggs and stages of death rates in indigenous and ross-308 broiler breeder chickens is presented on Table 1. As shown in table, after single insemination, the fertility rate in both Indigenous and Ross-308 chickens significantly increased from 1-2 day. In indigenous strain, fertility rate reached to maximum on 2 day and remained significantly constant till 3 day and then decreased slowly and reached to 0 on 19 day. In Ross strain, fertility rate reached to maximum on 3 day and remained significantly constant until 4 day and then dropped slightly and arrived to 0 on 17 day. The difference of fertility rate between two strains was significant on 2, 7, 8, 10, 12, 13 and 14 days ($p<0.01$) and 9, 11, 15, 17 and 18 days ($p<0.05$). Total hatch rate in Indigenous chickens reached to peak on 2 day and significantly bide constant until 6 day and then decreased slowly and reached to zero on 16 day. In Ross chickens total hatch arrived to maximum on 3 day and significantly constant until 4 day and then decreased slowly, reached

Table 1: Comparison of oviductal sperm age on Fertility, Total hatch, Hatch of fertile eggs, Death of fertile eggs and stages of death rates in indigenous and ross-308 broiler breeder chickens

		Variables (%)						
D.[A]	S.[B]	Fertility	Total hatch	Hatch of fertile eggs	Death of fertile eggs	Early deathC	Mid deathD	Late deathE
1	I	83.34±1.07^{a}	**75±0.86^{a}	90±0.12^{af}	10±0.12^{abc}	0±0^{a}	0±0^{a}	10±0.12^{b}
	R	78.58±0.88^{a}	71.43±1.11^{ae}	90.89±0.40^{ae}	13.27±3.83^{a}	0±0^{a}	10.24±6.04^{a}	3.03±3.03^{a}
2	I	**99.4±0.59^{b}	*91.67±1.24^{b}	92.23±1.75^{af}	7.77±1.75^{abc}	7.77±1.75^{ab}	0±0^{a}	0±0^{a}
	R	81.82±0.82^{b}	72.63±1.05^{a}	88.79±0.39^{ab}	11.20±0.40^{ab}	11.20±0.40^{ab}	0±0^{a}	0±0^{a}
3	I	99.28±0.72^{b}	90.90±0.17^{b}	*91.57±0.53^{af}	*8.43±0.53^{abc}	0±0^{a}	2.93±2.93^{a}	5.49±2.80^{ab}
	R	98.43±0.81^{c}	92.30±0.62^{b}	93.78±0.86^{cd}	6.22±0.86^{cd}	0±0^{a}	0±0^{a}	6.22±0.86^{a}
4	I	93.33±1.02^{c}	86.66±1.76^{bc}	92.18±1.46^{af}	6.99±0.71^{a}	6.99±0.71^{ab}	0±0^{a}	0±0^{a}
	R	97.93±1.03^{c}	93.57±0.93^{b}	95.56±1.44^{d}	4.42±1.43^{c}	4.42±1.43^{a}	0±0^{a}	0±0^{a}
5	I	91.82±1.20^{c}	88.24±1.05^{b}	93.77±0.51^{f}	6.23±0.51^{a}	0±0^{a}	0±0^{a}	6.23±0.51^{ab}
	R	94.11±1.16^{e}	88.23±1.67^{c}	93.74±0.62^{cd}	6.26±0.62^{cd}	0±0^{a}	6.26±0.62^{a}	0±0^{a}
6	I	93.33±2.15^{c}	86.67±1.97^{bc}	92.86±0.03^{af}	7.13±0.03^{ab}	0±0^{a}	7.13±0.03^{ab}	0±0^{a}
	R	87.50±1.10^{f}	81.25±0.23^{d}	92.88±0.90^{ce}	7.12±0.90^{cd}	1.85±1.85^{a}	0±0^{a}	5.27±2.67^{a}
7	I	**90.90±2.1^{e}	81.81±1.96^{c}	90±0.01^{af}	10±0.01^{abc}	10±0.01^{ab}	0±0^{a}	0±0^{a}
	R	75.09±1.98^{g}	68.71±2.27^{e}	91.58±0.61^{ce}	8.39±0.68^{bd}	0±0^{a}	0±0^{a}	8.40±0.61^{a}
8	I	**90.91±1.1^{e}	**81.82±0.8^{e}	**90±0.13^{af}	*10.03±0.16^{abc}	10.03±0.16^{ab}	0±0^{a}	0±0^{a}
	R	63.64±1.56^{h}	54.54±1.54^{f}	85.69±0.31^{f}	14.31±0.32^{a}	0±0^{a}	4.59±4.59^{a}	9.72±4.86^{a}
9	I	*78.58±1.66^{a}	**71.42±1.7^{a}	**90.88±0.25^{af}	**9.11±0.25^{abc}	0±0^{a}	9.11±0.25^{ab}	0±0^{a}
	R	61.54±0.33^{hi}	53.84±0.34^{f}	87.49±0.08^{bf}	12.51±0.08^{a}	4.17±4.17^{a}	13.27±0.76^{a}	0±0^{a}
10	I	**72.73±1.7^{d}	63.64±1.80^{d}	87.48±0.31^{a}	12.51±0.32^{bc}	8.52±4.26^{ab}	3.99±3.99^{a}	0±0^{a}
	R	59.73±1.62^{i}	53.33±1.84^{f}	88.85±0.68^{ab}	11.15±0.68^{ab}	7.04±3.53^{ab}	4.11±4.11^{a}	0±0^{a}
11	I	*66.67±2.01^{e}	58.33±2.79^{e}	87.40±1.56^{a}	12.60±1.56^{c}	0±0^{a}	0±0^{a}	12.60±1.5^{b}
	R	53.33±0.52^{j}	46.67±1.25^{g}	87.48±1.50^{bf}	12.52±1.50^{a}	4.17±4.17^{a}	8.35±4.43^{a}	0±0^{a}
12	I	**64.29±1.6^{e}	*57.14±1.40^{e}	88.89±0.12^{af}	11±0.12^{abc}	3.70±3.70^{a}	7.41±3.70^{ab}	0±0^{a}
	R	36.20±0.70^{k}	30.92±0.72^{h}	85.42±1.70^{f}	14.58±1.70^{a}	9.30±4.91^{ab}	0±0^{a}	5.28±5.28^{a}
13	I	**55.55±2.1^{f}	*44.44±2.20^{f}	79.93±0.90^{b}	20.07±0.90^{d}	0±0^{a}	13.89±6.96^{ab}	6.18±6.18^{ab}
	R	35.71±0.87^{k}	28.57±1.08^{h}	79.95±1.07^{g}	20.05±1.07^{e}	13.98±7.01^{ab}	6.07±6.07^{a}	0±0^{a}
14	I	**40±2.14^{g}	*20±3.23^{g}	*49.42±5.47^{c}	*50.58±5.47^{e}	18.39±9.32^{ab}	25.29±2.73^{ab}	6.90±6.90^{ab}
	R	8.3±1.04^{l}	0±0^{j}	0±0^{h}	100±0^{f}	0±0^{a}	33.33±33.3^{a}	66.67±33.3^{b}
15	I	*27.27±2.8^{h}	*9.09±1.85^{h}	*32.62±6.06^{d}	*67.36±3.52^{f}	33.68±1.76^{b}	33.68±1.76^{b}	0±0^{a}
	R	7.66±0.72^{l}	0±0^{j}	0±0^{h}	100±0^{f}	100±0^{c}	0±0^{a}	0±0^{a}
16	I	9.09±3.30^{j}	0±0^{j}	0±0^{e}	100±0^{g}	0±0^{a}	100±0^{c}	0±0^{a}
	R	7.14±1.35^{l}	0±0^{j}	0±0^{h}	100±0^{f}	33.33±33.3^{b}	33.33±33.3^{a}	0±0^{a}
17	I	*8.33±1.03^{i}	0±0^{j}	0±0^{e}	100±0^{g}	100±0^{d}	0±0^{a}	0±0^{a}
	R	0±0^{m}	-	-	-	-	-	-
18	I	*9.97±1.89^{i}	0±0^{j}	0±0^{e}	100±0^{g}	66.67±33.3^{c}	33.33±33.3^{b}	0±0^{a}
	R	0±0^{m}	-	-	-	-	-	-
19	I	0±0^{j}	-	-	-	-	-	-
	R	0±0^{m}	-	-	-	-	-	-

[A]One day is the 1st day of fertile eggs (2nd day after insemination). [B]Strain, i = Indigenous, R = Ross-308. [C,D,E]The time of embryonic death was assigned to one of three categories: Early death = 1-7 days, Mid death =8 -18 days, Late death = 19-21 days, [a-m]For each strain, means within a column with different superscripts differ significantly ($p<0.05$). *Means within a column between strains for each variable of Fertility, Total hatch, Hatch of fertile and Death of fertile differ significantly ($p<0.05$). **Means within a column between strains for each variable of Fertility, Total hatch, Hatch of fertile and Death of fertile differ significantly ($p<0.01$)

to 0 on 14 day. The difference in total hatch rate between 2 strains was significant on 1, 8 and 9 days ($p<0.01$) and 2, 12, 13, 14 and 15 days ($p<0.05$). Hatch of fertile eggs rates between Indigenous and Ross chickens was significant on 8, 9 days ($p<0.01$) and 3, 14, 15 days ($p<0.05$). The death of fertile eggs rates in both strains negatively followed the fertility rate and reached to 100% on 15 days (In Ross strain) and 16 (In indigenous strain). The difference of death of fertile eggs rates between 2 strains was significant on 9 days ($p<0.01$) 3, 8, 14 and 15 days ($p<0.05$). For both strains, the stages of embryonic mortality had fluctuations on consecutive days. The correlation between fertility and death rates was negatively significant in both indigenous ($p<0.01$, $r = -0.96$) and Ross ($p<0.01$, $r = -0.91$) chickens. The correlation between hatch of fertile eggs and fertility rates was positively significant in both Indigenous ($p<0.01$, $r = 0.96$) and Ross ($p<0.01$, $r = 0.91$) chickens.

In this study, the results of indigenous and ross chickens eggs that were collected daily after a single insemination, showed a significant increase in fertility on the day after the first day of fertile eggs. This is in accordance with data of Lodge *et al.* (1971) on leghorn chickens. Salisbury and Hart (1970) showed that in cattle as the duration of in vitro sperm storage prolonged, there was an initial increase in the fertility of samples as estimated by the frequency of non return rates to service. Non returns reached a maximum on a 2nd day of storage at 4°C and subsequently exhibited a linear decline with increased storage time. Studies with rabbits (Lodge *et al.*, 1971) have shown an initial increase in fertility to a maximum followed by a decreased fertility and

increased embryonic loss with *in vitro* aged sperm. Perhaps the single most unanticipated finding in these studies was that spermatozoa fertility increased for a time after storage. One theory in cattle assumed that immediately after ejaculation, spermatozoa containing aberrant chromatin, or those which are otherwise abnormal, may compete effectively with normal sperm for fertilization sites. Later, they are unable to do so and higher proportions of the available ova are then fertilized by more normal spermatozoa, resulting in higher fertility before aging of spermatozoa and reducing fertility (Salisbury and Hart, 1970). The findings of this study, in both indigenous and ross chickens concurs with the observation of other study who stated that in chicken spermatozoa aging result in gradual loss with time in the capacity of the gamete to effect fertilization and to support normal embryological development (Lodge *et al*., 1971). In this study, there were a positive correlation between fertility and hatchability of fertile eggs in both Indigenous ($p<0.01$, $r = 0.96$) and Ross ($p<0.01$, $r = 0.91$) chickens, that was similar to result of Beaumont *et al*. (1997) on leghorn hens and Islam *et al*. (2002) on white Leghorn, Rode Island red and New Hampshire strains. In the present study, the fertility rate of Indigenous chickens reached to zero on 19 day after single insemination, while it was on 17 day for Ross chickens. In one study by Bramwell (2002), on broiler breeder flock, the fertility reached to zero on 22 day after single insemination, but the strain of flock were unknown. As show in table, the fertility, total hatch and hatch of fertile eggs rates of Indigenous chickens in most of the days were significantly higher than Ross chickens and for all days, there was not any significant increasing in fertility and totl hatch of Ross chickens as compared with Indigenous chickens. Hatch of fertile rate of Ross chickens on 3 day was significantly higher than indigenous chickens. In present study, there was a negative correlation between fertility and death of fertile eggs rate for both Indigenous ($p<0.01$, $r = - 0.96$) and Ross ($p<0.01$, $r = - 0.91$) chickens, that was similar to results of Beaumont *et al*. (1997) on Laying hens and Fairchild *et al*. (2002) on turkey. In this study, death of fertile rate on Ross chickens on 8, 9, 14 and 15 days was significantly higher than Indigenous chickens. Only on 3 day, the death of fertile egg rate for Indigenous chickens was significantly higher than Ross chickens. Bramwell (2002) indicated that old and stale sperm in the oviduct is associated with poor chick quality and early embryonic death. In present study, with increasing oviduct sperm storage time (aging of sperms) in both strains, mortality rate increased but embryonic death stage has not special arrangement in consecutive days and there was no trend toward the embryos dying at a younger age with increasing age of the spermatozoa.

CONCLUSION

It is concluded that: following single insemination, maximum fertility for Ross and Indigenous chickens were on 3 and 2 days of fertility, respectively, not on 1 day of fertility, following single insemination for consecutive days, the fertility, total hatch and hatch of fertile eggs rates in Indigenous chickens were significantly higher than Ross chickens, in both strains, there was a significantly positive correlation between fertility and hatch of fertile egg rates and negative correlation between fertility and embryonic mortality rates and this study demonstrated that there was no trend toward the embryonic mortality at a younger age with increasing age of the spermatozoa.

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