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Original Research Paper

Effect of season and age on Indian red jungle fowl (*Gallus gallus murghi*) semen characteristics: A 4-year retrospective study

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Running Title: Effect of season and age on semen characteristics of Indian Red Jungle Fowl

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ABSTRACT

The reproductive potential of the adult males is expected to vary with age/season and largely differ not only in closely related avian species but even in subspecies, breeds and/or strains of the same species. Thus, it is pre-requisite to have knowledge of seminal parameters to achieve maximum production potential of at-risk species for *ex situ in vitro* conservation programs. A 4-year study was designed to evaluate the effect of age and season (spring, summer, autumn and winter) on semen characteristics of Indian red jungle fowl (*Gallus gallus murghi*) in retrospective manner. Semen ejaculates (n=1148) were regularly collected from eight adult Indian red jungle fowls from 6 to 54 months of age. Quantitative and qualitative semen parameters viz; volume (µL), concentration ($1x10^9$ mL^{-1}), total sperm number per ejaculate ($1x10^9$ mL^{-1}), motility (%), viability (%), plasma membrane integrity (%), acrosome integrity (%) and semen quality factor were recorded. A chronological increasing trend with age of most sperm quantitative and qualitative traits (semen volume, sperm concentration, total sperm number per ejaculate, plasma membrane integrity, viability, acrosomal integrity, semen quality factor) was observed. The highest values were observed at four years of age ($P < 0.05$) with the exception of sperm motility that was not affected by the age. Spring was the best season for sperm parameters viz; volume, motility, plasma membrane integrity, viability and acrosomal integrity ($P < 0.05$), however a remarkable sperm production was noticed all over the year. It is concluded that Indian red jungle fowl exhibit an evolution of sperm production that greatly differs in many points from other fowl sub-species. It is suggested that semen ejaculates of highest quality achieved for semen banking at the age of four year in the spring season.

Keywords: Semen characteristics; Age; Season; Quantitative semen quality; Qualitative semen quality; Indian red jungle fowl

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1. Introduction

The red jungle fowl, a member of family Phasianidae and genus *Gallus* is considered one of the ancestors of the domestic fowl [1-3]. The red jungle fowl is enlisted as least concern species in IUCN Red List of Threatened Species [4] but its population is continuously declining in its native habitat [2,5]. Red jungle fowl is considered as endangered in Singapore list of Threatened Plant and Animal Species, facing high risk of extinction in the wild [6]. From five fowl subspecies, Indian red jungle fowl (*Gallus gallus murghi*) is native to Northern India and Azad Jammu and Kashmir, Pakistan [7-10]. The Indian red jungle fowl population is found in restricted areas in fragments, and is more likely to collapse of genetic diversity and population decline due to sudden unexpected threats [2,11-13]. The factors contributing in population reduction are habitat destruction, egg collection, predation and genetic hybridization with domestic fowl [7,13].

The decline in Indian red jungle fowl needs special attention; several reports suggest adopting appropriate *ex situ* conservation strategies [2,11,12]. For *ex situ in vitro* conservation, semen banking is considered one of the most applicable options for bird species that are facing continuous decline in their natural habitat and the threats cannot be easily mitigated and/or ameliorated [14-20]. Cryo-banking of valuable male germplasm could be an important assisted reproductive biotechnology to maintain genetic diversity and *in vitro ex situ* conservation program [8,9]. It is well established that avian semen can be stored in frozen state for infinite period of time without significant decline in fertilizing potential [17,21,22]. The characteristics of the semen ejaculate viz; volume, concentration, motility, viability, plasma membrane and acrosome integrity, semen quality factor determine the reproductive potential of the cocks either used in natural or artificial breeding program [8,9,23-25]. Nevertheless, semen quality varies with age and season of collection in birds that may affect fertilizing capacities accordingly [26-28]. The success of semen banking depends

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on successful cryopreservation and acceptable frozen thawed semen quality and fertility outcomes that depend on the initial quality of the ejaculate to be used in processing for cryopreservation [17,20,29]. The reproductive performance of the male fowl varies with age and seasons; largely differ not only in closely related bird species but also even in subspecies, breeds and/or strains of the same species [30]. Scarce available literature depicted immense variations in semen characteristics of five subspecies of red jungle fowl [8,31]. The knowledge about retrospective seminal parameters is therefore the perquisite to achieve maximum production potential of at-risk species for *ex situ in vitro* conservation.

Therefore, present, 4-year retrospective study was designed to elucidate the effect of age and season (autumn, spring, summer and winter) on semen characteristics (motility, semen volume, sperm concentration, total sperm number per ejaculate, viability, semen quality index, plasma membrane and acrosome integrity) of Indian red jungle fowl.

2 Materials and methods

2.1 Experimental birds

The chicks of Indian red jungle fowl were reared at Avian Research Center, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. The Rawalpindi region (33° 36' 0" North, 73° 4' 0" East) is located in Southern slopes of the North-Western Himalayas including large mountain tracks with rich valleys traversed by mountain rivers. This region falls in humid and subtropical region having four seasons; autumn (13.9°C to 33.5°C), spring (10.1°C to 23.7°C), summer (21.5°C to 46.5°C) and winter (-2.8°C to 20.0°C). The average annual rainfall of this region is about 1,249 millimeters. The average rainfall in each season was 73.13 mm/autumn, 39.2 mm/spring, 224.73 mm/summer and 37.3 mm/winter recorded during the study period. The average temperature of the area ranges from -3.9°C to 48.3°C (Pakistan Metrological Department, 2012-2015). The chicks were kept in individual pens

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106.68 cm x 121.92 cm, exposed to natural day-light and standardized environmental conditions without any significant changes during the entire 4-year study period. The birds were offered commercially available poultry cock breeder feed (100g/day). Fresh water was available to the birds all the daylong throughout the experimental period. The birds were subjected to abdominal massage and were trained for semen collection until the birds were able to donate neat and clean semen without fecal contamination. This training was continued until we were able to get sufficient semen for further processing and experimental work. Semen collection of eight cocks was performed on alternate days, depending on individual cock quality and libido.

2.2. Semen collection and quantitative evaluation

The birds were subjected to semen collection training through abdominal massage as described by Burrows and Quinn [8, 32,33]. The successful ejaculate was collected after 4 weeks of training. Semen was collected from individual birds in a graduated plastic tube. A total of 1148 ejaculates were studied during the 4-year study period (2012-2015). Semen volume was measured in microlitres using micropipette.

2.2.1 Sperm progressive motility

Sperm motility of each ejaculate was determined as described by Zemjanis [34] by mixing 10 µL of semen samples in 500 µL of phosphate buffer saline (pH 7.2, 300 mOsm/kg). The percentage of motile sperm was subjectively evaluated on a scale ranging from 0 to 100% by putting a drop of semen sample on a pre-warmed glass slide (37°C) under phase contrast microscope (x400, Olympus BX20, Japan).

2.2.2 Sperm concentration

Sperm concentration was measured by taking 1µL of semen and 200 µL of formal citrate solution (1mL of 37% formaldehyde in 99 mL of 2.9% (w/v) sodium citrate) with

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Neubauer haemocytometer (Marienfeld, Germany) under phase contrast microscope (x400, Olympus BX20, Japan) throughout the 4-year study period [8].

2.2.3. Total sperm number per ejaculate

Total sperm number per ejaculate was obtained by multiplying the total volume with the concentration of ejaculates obtained during the study period [8, 35].

2.2.4 Plasma membrane integrity

Plasma membrane integrity of spermatozoa was assessed by using hypo-osmotic swelling test (HOS) as described by Santiago-Moreno et al. [36]. The HOS solution was prepared by adding 1 g of sodium citrate to 100 mL of distilled water. Semen (25 µL) was mixed with 500 µL of HOS solution (100 mOsm/kg) and incubated at 37°C for 30 minutes. A drop of incubated solution was placed on a pre warmed (37°C) slide and fixed in buffered 2% glutaraldehyde. The spermatozoa showing swollen heads, swollen and coiled tails were classified as normal spermatozoa having intact plasma membrane. A total of 200 spermatozoa were counted at four separate fields under a phase-contrast microscope (1000x with oil immersion).

2.2.5 Sperm viability

Viability (% Live / total sperm) of Indian Red Jungle Fowl spermatozoa was examined by adding eosin-nigrosin to the lake glutamate solution [37]. Twelve drops of stain were mixed with 1 drop of semen. A smear was made on a glass slide, fixed and air dried. A total of 200 spermatozoa were studied per slide under a phase-contrast microscope (1000x with oil immersion). The mixture provides a clear background in the smear to enhance the contrast of white and pink. Unstained and straight sperm were considered as “live and morphologically normal” while the pinkish stained as “dead ” sperm.

2.2.6 Acrosomal integrity

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Acrosomal integrity of Indian Red Jungle Fowl spermatozoa was assessed through Giemsa stain [38]. The stain was prepared by adding Giemsa (3g) and phosphate buffer saline at pH 7.0 (2 mL) into 35 mL water. Smear was prepared by taking a drop of semen sample on a clean glass slide, dried and fixed in neutral formal-saline (5% formaldehyde) for 30 min. Fixed slides were kept in Giemsa stain for 1.5 hrs. Sperm with normal acrosome appeared to be evenly stained; abnormal spermatozoa were unevenly stained while spermatozoa having ruptured acrosome remained unstained. A total of 200 spermatozoa were observed at least in four separate fields under a phase-contrast microscope (x400; Olympus BX20, Japan) at magnification of1000x with oil immersion.

2.2.7 Sperm quality factor

Sperm quality factor (SQF) of each semen ejaculate was calculated to check the number of spermatozoa potentially capable of fertilization in ejaculate. Sperm quality factor was calculated by the following formula [24].

$$\mathrm{SQF} = \frac{\text{Sperm concentration } \left(\frac{\text{billion}}{\text{mL}}\right) \text{ x ejaculate volume (mL) x normal livespermatozoa}}{100}$$

2.3 Statistical analysis

The data were analyzed with statistical software Megastat®version 7.25 Mc-Graw-Hill New Media, New York, for excel. The descriptive statistical parameters (mean, standard error of mean) were calculated for different age groups and seasons. To compare differences between seasons and age groups, data were analyzed using analysis of variance in completely randomized design with unequal replications. Post hoc comparisons between the means were done though least significant difference test to determine differences among individual groups at different ages and seasons. Differences were considered statistically different when $P<0.05$, and presented as Mean ± SEM.

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3. Results

The data on ejaculate volume, sperm concentration and total sperm number per ejaculate of red jungle fowl from 6 to 54 months of age are given in Table 1. Semen volume, sperm concentration and total sperm number per ejaculate was recorded significantly (P<0.05) higher at 43-54 months of age followed by 31-42, 19-30 and 6-18 months of age. The highest (P<0.05) semen ejaculate volume, sperm concentration and total sperm number were recorded in spring season followed by winter, autumn and summer from 6 to 54 months of age.

The data on qualitative semen quality parameters viz; motility, plasma membrane integrity, viability, acorosme integrity and semen quality factor were given in Table 2. Sperm motility did not differ (P>0.05) with increase in age from 6 to 54 months of age. Nevertheless, an increase was observed in plasma membrane integrity, viability, acrosome integrity and semen quality factor with the increasing age. The highest (P<0.05) values for aforementioned parameters were recorded during 43-54 months of age compared to 31-42, 19-30 and 6-18 months of age. A highly positive (P<0.05) effect of spring season was recorded on most of the quality parameters with the exception of motility that was equivalently efficient in autumn, spring and summer and only altered in winter.

4. Discussion

In conservation programs, the success of AI and semen cryopreservation depends on the initial semen quality of ejaculate [17]. Semen evaluation, based on *in vitro* measurement of sperm motility, plasma membrane integrity, viability, acrosomal integrity and semen quality factor (SQF) provides an indirect clue about the fertility potential of the males [25,8,23,24]. The production of high quality semen ejaculates depends on the reproductive cycle of the species; furthermore, species or subspecies specific variations depend on the birds, their age, their environment including climate changes such as seasons [30, 36,39-46].

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In contrast to domestic fowl, we showed for the first time in the present study that the highest production and quality of semen of Indian red jungle fowl is not reached until the fourth year of age and spring stays the best season.

Our results greatly differ from the observations made in most domestic fowl as Long et al. [47] reported a decrease in sperm concentration from 1 to 1.5 years of age, a well-known fact in the breeding practice. Nevertheless, this situation would be modulated in local breeds of tropical area as Chotesangasa [48] and Sonseeda et al. [45] found an increase in Thai indigenous chicken sperm concentration between 1 and 2 years of age. However, our results with Indian red jungle fowl appeared to be “extreme” as 4 years of age is the best in terms of sperm concentration and volume. Semen volume has already been reported to differ with the age in Leghorns [30]. Contrary to our findings, broiler males showed maximum semen volume in the first year of age and then decreased with age, a difference with here, one of the presumed ancestors, the red jungle fowl. As a way of consequence, the total number of sperm per ejaculate increased with the age, and was observed highest when the Indian red jungle fowls were 4 years old. This leads to suggest an increase in the efficiency of sperm production with the age in this wild fowl. Many hypotheses may arise from this observation including suggestion of increasing adaptation of the species to its environment, nutrition, and management conditions. However, due to low mean volume of semen, total sperm number per ejaculate (billion) were still lower in Indian red jungle fowl compared to Malaysian red jungle fowl [31] and domestic fowls [47,49]. Would there be a sort of “compensation” of this low daily sperm production through and increasing of the longevity of sperm production?

In addition to the increase of sperm production with the age, it was evident that the functionality of the sperm produced by Indian red jungle fowl increased with the age to reach a maximum at 4 years as most of the quality parameter measured here (Hypo-osmotic test, livability, acrosome integrity) were at their optimum at 43-54 months of age.

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More precisely, Indian red jungle fowl spermatozoa showed greater resistance to the hypo-osmotic solution at 37°C at 4 years of age. These changes would indicate that sperm plasma membrane increases its "plasticity" with the age and thus its functionality. Indeed, the response of spermatozoa towards hypo-osmotic solution in terms of swollen head and coiled tail is due to the movement of physical and biochemical compounds which play essential roles in maintaining the viability and fertilizing ability of sperm [50-52]. Shanunagum et al. [44] reported that response of spermatozoa to the hypo-osmotic environment at one year of age was maximum compared to 6 months of age; and described that age and genetic factors contribute to the quality of semen in naked neck and dwarf chicken. But none of these studies examined 3 or 4 years old fowl, thus limiting the potential comparison with our study.

Sperm viability and the integrity of the sub-cellular fraction of sperm such as the acrosome are of absolute significance for the success of fertilization. In the present study, viability and acrosome integrity were highest at the age of 43-54 months, a fact that is opposite to the results described in previous reports. Indeed, decreases in viability and sperm morphological integrity have previously been reported to coincide with the end of a reproductive period between 1 and 1.5 years of age in free-range poultry [44], turkeys [53] and roosters [54,55]. Such decrease has been related to changes in lipid composition and decrease in fertility [55]. However, for these parameters also, the comparison between the literature results and our results is limited since older ages were not explored in earlier studies. One hypothesis to explain results could be due to late optimal sexual maturity in Indian red jungle fowls. The SQF is dependent on the quantitative parameters of semen and on one qualitative parameter, the number of morphologically normal and live spermatozoa. In the present study, the SQF was significantly higher at 43-54 months of age compared to other ages. As discussed earlier, the Indian red jungle fowl is a resident of Temperate and Tropical forest and developed a high tolerance to environmental variations that could be "cost-

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intensive" for the animal metabolism and consequently the sexual maturation. We suggest that the Indian red jungle fowl would get fully mature at later ages compared to other fowls [31].

Sperm motility was the only quality factor that was not affected by the age in the present study, partially confirming the results obtained by Sonseeda et al. [45] on Thai indigenous chicken of over 30 months. Sperm motility is a predictive parameter of viability and quality of semen. However, in the present study, this parameter did not follow the evolution of the viability measurements. This could indicate a specific reproductive maturation process where the different functions of the sperm do not reach the maturity at the same time; the signaling pathways and flagellar structures related to motility being fully mature much earlier than plasmatic membranes and acrosome attributes.

The sperm production, volume and concentration of sperm were higher in spring and low in the other seasons with a remarkably low semen volume in summer. The sperm production is highly species-specific and even varies among different strains or breeds [46], and may vary considerably throughout the year and seasons among avian species [30,40]. Environmental factors such as photoperiod and temperature affect semen production. In mild climates with large range of photoperiods/temperatures, domestic fowl sperm production is totally stopped for some months during autumn/winter and reaches maximum during spring. In subtropical desert and in Saharan climates with a smaller range of annual photoperiods, sperm production may be severely affected by the extremely hot temperatures and lack of precipitation of the summer [30,56]. These environmental cues have also been expressed in a breed specific manner. Some breeds show consistent semen production throughout the year as in Thai chicken held in tropical humid climates [45,57,58].

Our study shows that the seasonal sperm production of Indian red jungle fowl is not so far from the Thai indigenous chicken "model", but also shows characteristics close to the

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"Saharan model" with summer variations potentially related to the high temperatures of the Pakistan summer. Indeed, deleterious effects of high temperatures on germ cells have already been described [59]. The results of the present study are in agreement with previous studies on Northern pintail ducks and Nigerian local domestic chicken in which high summer temperatures caused temporary reduction in sperm production [40,43,59]. The low volume of semen in hot summer could tentatively be related to the absence of cutaneous heat evaporation that cannot mitigate the stressful events of heat disruption in summer season [60].

In our study, spring was identified as the best season for the SQF that mixes quantitative and viability sperm traits and for many other sperm quality traits (motility, viability, acrosome integrity). In mild climates, the lengthening of photoperiod in spring season acts as an environmental cue, stimulates the secretion of FSH, LH and testosterone, which play important role in spermatogenesis and semen production [61]. Photoperiod increase has previously been reported to increase the number of morphologically normal and live sperm as well as motility and SQF in domestic fowls such as free range poultry, Leghorns and others [30,62,63]. At the opposite, poor livability and viability of sperm in winter or autumn have directly been associated with the shortening of the photoperiod in free-range poultry [64] and turkey [53]. Poor motilities were also reported in correlation with loss of energy and mitochondrial integrity in shortened photoperiod compared to spring collected semen [65]. Seasonal differences in mitochondrial permeability [66] and its membrane phospholipids composition [67] could also contribute to explain the greater sensitivity of sperm in time of short photoperiod [64].

In the "Saharan climate," the semen quality of Nigerian shika brown in terms of live and normal sperm was reported maximum in winter season, which is in contrast to the present study [68]. The reason of these differences could possibly be attributed to heterogeneity of

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the semen quality among breeds, strains and species-specificity [47], but also to the specific Saharan climate. The terrible dry and hot summer would have a significant role in the adaptation of the reproductive season, the best season of reproduction being consequently moved to winter. Regarding this parameter, the "Pakistan" environment of the Indian red jungle fowl presents a more classical but "mixed" seasonal effect since sperm production is present all over the year.

It is concluded that Indian red jungle fowls exhibit better semen quality in spring season and chronological increasing trend with maximal quality at the age of 43-54 months in terms of volume, sperm concentration, total sperm number per ejaculate, motility, viability, semen quality factor, plasma membrane and acrosome integrity. It is suggested that semen ejaculates of maximal quality can be achieved at the age of four years in the spring season. These elements are very important in order to build an AI program for the conservation of genetic diversity of the Indian red jungle fowl.

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Table 1: Effect of season and age (Mean ±SE) on quantitative efficiency of spermatogenesis in Indian red jungle fowl: A 4-year retrospective study (n=8cocks/1148ejaculates)

		Age in months			
	Season	6-18	19-30	31-42	43-54
Semen volume (µL)	Autumn	$41.9 \pm 5.1^{c,z}$	$56.5 \pm 4.4^{b,y}$	$68.0 \pm 7.5^{b,y}$	$73.4 \pm 2.9^{c,x}$
	Spring	$88.7 \pm 9.5^{a,z}$	$78.5 \pm 7.6^{a,y}$	$106.6 \pm 6.8^{a,x}$	$160.1 \pm 8.2^{a,w}$
	Summer	$25.3 \pm 4.3^{d,z}$	$26.4 \pm 2.8^{d,y}$	$25.6 \pm 1.9^{c,x}$	$39.9 \pm 3.6^{d,w}$
	Winter	$41.1 \pm 4.1^{b,y}$	$44.2 \pm 2.9^{c,y}$	$63.3 \pm 5.4^{b,x}$	$93.8 \pm 4.2^{b,w}$
Sperm concentration ($1x10^9$ mL^{-1})	Autumn	$0.45 \pm 0.1^{b,z}$	$0.95 \pm 0.2^{b,y}$	$2.09 \pm 0.3^{a,x}$	$3.00 \pm 0.2^{b,w}$
	Spring	$1.11 \pm 0.2^{a,z}$	$1.83 \pm 0.3^{a,y}$	$2.50 \pm 0.2^{a,x}$	$3.41 \pm 0.3^{a,w}$
	Summer	$0.86 \pm 0.2^{a,z}$	$0.91 \pm 0.1^{b,y}$	$2.00 \pm 0.3^{a,x}$	$2.54 \pm 0.1^{b,w}$
	Winter	$0.53 \pm 0.1^{b,z}$	$1.14 \pm 0.1^{b,y}$	$2.37 \pm 0.2^{a,x}$	$3.01 \pm 0.2^{b,w}$
Total sperm per ejaculate ($1x10^9$)	Autumn	0.03b,y	0.06b,y	0.14b,x	0.22b,w
	Spring	0.12a,z	0.15a,y	0.21a,x	0.37a,w
	Summer	0.02b,x	0.04b,x	0.07b,x	0.13c,w
	Winter	0.02b,y	0.04b,y	0.14b,x	0.25b,w

The values (mean ± SE) for a given semen parameter in a column (season = a, b, c, and d) and row (age = w, x, y and z) with different superscript differ significantly ($P<0.05$).

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Table 2: Effect of season and age (Mean ±SE) on qualitative semen parameters in Indian red jungle fowl: A 4-year retrospective study (n=8 cocks/ 1148 ejaculates)

		Age in months			
	Season	6-18	19-30	31-42	43-54
Sperm motility (%)	Autumn	$74.8 \pm 1.7^{b,x}$	$75.7 \pm 1.7^{b,x}$	$76.9 \pm 2.9^{b,x}$	$82.7 \pm 3.4^{a,x}$
	Spring	$77.8 \pm 2.4^{a,y}$	$82.1 \pm 2.7^{a,y}$	$83.9 \pm 5.0^{a,y}$	$88.9 \pm 1.8^{a,x}$
	Summer	$61.9 \pm 2.2^{c,x}$	$66.8 \pm 2.2^{c,x}$	$71.5 \pm 4.4^{b,x}$	$78.0 \pm 4.8^{b,x}$
	Winter	$36.0 \pm 1.1^{d,z}$	$42.1 \pm 5.5^{d,y}$	$46.9 \pm 3.4^{c,y}$	$58.8 \pm 3.1^{c,x}$
Sperm plasma membrane integrity (%)	Autumn	$16.7 \pm 1.9^{b,z}$	$27.3 \pm 3.0^{c,y}$	$48.3 \pm 5.1^{b,x}$	$84.7 \pm 1.3^{c,w}$
	Spring	$44.0 \pm 3.4^{a,y}$	$45.8 \pm 3.8^{a,y}$	$56.9 \pm 2.8^{a,x}$	$95.3 \pm 1.5^{a,w}$
	Summer	$21.3 \pm 1.8^{b,z}$	$29.5 \pm 4.1^{c,y}$	$44.9 \pm 3.5^{b,x}$	$90.9 \pm 0.8^{b,w}$
	winter	$17.8 \pm 2.4^{b,z}$	$38.1 \pm 3.9^{b,y}$	$47.7 \pm 6.7^{b,x}$	$67.8 \pm 4.4^{d,w}$
Sperm livability (%)	Autumn	$19.9 \pm 3.1^{b,z}$	$39.6 \pm 7.0^{b,y}$	$51.4 \pm 2.9^{b,x}$	$66.1 \pm 5.6^{b,w}$
	Spring	$61.9 \pm 6.0^{a,y}$	$69.5 \pm 4.3^{a,y}$	$84.7 \pm 1.5^{a,x}$	$88.6 \pm 1.7^{a,w}$
	Summer	$26.8 \pm 4.4^{b,y}$	$28.0 \pm 4.7^{b,y}$	$43.6 \pm 5.0^{b,x}$	$90.3 \pm 0.9^{a,w}$
	winter	$23.9 \pm 3.0^{b,z}$	$31.3 \pm 4.6^{b,y}$	$44.2 \pm 5.9^{b,x}$	$66.0 \pm 4.7^{b,w}$
Sperm acrosomal integrity (%)	Autumn	$48.7 \pm 7.2^{a,x}$	54.9 ± 7.1^{x}	51.7 ± 6.5^{x}	74.9 ± 2.3^{w}
	Spring	$52.8 \pm 4.6^{a,y}$	64.4 ± 4.2^{x}	51.7 ± 4.4^{y}	79.5 ± 1.4^{w}
	Summer	$17.8 \pm 2.7^{c,z}$	57.1 ± 6.3^{x}	51.0 ± 4.5^{y}	73.9 ± 2.8^{w}
	winter	$41.9 \pm 3.7^{b,x}$	47.3 ± 5.6^{x}	47.5 ± 7.0^{x}	75.1 ± 2.9^{w}
Semen quality factor	Autumn	$25.4 \pm 11.3^{b,z}$	22.0 ± 7.1b,y	$71.6 \pm 10.3^{b,x}$	$143.5 \pm 11.7^{c,w}$
	Spring	$81.8 \pm 18.0^{a,z}$	$109.0 \pm 26.8^{a,y}$	$179.6 \pm 36.0^{a,x}$	$326.4 \pm 26.9^{a,w}$
	Summer	$6.2 \pm 1.6^{c,z}$	$7.0 \pm 1.3^{d,y}$	$17.5 \pm 2.4^{c,x}$	$122.2 \pm 16.5^{d,w}$
	winter	$6.3 \pm 1.2^{c,z}$	$14.1 \pm 2.6^{c,y}$	$64.2 \pm 10.1^{b,x}$	$168.0 \pm 14.2^{b,w}$

The values (mean ± SE) for a given semen parameter in a column (season = a, b, c, and d) and row (age = w, x, y and z) with different superscript differ significantly (P<0.05).

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HIGHLIGHTS

- Retrospective seminal parameters with respect to age/season of Indian red jungle fowl (IJRF) were studied over the 4-year period.
- Semen quantitative and qualitative traits increased with the increasing age.
- Spring was the best season for the production of high quality ejaculate at 4-years of age.
- The reproductive potential of IJRF is highly variable in many points from its descendants.

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