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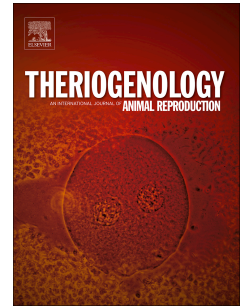
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1 **Original Research Paper**2 **Effect of season and age on Indian red jungle fowl (*Gallus gallus murghi*) semen**  
3 **characteristics: A 4-year retrospective study**4  
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16 **Running Title:** Effect of season and age on semen characteristics of Indian Red Jungle Fowl17  
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## 26 ABSTRACT

27 The reproductive potential of the adult males is expected to vary with age/season and largely  
28 differ not only in closely related avian species but even in subspecies, breeds and/or strains of  
29 the same species. Thus, it is pre-requisite to have knowledge of seminal parameters to  
30 achieve maximum production potential of at-risk species for *ex situ in vitro* conservation  
31 programs. A 4-year study was designed to evaluate the effect of age and season (spring,  
32 summer, autumn and winter) on semen characteristics of Indian red jungle fowl (*Gallus*  
33 *gallus murghi*) in retrospective manner. Semen ejaculates (n=1148) were regularly collected  
34 from eight adult Indian red jungle fowls from 6 to 54 months of age. Quantitative and  
35 qualitative semen parameters viz; volume ( $\mu\text{L}$ ), concentration ( $1 \times 10^9 \text{ mL}^{-1}$ ), total sperm  
36 number per ejaculate ( $1 \times 10^9 \text{ mL}^{-1}$ ), motility (%), viability (%), plasma membrane integrity  
37 (%), acrosome integrity (%) and semen quality factor were recorded. A chronological  
38 increasing trend with age of most sperm quantitative and qualitative traits (semen volume,  
39 sperm concentration, total sperm number per ejaculate, plasma membrane integrity, viability,  
40 acrosomal integrity, semen quality factor) was observed. The highest values were observed at  
41 four years of age ( $P < 0.05$ ) with the exception of sperm motility that was not affected by the  
42 age. Spring was the best season for sperm parameters viz; volume, motility, plasma  
43 membrane integrity, viability and acrosomal integrity ( $P < 0.05$ ), however a remarkable  
44 sperm production was noticed all over the year. It is concluded that Indian red jungle fowl  
45 exhibit an evolution of sperm production that greatly differs in many points from other fowl  
46 sub-species. It is suggested that semen ejaculates of highest quality achieved for semen  
47 banking at the age of four year in the spring season.

48

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

## 51 1. Introduction

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

63 The decline in Indian red jungle fowl needs special attention; several reports suggest  
64 adopting appropriate *ex situ* conservation strategies [2,11,12]. For *ex situ in vitro*  
65 conservation, semen banking is considered one of the most applicable options for bird species  
66 that are facing continuous decline in their natural habitat and the threats cannot be easily  
67 mitigated and/or ameliorated [14-20]. Cryo-banking of valuable male germplasm could be an  
68 important assisted reproductive biotechnology to maintain genetic diversity and *in vitro ex*  
69 *situ* conservation program [8,9]. It is well established that avian semen can be stored in  
70 frozen state for infinite period of time without significant decline in fertilizing potential  
71 [17,21,22]. The characteristics of the semen ejaculate viz; volume, concentration, motility,  
72 viability, plasma membrane and acrosome integrity, semen quality factor determine the  
73 reproductive potential of the cocks either used in natural or artificial breeding program  
74 [8,9,23-25]. Nevertheless, semen quality varies with age and season of collection in birds that  
75 may affect fertilizing capacities accordingly [26-28]. The success of semen banking depends

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

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

## 89 2 Materials and methods

### 90 2.1 Experimental birds

91 The chicks of Indian red jungle fowl were reared at Avian Research Center, Pir Mehr  
92 Ali Shah Arid Agriculture University Rawalpindi. The Rawalpindi region (33° 36' 0" North,  
93 73° 4' 0" East) is located in Southern slopes of the North-Western Himalayas including large  
94 mountain tracks with rich valleys traversed by mountain rivers. This region falls in humid  
95 and subtropical region having four seasons; autumn (13.9°C to 33.5°C), spring (10.1°C to  
96 23.7°C), summer (21.5°C to 46.5°C) and winter (-2.8°C to 20.0°C). The average annual  
97 rainfall of this region is about 1,249 millimeters. The average rainfall in each season was  
98 73.13 mm/autumn, 39.2 mm/spring, 224.73 mm/summer and 37.3 mm/winter recorded  
99 during the study period. The average temperature of the area ranges from -3.9°C to 48.3°C  
100 (Pakistan Metrological Department, 2012-2015). The chicks were kept in individual pens

101 106.68 cm x 121.92 cm, exposed to natural day-light and standardized environmental  
102 conditions without any significant changes during the entire 4-year study period. The birds  
103 were offered commercially available poultry cock breeder feed (100g/day). Fresh water was  
104 available to the birds all the daylong throughout the experimental period. The birds were  
105 subjected to abdominal massage and were trained for semen collection until the birds were  
106 able to donate neat and clean semen without fecal contamination. This training was continued  
107 until we were able to get sufficient semen for further processing and experimental work.  
108 Semen collection of eight cocks was performed on alternate days, depending on individual  
109 cock quality and libido.

## 110 2.2. *Semen collection and quantitative evaluation*

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

### 116 2.2.1 *Sperm progressive motility*

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

### 122 2.2.2 *Sperm concentration*

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

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

### 127 2.2.3. *Total sperm number per ejaculate*

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

### 130 2.2.4 *Plasma membrane integrity*

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

### 140 2.2.5 *Sperm viability*

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

### 148 2.2.6 *Acrosomal integrity*



149 Acrosomal integrity of Indian Red Jungle Fowl spermatozoa was assessed through  
150 Giemsa stain [38]. The stain was prepared by adding Giemsa (3g) and phosphate buffer saline  
151 at pH 7.0 (2 mL) into 35 mL water. Smear was prepared by taking a drop of semen sample on  
152 a clean glass slide, dried and fixed in neutral formal-saline (5% formaldehyde) for 30 min.  
153 Fixed slides were kept in Giemsa stain for 1.5 hrs. Sperm with normal acrosome appeared to  
154 be evenly stained; abnormal spermatozoa were unevenly stained while spermatozoa having  
155 ruptured acrosome remained unstained. A total of 200 spermatozoa were observed at least in  
156 four separate fields under a phase-contrast microscope (x400; Olympus BX20, Japan) at  
157 magnification of 1000x with oil immersion.

158

### 159 2.2.7 Sperm quality factor

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

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

### 163 2.3 Statistical analysis

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

172

### 173 3. Results

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

181 The data on qualitative semen quality parameters viz; motility, plasma membrane  
182 integrity, viability, acrosome integrity and semen quality factor were given in Table 2. Sperm  
183 motility did not differ ( $P>0.05$ ) with increase in age from 6 to 54 months of age.  
184 Nevertheless, an increase was observed in plasma membrane integrity, viability, acrosome  
185 integrity and semen quality factor with the increasing age. The highest ( $P<0.05$ ) values for  
186 aforementioned parameters were recorded during 43-54 months of age compared to 31-42,  
187 19-30 and 6-18 months of age. A highly positive ( $P<0.05$ ) effect of spring season was  
188 recorded on most of the quality parameters with the exception of motility that was  
189 equivalently efficient in autumn, spring and summer and only altered in winter.

### 190 4. Discussion

191 In conservation programs, the success of AI and semen cryopreservation depends on  
192 the initial semen quality of ejaculate [17]. Semen evaluation, based on *in vitro* measurement  
193 of sperm motility, plasma membrane integrity, viability, acrosomal integrity and semen  
194 quality factor (SQF) provides an indirect clue about the fertility potential of the males  
195 [25,8,23,24]. The production of high quality semen ejaculates depends on the reproductive  
196 cycle of the species; furthermore, species or subspecies specific variations depend on the  
197 birds, their age, their environment including climate changes such as seasons [30, 36,39-46].

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

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

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

223 More precisely, Indian red jungle fowl spermatozoa showed greater resistance to the  
224 hypo-osmotic solution at 37°C at 4 years of age. These changes would indicate that sperm  
225 plasma membrane increases its “plasticity” with the age and thus its functionality. Indeed, the  
226 response of spermatozoa towards hypo-osmotic solution in terms of swollen head and coiled  
227 tail is due to the movement of physical and biochemical compounds which play essential  
228 roles in maintaining the viability and fertilizing ability of sperm [50-52]. Shanunagum et al.  
229 [44] reported that response of spermatozoa to the hypo-osmotic environment at one year of  
230 age was maximum compared to 6 months of age; and described that age and genetic factors  
231 contribute to the quality of semen in naked neck and dwarf chicken. But none of these studies  
232 examined 3 or 4 years old fowl, thus limiting the potential comparison with our study.

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

248 intensive” for the animal metabolism and consequently the sexual maturation. We suggest  
249 that the Indian red jungle fowl would get fully mature at later ages compared to other fowls  
250 [31].

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

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

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

273 “Saharan model” with summer variations potentially related to the high temperatures of the  
274 Pakistan summer. Indeed, deleterious effects of high temperatures on germ cells have already  
275 been described [59]. The results of the present study are in agreement with previous studies  
276 on Northern pintail ducks and Nigerian local domestic chicken in which high summer  
277 temperatures caused temporary reduction in sperm production [40,43,59]. The low volume of  
278 semen in hot summer could tentatively be related to the absence of cutaneous heat  
279 evaporation that cannot mitigate the stressful events of heat disruption in summer season  
280 [60].

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

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

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

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

## 312 References

- 313 [1] Delacour J. The pheasants of the world. Country Life Ltd, London 1951.
- 314 [2] Gautier Z. "Gallus gallus" (On-line), Animal Diversity Web 2002. Accessed May 31,  
315 2016 at [http://animaldiversity.org/accounts/Gallus\\_gallus/](http://animaldiversity.org/accounts/Gallus_gallus/)
- 316 [3] Eriksson J, Larson G, Gunnarsson U, Bedhom B, Tixier-Boichard M, Strömstedt L,  
317 Wright D, Jungerius A, Vereijken A, Randi E, Jensen P, Andersson L Identification of  
318 the yellow skin gene reveals a hybrid origin of the domestic chicken. PloS Gen  
319 2008;4: e1000010.
- 320 [4] IUCN (2015) IUCN Red list of threatened species 2015-4.  
321 <http://www.iucnredlist.org/details/22679199/0>.

- 322 [5] Birdlife International (2016) *Gallus gallus*. The IUCN Red List of Threatened Species  
323 2016: <http://dx.doi.org/10.2305/IUCN.UK.2012-1.RLTS.T22679199A40112723.en>.
- 324 [6] Wang LK, Hails CJ. An annotated checklist of birds of Singapore. *Raffles BullZool*  
325 2007;15:1–179.
- 326 [7] Subhani A, Awan MS, Anwar M, Ali U, Dar NI. Population status and distribution  
327 pattern of red jungle fowl (*Gallus gallus murghi*) in Deva Vatala National Park, Azad  
328 Jammu & Kashmir, Pakistan: A pioneer study. *Pak J Zool* 2010;42:701-706.
- 329 [8] Rakha BA, Ansari MS, Hussain I, Malik MF, Akhter S, Blesbois E. Semen  
330 characteristics of the Indian Red Jungle Fowl (*Gallus gallus murghi*). *Eur J Wildl Res*  
331 2015a;61:379-386.
- 332 [9] Rakha BA, Hussain I, Malik MF, Akhter S, Ansari MS. Impact of ejaculate  
333 frequencies on the quality of Red Jungle Fowl (*Gallus gallus murghi*) semen. *Avian*  
334 *Biol Res* 2015b;8:109-112.
- 335 [10] Rakha BA, Ansari MS, Akhter S, Hussain I, Blesbois E. Cryopreservation of Indian  
336 red jungle fowl (*Gallus gallus murghi*) semen. *Anim Reprod Sci* 2016a;174:45-55.
- 337 [11] Peterson AT, Brisbin IL. Genetic endangerment of wild Red Jungle fowl *Gallus*  
338 *gallus*?. *Bird Cons Int* 1998;8:387-394.
- 339 [12] Mukesh SKS, Shukla M, Sharma LK, Mohan N, Goyal SP, Sathyakumar S.  
340 Identification of galliformes through forensically informative nucleotide sequencing  
341 (FINS) and its implication in wildlife forensics. *J Forensic Res* 2013;4:195  
342 doi:10.4172/2157-7145.1000195.
- 343 [13] Kumar V, Shukla SK, Mathew J, Sharma D. Genetic diversity and population  
344 structure analysis between Indian red jungle fowl and domestic chicken using  
345 microsatellite markers. *Anim Biotech* 2015;26:201-210.



- 346 [14] Blanco JM, Gee G, Wildt DE, Donoghue AM. Species variation in osmotic,  
347 cryoprotectant, and cooling rate tolerance in poultry, eagle, and peregrine falcon  
348 spermatozoa. *Biol Reprod* 2000;63:1164-1171.
- 349 [15] Łukaszewicz E, Chrzanowska M, Jerysz A, Chełmońska B. Attempts on freezing  
350 the Greylag (*Anser anser L.*) gander semen. *Anim Reprod Sci* 2004;80:163-173.
- 351 [16] Blesbois E, Seigneurin F, Grasseau I, Limouzin C, Besnard J, Gourichon D, Tixier-  
352 Boichard M. Semen cryopreservation for ex situ management of genetic diversity in  
353 chicken: creation of the French avian cryobank. *Poul Sci* 2007;86:555-564.
- 354 [17] Blesbois E, Grasseau I, Seigneurin F, Mignon-Grasteau S, Saint Jalme M, Mialon-  
355 Richard MM. Predictors of success of semen cryopreservation in chickens.  
356 *Theriogenology*2008;69:252-261.
- 357 [18] Blanco JM, Long JA, Gee G, Wildt DE, Donoghue AM Comparative  
358 cryopreservation of avian spermatozoa: benefits of non-permeating  
359 osmoprotectants and ATP on turkey and crane sperm cryosurvival. *Anim Reprod*  
360 *Sci* 2011;123:242-248.
- 361 [19] Blanco JM, Long JA, Gee G, Wildt DE, Donoghue AM. Comparative  
362 cryopreservation of avian spermatozoa: effects of freezing and thawing rates on  
363 turkey and sandhill crane sperm cryosurvival. *Anim Reprod Sci* 2012;131:1-8.
- 364 [20] Rakha BA, Hussain I, Akhter S, Ullah N, Andrabi SMH, Ansari MS. Evaluation of  
365 Tris–citric acid, skim milk and sodium citrate extenders for liquid storage of Punjab  
366 Urial (*Ovis vignei punjabiensis*) spermatozoa. *Reprod Biol* 2013;13:238-242.
- 367 [21] Holt WV. Basic aspects of frozen storage of semen. *Anim Reprod Sci* 2000;62:3-22.
- 368 [22] Blesbois E. Freezing avian semen. *Avian Biol Res*2011;4:52-58.
- 369 [23] Saacke RG. Semen quality in relation to semen preservation. *J Dairy Sci*  
370 1983;66:2635-2644.

- 371 [24] Siudzińska A, Łukaszewicz E. Effect of semen extenders and storage time on sperm  
372 morphology of four chicken breeds. J Appl Poul Res 2008;17:101-108.
- 373 [25] Lierz M, Reinschmidt M, Müller H, Wink M, Neumann D. A novel method for semen  
374 collection and artificial insemination in large parrots (*Psittaciformes*). Sci Rep2013;3.
- 375 [26] Sexton KJ, Renden JA, Marple DN, Kempainen RJ. Effects of dietary energy on  
376 semen production, fertility, plasma testosterone, and carcass composition of broiler-  
377 breeder males in cages. Poul Sci1989;68:1688-1694.
- 378 [27] Anderson J. The semen of animals and its use for artificial insemination. Greenworld  
379 Publishers2001.
- 380 [28] Oguntunji AO, Aderemi FA, Lawal TE, Alabi OM. The influence of seasonal  
381 variation on performance of a commercial laying strain in a derived savanna  
382 environment in Nigeria. Nigerian Poul Sci J 2008;5:75-82.
- 383 [29] Ansari MS, Rakha BA, Andrabi SMH, Ullah N, Iqbal R, Holt WV, Akhter S.  
384 Glutathione-supplemented tris-citric acid extender improves the post-thaw quality and  
385 in vivo fertility of buffalo (*Bubalus bubalis*) bull spermatozoa. Reprod Biol  
386 2012;12:271-276.
- 387 [30] Elagib HAA, Musharaf NA, Makawi SA, Mohamed HE. The effects of age and  
388 season on semen characteristics of white leghorn cocks under Sudan conditions. Int J  
389 Poult Sci 2012;11:47-49.
- 390 [31] Malik A, Haron AW, Yusoff R, Nesa M, Bukar M, Kasim A. Evaluation of the  
391 ejaculate quality of the red jungle fowl, domestic chicken, and bantam chicken in  
392 Malaysia. Turk J Vet Anim Sci2013;37:564-568.
- 393 [32] Burrows WH, Quinn JP. A method of obtaining spermatozoa from the domestic fowl.  
394 Poul Sci1935;14:251-253.

- 395 [33] Rakha BA, Ansari MS, Hussain I, Anwar M, Akhter S, Blesbois E. Comparison of  
396 extenders for liquid storage of Indian red jungle fowl (*Gallus gallus murghi*)  
397 spermatozoa. Avian Biol Res 2016b;9:207-2012.
- 398 [34] Zemjanis R. Diagnostic and therapeutic technique in animal reproduction. Baltimore,  
399 Williams and Wilkins Company 1970.
- 400 [35] De Reviers M, Williams J, Garreau M, Brillard JP. Predicting the adult daily sperm  
401 output after the first ejaculates in cockerels raised under different photoschedules.  
402 Reprod Nut Dévelop 1981;21:1113-1124.
- 403 [36] Santiago-Moreno J, Castaño C, Coloma MA, Gómez-Brunet A, Toledano-Díaz A,  
404 López-Sebastián A, Campo JL. Use of the hypo-osmotic swelling test and aniline blue  
405 staining to improve the evaluation of seasonal sperm variation in native Spanish free-  
406 range poultry. Poul Sci 2009;88:2661-2669.
- 407 [37] Bakst MR, Cecil HC. Determination of sperm concentration II. Establishing a  
408 standard curve. Techniques for semen evaluation, semen storage, and fertility  
409 determination Ed. Bakst MR, Cecil HC 1997;11-19.
- 410 [38] Jianzhong L, Yiling Z. Methods and effects of Hongshan cock spermatozoa  
411 cryopreservation. Wuhan Uni J Nat Sci 2006;11:447-450.
- 412 [39] Santiago-Moreno J, Castaño C, Toledano-Díaz A, Esteso MC, López-Sebastián A,  
413 Gañán N, Blesbois E. Characterization of red-legged partridge (*Alectoris rufa*) sperm:  
414 seasonal changes and influence of genetic purity. Poul Sci 2015;94:80-87.
- 415 [40] Bah GS, Chaudhari SUR, Al-Amin JD. Semen characteristics of local breeder cocks  
416 in the Sahel region of Nigeria. Revue Élev Méd Vét Trop 2001;54:153-158.
- 417 [41] Dumpala PR, Parker HM, McDaniel CD. The effect of semen storage temperature and  
418 diluent type on the sperm quality index of broiler breeder semen. Int J Poult Sci  
419 2006;5:838-845.

- 420 [42] Obidi JA, Onyeanusi BI, Rekwot PI, Ayo JO, Dzenda T. Seasonal variations in  
421 seminal characteristics of Shikabrown breeder cocks. *Int J Poul Sci*2008;7:1219-1223.
- 422 [43] Tabatabaei S, Chaji M, Mohammadabadi T. Correlation between age of rooster and  
423 semen quality in Iranian indigenous broiler breeder Chickens. *J Anim Vet Adv*  
424 2010;9:195-198.
- 425 [44] Shanmugam M, Rajkumar U, Reddy MR, Rao SR. Effect of age on semen quality in  
426 naked neck and dwarf chicken under tropical climatic conditions. *Anim Prod Sci*  
427 2012;52:964-968.
- 428 [45] Sonseeda P, Vongpralub T, Laopaiboon B. Effects of Environmental Factors, Ages  
429 and Breeds on Semen Characteristics in Thai Indigenous Chickens: A One-year  
430 Study. *The Thai J Vet Med*2013;43:347.
- 431 [46] Churchil RR, Praveena PE, Sharma D. Semen quality parameters, their inter-  
432 relationship and post-washing sperm attributes of Rhode Island Red roosters. *Vet*  
433 *World* 2014;7: 1117-1122.
- 434 [47] Long JA, Bongalhardo DC, Pelaez J, Saxena S, Settar P, O'Sullivan NP, Fulton JE.  
435 Rooster semen cryopreservation: effect of pedigree line and male age on postthaw  
436 sperm function. *Poul Sci*2010;89:966-973.
- 437 [48] Chotesangasa R. Effects of mating ratio, cock number in the flock and breeder age  
438 on fertility in Thai native chicken flock. *Nat Sci*2001;35:122-131.
- 439 [49] Hafez B, Hafez ESE. *Reproduction in Farm Animals*. 7th ed. New York. Lippincott  
440 Williams and Wilkins, USA2000.
- 441 [50] Zavos PM, Graham EF. Effects of various degrees of supercooling and nucleation  
442 temperatures on fertility of frozen turkey spermatozoa. *Cryobiol*1983;20:553-559.

- 443 [51] Daader AH, Seleem TST. Response of spermatozoa of different breeds of rabbits to  
444 hypo osmotic swelling test. In Proc: The 4<sup>th</sup> Inter. Con. on Rabbit Prod. in Hot Clim.,  
445 Sharm El-Sheikh, Egypt, 2005;177-181.
- 446 [52] Ramu S, Jeyendran RS. The hypo-osmotic swelling test for evaluation of sperm  
447 membrane integrity. *Spermatogenesis: Methods and Protocols*, 2013;21-25.
- 448 [53] Douard V, Hermier D, Magistrini M, Blesbois E. Reproductive period affects lipid  
449 composition and quality of fresh and stored spermatozoa in turkeys. *Theriogenology*  
450 2003;59:753-764.
- 451 [54] Kelso KA, Cerolini S, Noble RC, Sparks NC, Speake BK. Lipid and antioxidant  
452 changes in semen of broiler fowl from 25 to 60 weeks of age. *J Reprod Fertil*  
453 1996;106:201-206.
- 454 [55] Cerolini S, Kelso KA, Noble RC, Speake BK, Pizzi F, Cavalchini LG. Relationship  
455 between spermatozoan lipid composition and fertility during aging of chickens. *Biol*  
456 *Reprod*1997;57:976-980.
- 457 [56] Peltoniemi OAT, Love RJ, Heinonen M, Tuovinen V, Saloniemi H. Seasonal and  
458 management effects on fertility of the sow: a descriptive study. *Anim Reprod Sci*  
459 1999;55:47-61.
- 460 [57] Aengwanich W. Effects of high environmental temperature on blood indices of Thai  
461 indigenous chickens, thai indigenous chickens crossbred and broilers. *Int J Poul Sci*  
462 2007;6:427-430.
- 463 [58] Penfold LM, Wildt DE, Herzog TL, Lynch W, Ware L, Derrickson SE, Monfort SL.  
464 Seasonal patterns of LH, testosterone and semen quality in the Northern pintail duck  
465 (*Anas acuta*). *Reprod Fertil Develop*2001;12:229-235.
- 466 [59] Duangduen C, Duangjinda M, Katavatin S, Aengwanich W. Effect of hsp70  
467 Genotypes on Thermotolerance in Thai Native Chicken and Broilers. In Proceedings

- 468 of the 4th Symposium on Thermotolerance in Domestic Animals. Khon Kaen.  
469 2008;241-245.
- 470 [60] Bianca W. The significance of meteorology in animal production. Int J Biomet  
471 1976 ;20:139-156.
- 472 [61] Thurston RJ, Korn N. Spermiogenesis in commercial poultry species: anatomy and  
473 control. Poul Sci2000;79:1650-1668.
- 474 [62] Sexton TJ, Fewlass TA A New Poultry Semen Extender 2. Effect of the Diluent  
475 Components on the Fertilizing Capacity of Chicken Semen Stored at 5° C. Poul Sci  
476 1978;57:277-284.
- 477 [63] Giesen AF, Sexton TJ. Beltsville poultry semen extender. 9. Effect of storage  
478 temperature on turkey semen held eighteen hours. Poul Sci1983;62:1305-1311.
- 479 [64] Santiago-Moreno J, Castaño C, Toledano-Díaz A, Coloma MA, López-Sebastián A,  
480 Prieto MT, Campo JL. Influence of Season on the Freezability of Free-Range Poultry  
481 Semen. Reprod Domest Anim 2012;47:578-583.
- 482 [65] Long JA. Avian semen cryopreservation: what are the biological challenges?. Poul  
483 Sci2006;85:232-236.
- 484 [66] Savina MV, Emelyanova LV, Belyaeva EA. Bioenergetic parameters of lamprey and  
485 frog liver mitochondria during metabolic depression and activity. Comparative  
486 Biochemistry and Physiology Part B: Biochem Mol Biol2006;145:296-305.
- 487 [67] Martin N, Kraffe E, Guderley H. Effect of day length on oxidative capacities of  
488 mitochondria from red muscle of rainbow trout (*Oncorhynchus mykiss*). Comparative  
489 Biochemistry and Physiology Part A: Mol Integr Physiol2009;152:599-603.
- 490 [68] Rekwot PI, Abubakar YU, Anyam AA, Sekoni VO, Jatau J, Magaji S. Spermogram  
491 of Rhode Island Red breeder cocks. In: Nigerian Veterinary Medical Association  
492 Conference. 2005;1-10.

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

	Season	Age in months			
		6-18	19-30	31-42	43-54
Semen volume ( $\mu$ L)	Autumn	41.9 $\pm$ 5.1 <sup>c,z</sup>	56.5 $\pm$ 4.4 <sup>b,y</sup>	68.0 $\pm$ 7.5 <sup>b,y</sup>	73.4 $\pm$ 2.9 <sup>c,x</sup>
	Spring	88.7 $\pm$ 9.5 <sup>a,z</sup>	78.5 $\pm$ 7.6 <sup>a,y</sup>	106.6 $\pm$ 6.8 <sup>a,x</sup>	160.1 $\pm$ 8.2 <sup>a,w</sup>
	Summer	25.3 $\pm$ 4.3 <sup>d,z</sup>	26.4 $\pm$ 2.8 <sup>d,y</sup>	25.6 $\pm$ 1.9 <sup>c,x</sup>	39.9 $\pm$ 3.6 <sup>d,w</sup>
	Winter	41.1 $\pm$ 4.1 <sup>b,y</sup>	44.2 $\pm$ 2.9 <sup>c,y</sup>	63.3 $\pm$ 5.4 <sup>b,x</sup>	93.8 $\pm$ 4.2 <sup>b,w</sup>
Sperm concentration ( $1 \times 10^9$ mL <sup>-1</sup> )	Autumn	0.45 $\pm$ 0.1 <sup>b,z</sup>	0.95 $\pm$ 0.2 <sup>b,y</sup>	2.09 $\pm$ 0.3 <sup>a,x</sup>	3.00 $\pm$ 0.2 <sup>b,w</sup>
	Spring	1.11 $\pm$ 0.2 <sup>a,z</sup>	1.83 $\pm$ 0.3 <sup>a,y</sup>	2.50 $\pm$ 0.2 <sup>a,x</sup>	3.41 $\pm$ 0.3 <sup>a,w</sup>
	Summer	0.86 $\pm$ 0.2 <sup>a,z</sup>	0.91 $\pm$ 0.1 <sup>b,y</sup>	2.00 $\pm$ 0.3 <sup>a,x</sup>	2.54 $\pm$ 0.1 <sup>b,w</sup>
	Winter	0.53 $\pm$ 0.1 <sup>b,z</sup>	1.14 $\pm$ 0.1 <sup>b,y</sup>	2.37 $\pm$ 0.2 <sup>a,x</sup>	3.01 $\pm$ 0.2 <sup>b,w</sup>
Total sperm per ejaculate ( $1 \times 10^9$ )	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

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

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

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

15 The values (mean  $\pm$  SE) for a given semen parameter in a column (season = a, b, c, and d) and  
 16 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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