Abstract—In this study, three different groups of male broodstocks according to their age (4, 5 and 6 respectively) were used to fertilizing of eggs which obtained from nine females. The results showed that 6 years old males had maximum body weight (1766.67 g), total length (56.33 cm) and sperm volume (31.83 ml). Our results did not show significant difference in values of spermatocrit and spermatozoa concentration among male broodstocks (P>0.05). The present study showed maximum fertilization percentage (98.5 %), survival rate until eyeing percentage (91.17 %), hatching percentage (94.5 %) and survival rate until absorption of yolk sack stage (97.16 %) when sperm of 4 years old males was used to inseminate of eggs. Some positive relationships were detected between sperm production characteristics (spermatozoa concentration, spermatocrit and sperm volume) and fertilization parameters. Based on our results, can be concluded that 4 years old males had high efficiency for achieve fertilization success.

Keywords— Age, Fertility ability, Fertilization rate, Caspian brown trout, Male.

I. INTRODUCTION

The Caspian brown trout, *Salmo trutta caspius*, is a vulnerable anadromous fish, which considered to biological conservation program in southern part of Caspian Sea [12, 18]. Since 1999, the International Union for Conservation of Nature (IUCN) registered this fish as a species in danger of extinction, so the Iranian Fisheries Organization has been conducted artificial reproduction of this species. This organization also has been grown fry to the smolt level and releases them into the sea [17]. The Caspian brown trout enters to northern part rivers such as Shirood and Cheshme kileh for spawning. The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of viable larvae [13]. Semen quality is a key issue in fertilization success [7, 22]. Techniques used to assess sperm quality in fish include monitoring sperm density and motility and fertilization success [1, 24]. Male’s success may depend on the timing and position of sperm release, ability of the sperm to compete with other males and the number of sperm released [7]. The sperm to eggs ratio, fertilization technique [6] size and age of males [5] are important factors that have been evaluated for maximizing fertilization rate. Liley et al. [14] studied effect of male broodstocks age, social experience and spermatozoa concentration and motility on in vitro fertilization parameters of rainbow trout (*Oncorhynchus mykiss*). Also, spermatological properties of rainbow trout were investigated at different age classes [24]. Same research about influence different males ages on sperm characteristics and fertilization capacity was carried out by Lorestany et al. [15] in rainbow trout. Other studied were performed on the reproductive performance, sex ratio, growth and survival rate of rainbow trout in 3 to 5 years-old males respectively [11, 21]. The aim of this investigation was to determine the relationships between different male broodstocks ages and reproduction efficiency in *Salmo trutta caspius*.

II. MATERIALS AND METHODS

The experiment was carried out at the Kalardasht Salmonids Reproduction Center (KSRC), Iran, during spawning season at 2008-2009 (December to March). The broodstock were captured from Cheshme Kileh River during upstream migration and then transferred to KSRC. A total of nine mature males and females were selected and transferred to hatchery for collecting by genital materials. All broodes were anaesthetized in 100 ppm of MS222 (tricaine methane
sulfonate) for subsequent experiments. Scales of Caspian brown trout were used to age determination according to method suggested by Heinimaa and Heinimaa [8]. Sperm and eggs were collected by manual stripping. The sperm was collected in graded tubes for each male and expressed as ml [3]. Amount of 1.5 ml of obtained sperm from each male separately transferred to laboratory under cold conditions (7-10 °C) for measurement sperm quality parameters (spermatocrit concentration and spermatocrit). The spermatocrit was defined as the ratio of volume of white packed material to the total volume of semen ×100 [20].

Microhaematocrit capillary tubes (75mm length, 1.1–1.2 mm diameter) were filled with semen and one end of each tube was sealed with clay. The capillary tubes were centrifuged at 3000 rpm for 8 min (Sigma, 13 USA). Spermatocrit concentration was measured by counting the number of spermatocrit in a sample diluted with D sodium bicarbonate (5 g Na2Hco3, 10 cc Formalin, 100 cc water) in a hemocytometer, under 400 X magnification [23].

After females stripping, eggs were divided to three equal parts and then eggs inseminated with equal condition for all treatments. Sperm mixture of male broods based on age was added to eggs. Eggs and sperms were fertilized according to dry fertilization method. After hardness (for 45 min), fertilized eggs were delivered to hatchery. For prevention of disorder, each treatment was divided to three parts and was set in three trays; in fact, there were nine trays. The hatchery trays were covered with a red plastic plate to protect the eggs from sun light. These trays were placed in incubators with cold running water (8°C) until fertilization (6-7 days), eyeing (14-15 days), hatching (30-35 days) and absorption of yolk sack stage (55-60 days). The fertilization rate was determined according to Bromage and Cumaranatunga [4] method where the 80 eggs were randomly sampled from each tray and then transferred to laboratory under cold conditions (7-10 °C) for measurement sperm quality parameters.

The relationship between sperm production characteristics and fertilization parameters are given in Figure 1–5 respectively. There was positive significant relationships between the male spermatocrit concentration and spermatocrit with fertilization rate (Fig. 1 and 2). Maximum spermatocrit concentration and spermatocrit found to be 7.77 ± 17.6, 10.44 ± 38.0, which belonged to 4-year-old male broods respectively. But, spermatocrit concentration and spermatocrit did not show statistically significant change at different age classes.

III. RESULTS

The mean of spermatocrit concentration and fertilization parameters are given in Table 1. The body weight, total length as well as sperm volume (Table 1, P<0.05) significantly increased with increasing of male broods age. As increased age of male broodstocks, sperm volume was increased (Table I).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>4-year</th>
<th>5-years</th>
<th>6-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight (g)</td>
<td>866.67 ± 57.74 b</td>
<td>1100 ± 0.0 b</td>
<td>1766.67 ± 152.75 a</td>
</tr>
<tr>
<td>Total length (cm)</td>
<td>1.53 ± 44.67 c</td>
<td>0.58 ± 50.33 b</td>
<td>2.08 ± 56.33 a</td>
</tr>
<tr>
<td>sperm volume (ml)</td>
<td>1.71 ± 17.74 b</td>
<td>2.87 ± 22.31 ab</td>
<td>6.22 ± 31.83 a</td>
</tr>
<tr>
<td>spermatocrit concentration</td>
<td>7.77 ± 17.6</td>
<td>19.6 ± 8.14</td>
<td>27.7 ± 16.16</td>
</tr>
<tr>
<td>Spermatocrit (%)</td>
<td>10.44 ± 38.0</td>
<td>10.12 ± 28.67</td>
<td>17.01 ± 35.33</td>
</tr>
</tbody>
</table>

The correlation between spermatozoa concentration and fertilization rate is shown in Figure 1–2. Maximum spermatozoa concentration and fertilization rate found to be 7.77 ± 17.6, 10.44 ± 38.0, which belonged to 4-year-old male broods respectively. But, spermatocrit concentration and spermatocrit did not show statistically significant change at different age classes.

![Fig. 1 Correlation Between Spermatozoa Concentration (× 109 /Ml) And Fertilization Rate In Caspian Brown Trout](image1)

![Fig. 2 Correlation Between Spermatocrit And Fertilization Rate In Caspian Brown Trout](image2)
The highest fertilization rate (98.5 %) belonged to 4 and 6 years old male broods (Table 2, P<0.05). Maximum eyeing, hatching and survival rates until absorption of yolk sack stage found to be 91.17%, 94.5% and 97.16%, respectively that related to 4 years-old male broodstocks (Table 2, P<0.05).

### IV. DISCUSSION

In various fish species, sperm volume is different according to fish species [16], sequential stripping, age, weigh and strain [10]. In present study, 6 years old broodstocks had more sperm volume then 4 and 5 years-old fish. These results are in agreement with reports on rainbow trout (*Oncorhynchus mykiss*) [21]. Tekin et al. [24] reported such positive relationship between sperm volume and age as well as length and weight of fish. In this study, 4 years old male broodstocks had higher values of spermatocrit and spermatozoa concentration then 5 and 6 years-old fish. Some authors confirmed that increasing in male broodstocks ages are leads to decrease in spermatocrit value [14, 24]. Also, in sockeye salmon similar findings observed between male age and spermatocrit [9]. In rainbow trout and sockeye salmon have been demonstrated that older male broodstocks has lower spermatozoa concentration [9, 14, 15, 24]. Our findings cleared a positive relationship between spermatocrit and sperm volume (figure 5). These results are in agreement with reports on Rainbow trout (*Onchorhynchus mykiss*) [15, 21], Atlantic Cod (*Gadus morhua*) [19] and Atlantic halibut (*Hippoglossus hippoglossus*) [25]. With increasing the age, sperm concentration will decrease; probably is due to increased sperm volume in older fish. Because of older fish have larger testis; the production of sperm volume will increase. As the relationship between sperm volume and spermatozoa concentration is reverse, therefore with increasing age, sperm volume will increase but its concentration will decreases [24]. The highest spermatocrit value was observed in 4 years old broodstocks and also the highest fertilization rate, eyeing, hatching rates were observed in these broodstocks. When the spermatocrit decreased, fertilization rate, eyeing, hatching rates decreased. Several published data revealed that positive correlation between fertilization success and sperm quality parameters (spermatocrit, spermatozoa concentration and sperm volume). For example, positive significant relationship was observed between spermatocrit and fertilization rate in Atlantic salmon.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>4-years</th>
<th>5-years</th>
<th>6-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate (%)</td>
<td>0.58 ±5.98 a</td>
<td>0.50± 96.66 b</td>
<td>0.50± 98.5 b</td>
</tr>
<tr>
<td>Eyeing (%)</td>
<td>91.17 ± 1.75 a</td>
<td>87.33 ± 0.29 b</td>
<td>91.16 ± 1.80 a</td>
</tr>
<tr>
<td>Hatching (%)</td>
<td>94.5 ± 1.5 a</td>
<td>86.33 ± 0.76 c</td>
<td>91.66 ± 0.28 b</td>
</tr>
<tr>
<td>Survival rate until absorption of yolk sack stage (%)</td>
<td>97.16 ± 0.77 a</td>
<td>92.33 ± 1.52 c</td>
<td>95.83 ± 0.28 b</td>
</tr>
</tbody>
</table>

Values marked with a similar letter are not significantly different p<0.05.
(Salmo salar) and rainbow trout respectively [1, 15]. Similarly, in Sockeye salmon and rainbow trout positive significant correlation was reported between spermatozoa concentration and fertilization parameters [9, 14]. In our experiment, there were positive relationships between sperm quality parameters and fertilization capacity, which accordancc with above mentioned researches. The effect of mating different age classes of broodstocks on reproductive performance, sex ratio, growth and survival rate of rainbow trout was investigated by Kayam [11] and Shamspour [21]. These authors noted that young male broods have greater reproductive potential than older male broods. The present study indicates 4 years old male broodstocks has high potential in terms of sperm quality parameters to influence on fertilization success. Thus, using 4 years old male broodstocks could be most appropriate to propagation programs of Caspian brown trout hatchery.

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