Do Contraceptive Rods Affect the Behaviour of African Lions (*Panthera leo*) in Captivity?

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**ABSTRACT**

Surplus animals in zoos and wildlife parks are handled differently across Europe. Some zoos use the "Breed and Cull"-method where animals are allowed to reproduce, and surplus animals culled when mature. Other zoos sterilize the animals or inject them with contraceptive rods to prevent reproduction. Naturally secreted hormones affect both behaviour and morphology, but the effect of the injected hormones has not been studied in lions. The aim of this observational study was to compare the behaviour in captive groups of female lions, that had either been injected with contraceptive rods or not. The study was made in eight different zoos in Denmark, Germany, Belgium and The Netherlands. We found no significant differences in the behaviour of the two groups (with and without rods), indicating that there was no effect of contraceptive rods. However, the small sample size, and unbalanced factors such as pride composition, age and weather could have blurred our results. In order to address the challenge of surplus animals, we suggest, future studies with a more optimal experimental design and larger sample sizes and the inclusion of physiological measurement in addition to quantitative behavioural recordings.

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**INTRODUCTION**

In Europe, zoos use different types of fertility controls. In Scandinavia zoos use a "Breed and Cull" strategy, where animal are allowed to reproduce naturally and perform parental behaviour, and surplus offspring are culled when they mature. Zoos in South Western Europe, have chosen another strategy and use contraceptives, which give the animals the opportunity to perform reproductive but not parental behaviour. Both strategies have advantages and disadvantages, and even ethical challenges. Behavioural needs are defined as highly motivated behaviour, which if prevented, results in abnormal behaviour and stress responses (Jensen and Pedersen, 2008). In captivity, where space and enrichment can be limited, behaviour related to conflict and frustration, such as displacement behaviour (Van Iersel and Tinbergen, 1948; Manning and Stamp Dawkins, 2009), redirected behaviour, or even stereotypic behaviour (Mason, 1991) is not uncommon, due to lack of fulfilment of the animals’ behavioural needs (Jensen and Toates, 1993). Allowing the animals to reproduce, provide them with the ability to fulfil some of these behavioural needs, but this strategy can be questioned, with reference due to the welfare concerns and ethical issues regarding the culling of surplus animals. On the contrary, animals that are prevented from reproducing due to hormonal treatment, are prevented from performing natural behaviour, potentially leading to the development of abnormal behaviour (Mason, 1991) and physiological symptoms of stress (Friend, 1989). Furthermore, animals treated with contraceptives can develop pathological changes, as have been shown in...
other felid species (Harrenstein, 1996) (Captive wild felids: Tiger, Lion, Jaquar, Leopard, cougar and Jungle cat) and (Munson, 1991) (Exotic felids in zoos: Tiger, Jaguar and African leopard)(Munson, 2002). The recommended contraceptive for female lions is the hormonal contraceptive, with the active agent Deslorelin acetate (EAZA Group on Zoo Animal Contraception, EGZAC, personal communication, 25. Oct. 2016) a Gonadotropin-releasing hormone agonist (Bertschinger et al., 2008) which functions as an implant injected subcutaneously Superlorin®; (Goericke-Pesch et al., 2011). There are over 200 Lions in zoos in Europe (Kim Skalborg, Personal communication 17. Jan. 2018), 98 of these are registered as injected individuals, either with implants to be renewed every 6th or 12th months (EGZAC, personal communication, 17. Jan. 2018).

The very first formal study in endocrinology showed that the hormones naturally released by the testes in cockerels have a major effect on their morphology and behaviour (Berthold, 1849), and now it is commonly known that hormones affect biological organisms in numerous ways. A well-known example is contraceptive pills for women, that work by mimicking the hormonal level of pregnancy and has been found to change their preference for males assessed using olfactory, visual and vocal cues (Alvergne and Lummaa, 2010).

Even though more than 200 lions are registered in European zoos (Kim Skalborg, Personal communication, 17. Jan. 2018), very few studies have been made of their behaviour in captivity, which is also the case for lions in nature. From observations of lions in nature, we know that when two lions meet, they greet each other, by circling and rubbing, where after they raise their tail and start to swirl around each other (Schaller, 1972). These behavioural elements, together with anal sniffing, support the suggestion that lions use olfactory cues to recognize and categorize pride members. Whether and how they use smell for individual recognition is not fully understood (Schaller, 1972). If pride members can identify each other by olfactory cues, each pride or even each lion must have a specific smell. It is generally recognized that chemical signals can be used to help discriminate between conspecifics and hetero-specifics, sex and dominance status (Thomas, 2010). If hormonal contraceptives change olfactory cues, like it has been seen in the ring-tailed lemurs (Crawford et al., 2010), this could disturb social interactions, group dynamics and general group structure in a pride, due to confusion about relations or due to confusion about sexual/estrus status. The aim of this study was to compare the behaviour of African lionesses, which are either injected with contraceptives or intact.

Based on the knowledge about the natural behaviour of lions and our knowledge about the effects of hormonal change, we expected that:

a. Injected females to spend more time resting compared to the intact females, due to the increased restlessness seen in estrous females (Schaller, 1972).

b. The intra-individual difference in the behaviour of injected females due to the lack of estrous would be smaller, due to less fluctuation in the hormonal balance caused by the rods.

MATERIALS AND METHODS

The Zoos: This study involved eight zoos with African lions situated in the northern part of Europe (Table 1). All zoos were members of the “European alliance of zoos and aquariums” (EAZA) and followed the same standards regarding care and breeding, which to some extent made the zoos comparable. The climate in different countries is very similar, reducing effects due to varying weather conditions. Four zoos used contraceptive rods and four did not. One pride from each of the eight zoos was included in the study. The groups of lionesses without contraceptive rods were used as control groups. The conditions of the prides varied according to enclosure size and group composition, two zoos had cubs, one of them only in the first round of observations (Table 1).

Observations/The prides

Every pride was observed in two periods, and each observation period lasted three days of 4.5 hours/day. The timing of the periods enabled us to compare the possible effects of variation in season and day length.

Data collection

Pilot study: Before the observations started, we performed a pilot study to ensure that all relevant behavioural elements were listed, and realistic to observe. These initial observations were made in Givskud Zoo and Copenhagen Zoo. EGZAG was consulted on the preliminary research report to make sure the study was practically relevant.

Behavioural registrations: The pride and not the individual was the statistical unit, as it was not possible to distinguish between the individual female lions. Behaviour and location of all adult females was registered, as was the distance to nearest lion and
distance to the male. We used scan sampling and behaviour sampling to collect behavioural data, and the ethograms are shown in Table 2 and 3. We recorded behavioural states such as resting sitting/standing, walking, running and eating by scan sampling (Table 2) in intervals of 5 minutes, starting in the first minute of the daily 4.5 hours observation period. The observation order of the animals was always from left to right. It was not always possible to see all lionesses, either because they were out of sight in the enclosure, or because the lions had been given access to a stable area, due to bad weather.

Table 1. Summarizing facts about each zoo.

<table>
<thead>
<tr>
<th>Zoo</th>
<th>Number (male, female)</th>
<th>Treatment (+Rods/Intact)</th>
<th>Type of rod</th>
<th>Country</th>
<th>Date of observation</th>
<th>Estimated enclosure size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlebnis-zoo Hannover</td>
<td>1,1</td>
<td>+</td>
<td>Superlorin</td>
<td>DE</td>
<td>13-15/11</td>
<td>Ca. 0.010 Ha.*</td>
</tr>
<tr>
<td>WILDLANDS adventure zoo, Emmen</td>
<td>1,5</td>
<td>4+/1-</td>
<td>Superlorin</td>
<td>NL</td>
<td>5-7/1</td>
<td>Ca. 1 Ha.* (Park)</td>
</tr>
<tr>
<td>Royal Burgers’ zoo, Arnhem</td>
<td>1,3</td>
<td>2+/1</td>
<td>Superlorin</td>
<td>NL</td>
<td>29/11- 1/12</td>
<td>Ca. 1 Ha.* (Park)</td>
</tr>
<tr>
<td>ZOO Antwerpen</td>
<td>1,1</td>
<td>+</td>
<td>Superlorin (Virbac)</td>
<td>BE</td>
<td>2-4/12</td>
<td>Ca. 0.035 Ha.* (City)</td>
</tr>
<tr>
<td>Copenhagen Zoo</td>
<td>1,2  (+ (2,1 in first round))</td>
<td>-</td>
<td>-</td>
<td>DK</td>
<td>7-9/12</td>
<td>Ca. 0.015 Ha.* (City)</td>
</tr>
<tr>
<td>Odense Zoo</td>
<td>1,3 (+ (1,2))</td>
<td>-(2 sterilized)</td>
<td>-</td>
<td>DK</td>
<td>1-3/10</td>
<td>Ca. 0.015 Ha.* (City)</td>
</tr>
<tr>
<td>Givskud Zoo</td>
<td>1,10</td>
<td>-</td>
<td>-</td>
<td>DK</td>
<td>2,3 &amp; 21/11</td>
<td>4 Ha.* (Park)</td>
</tr>
<tr>
<td>Ree Park Safari, Ebeltoft</td>
<td>1,3</td>
<td>- (only 1 mature)</td>
<td>-</td>
<td>DK</td>
<td>24, 25/11 &amp; 11/12</td>
<td>2 Ha.* (Park)</td>
</tr>
</tbody>
</table>

*aAdults. bCubs. *Sizes given from the zoo. #Measurements made on google maps. +Estimated from memory. Cityzoo (City), Animal parks (park).

Table 2. Ethogram showing definitions of each behaviour registered with scan sampling and the recombined variables used in the results.

<table>
<thead>
<tr>
<th>Scan samplings behaviour</th>
<th>Description</th>
<th>Recombined variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Sternal recumbency, lateral recumbency or on its back. No vocalization.</td>
<td>Resting</td>
</tr>
<tr>
<td>Sit/Stand</td>
<td>Front paws on the ground. Chest not touching the ground. Only head, eyes, ears and tail is moving.</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>Only the paws touch the ground. The lion is walking. The body position is relaxed; there is no attention towards a specific object.</td>
<td>Active</td>
</tr>
<tr>
<td>Running</td>
<td>Only the paws touch the ground. The lion is moving either in trot or gallop. Body position more tens. Eyes and ears focused towards an object or individual.</td>
<td></td>
</tr>
<tr>
<td>Eating</td>
<td>The tongue or the teeth are touching meat.</td>
<td>Eating</td>
</tr>
</tbody>
</table>

If a lioness was out of sight at the time of scanning this was noted. Furthermore, in every scan distance to nearest lion and distance to the male were noted, using the following categories: In physical contact, <1m, <5m or >5m. The average duration of a scan was 20 sec.

We used behaviour sampling in the intervals between the scans for more sporadically occurring behavioural elements, such as aggression, mating, mating prelude, playing, grooming, stereotyping, displacement behaviour and abnormal behaviour (Table 3). In this study, stereotypic behaviour was recorded when the behaviour was repeated at least five times without variation. In general, a new behavioural bout was registered after a pause of at least 5 seconds.
No special equipment was used. If a sudden change in the surroundings triggered a change in behaviour, this change in the surroundings was noted if e.g. a zookeeper passed the enclosure in a vehicle used for transporting food. In cases of bad weather leading to that no lionesses were in sight, the observations were stopped after 3 and not 4.5 hours.

**Statistical Analyses**

**Data processing:** To take into account the missing observations, due to animals being out of sight, the variables collected by scan sampling, including the distances to the other females and the male, were analyzed as the percentage of lions performing the behaviour per scan divided by the number of animals visible at the time of scanning. A new variable named 'active' was calculated as the sum of sit/stand, walking and running. The variables collected by behavioural sampling were analyzed as the average number of a given behavioural element performed per lion, per scan. A new variable named "sexual" was calculated as the sum of mating and mating prelude.

**Statistical analysis:** All statistical analyses were done in SAS (version 9.3, SAS Institute Inc., Cary, NC). The data did not follow a normal distribution and were analyzed with non-parametric statistics, (Siegel, 1988). A Friedmann Two-way Analysis of variance by ranks was used to test whether the observed behavioural elements varied within observation days, comparing data from individual hours of observation, and between individual observation days (Proc GLM). Difference between rounds was tested with the Wilcoxon Signed Ranks test (Proc UNIVARIATE). Both analyses took into account that data within day and round are dependent (repeated measures). The difference in behaviour between zoos that used contraceptive and those that did not was analyzed separately for each round of observation using the Wilcoxon-Mann-Whitney test for differences between independent samples (Proc NPAR1WAY). All the analyzes were two-tailed, and a P-values of 0.05 was used throughout. The results are presented as medians, 25%- and 75% quartiles, minimum and maximum.

**Table 3.** Ethogram showing definitions of each behaviour registered with behaviour sampling and the recombined variables used in the results.

<table>
<thead>
<tr>
<th>Behaviour samplings behaviour</th>
<th>Explanation</th>
<th>Result groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggression</td>
<td>Behaviour addressed toward conspecifics either followed by or appearing together with an open mouth, growling or “coughing”. Can involve physical contacts like punches, scratches or bites.</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Mating</td>
<td>The female is lying on her abdomen, her hind raised. The male is standing over her, with his hind legs on the ground, the front legs on both sides of the female.</td>
<td>Sexual</td>
</tr>
<tr>
<td>Mating prelude</td>
<td>The lioness gets the male attention either by slapping him gently, rubbing her head against him or just walking around him, with her tail raised and the rump orientated towards his head. When she is ready, she crouches, raises her rump and slides her tail to the side.</td>
<td></td>
</tr>
<tr>
<td>Playing</td>
<td>Is often seen between juveniles but is seen between juvenile and adults as well. It is seen as exaggerated movements that are repeated. It is mostly social play like fighting but none of the individuals involved are showing any signs of fear</td>
<td></td>
</tr>
<tr>
<td>Displacement behaviour</td>
<td>A misplaced behaviour, apparently irrelevant to the solution of the conflict motivating it.</td>
<td>Displacement behaviour</td>
</tr>
<tr>
<td>Yawn</td>
<td>The mouth is wide open, the tongue hanging outside, the teeth are showing, eyes closed. The behaviour is not intentionally directed towards another individual.</td>
<td>Yawn</td>
</tr>
<tr>
<td>Redirected behaviour</td>
<td>“A natural behaviour directed towards a “wrong” stimulus. Here it could be nursing of dead objects, like a stone.</td>
<td>Redirected behaviour</td>
</tr>
<tr>
<td>Stereotypes</td>
<td>Movement pattern that does not vary but is repeated and lacks an obvious target or function and is registered if repeated five times or more.</td>
<td>Stereotypes</td>
</tr>
<tr>
<td>Grooming</td>
<td>The lioness is licking herself or scratches with her paws or teeth.</td>
<td>Grooming</td>
</tr>
<tr>
<td>Social grooming</td>
<td>The lioness licks another individual or scratches it with her paws or teeth.</td>
<td>S. Grooming</td>
</tr>
</tbody>
</table>
RESULTS
Overall, there were no significant differences in the lions’ behaviour within or between individual days, between rounds or within rounds between the groups in zoos, with or without contraceptive rods. Figure 1 shows that the lions stayed either in contact or above 5 meters away from each other. The lionesses with rods tended to stay mostly above 5 meters from each other and the intact lionesses mostly in physical contact. However, no statistical differences were found to support this. The same tendencies seemed to apply to the distances between the injected females and the male. In round 2, there is a tendency for the injected females to stay more within 5m of the male, compared to intact females (P = 0.08) (Figure 2). Furthermore, in round two we saw a tendency, for more aggression (P=0.05) and more social grooming (P= 0.08) in the intact compared to the injected lionesses (Figure 3).

Figure 1(a +b). Percentage of lions per scan that were either in contact, <1m, <5m or >5m away from another lion in round1 and round2 for the two treatments.
Figure 2. a+b Percentage of females seen per scan that were in contact, <1m, <5m or >5m away from the male in the two rounds and with the two treatments. p = 0.0782 is indicated by #.
Figure 3a+b. Average per lion per interval observed performing Social behaviour or yawns in the two rounds and with the two treatments. p=0.05135 is indicated by * and p= 0.0782 is indicated by #.

DISCUSSION
This study is the first to estimate if contraceptive rods have an effect on the behaviour of female lions in captivity. Our results showed no significant differences between the intact lionesses and the lionesses injected with rods. There were no significant differences in activity, in time spent in contact with the male, in aggression or in any of the other behavioural elements we observed. Still, based on this study we cannot conclude that contraceptive rods do not affect behaviour. Due to the small sample size and the study design it is possible that we were not able to detect eventual effects caused by the injected rods. However, the findings are still interesting, as we found a tendency for intact females to be more aggressive compared to injected females, and a tendency for injected females to stay further away than 5 m from the male and to perform more social grooming compared to the intact females.
Looking at the activity level for the two treatments, particularly in round 1, we see a numerical difference in the activity level of the injected and control groups, indicating a higher activity level in the injected group. This contrasts with our hypothesis that intact females would be most active. Age can affect activity level and Ingram (2000) reviewed this topic and found the phenomenon of age-related decline in many different species including dogs, invertebrates, mice and monkeys. There was a considerable age variation in the groups of lionesses in our study (injected females 4-16 years, average: 8.7 years, intact females 2-10 years, average: 4.8 years), and furthermore, age seemed to be confounded with treatment. Due to this systematic difference between the injected and intact females' age, the effect of the contraceptives could not be separated in this study and should be studied further.

The activity can also be affected by group size. (Spangenberg, 2009) found a positive correlation between group size (of 2, 4 and 8) and social interactions and activity levels in laboratory rats (Rattus norvegicus). The composition of the groups used in our study are not balanced either, the average number of lionesses per pride for intact females is 4.5 lionesses per pride and only 2.5 for the prides with injected females. Again, we have confounding factors and difficulties with distinguishing the effects of injected rods and group size.

Besides age and group size, temperature could also affect lion behaviour (Dunston et al., 2017). Lions observed in the Addo Elephant National Park in South Africa had a significantly higher activity level on days in the autumn and winter compared to the hot summer months (Hayward and Hayward, 2006). The annual temperature difference in South Africa is approximately 8°C (holiday-weather.com). In our study, the temperature difference between the coldest and warmest day was 19°C, and we had differing weather types, ranging from snowstorms to warm weather. On days with snow, the lions were less active if they had access to shelter, and more active if no shelter was available, but not all prides were observed during periods with snow. This suggests that weather could have affected the results as well, and that future studies should be able to control for this factor.

To summarize, confounding factors, such as age, group size, pride composition with treatment, are a limitation of the present study, as these factors would be expected to affect the lions' behaviour, as would weather conditions. Lions societies are without harassment between the male and female, and high cooperation in intrapride encounters. We suggest that future studies take such factors into consideration in order to isolate the eventual effects of contraceptive rods.

The observed distances to other lions show that females in both treatments, stayed >5 meters away from the male most of the time, but the distance to nearest lion, although not statistically different, seemed to differ, at least numerically, between groups with different treatments. Numerically the intact females were more in contact with other lions compared to females injected with rods, whereas injected females seemed to spend more time >5 meters away from the nearest lion. That is quite interesting, especially as aggression tended to be higher in intact females compared to injected females. In nature lionesses in the same pride, rarely show aggression towards each other (Schaller, 1972). Most aggression is observed in connection to resource protection (Schaller, 1972). Captivity restricts the possibility to perform a range of behavioural elements, such as locomotion, ranging, dispersal and exploration but can also limit access to resources, such as proximity to mates, choice of mate, neighbours, feed and den/nest sites (Mason et al., 2013). The limitation in dispersal can be the cause of the aggression seen in captivity. In nature, if lionesses have small cubs they might exclude the sub-adults from the pride, and if the excluded sub-adults do not keep distance to the pride, they can be subjects to aggression (Schaller, 1972).

A general contributor to aggression in captivity is the limitation of resources (Hemsworth et al., 2013; Arey and Edwards, 1998; Manning and Stamp Dawkins, 2009). In social systems, were the same animals frequently interact, dominance hierarchy helps to avoid the high costs associated with aggression (Manning and Stamp Dawkins, 2009). There is no hierarchy between same size individuals in a lion pride (Packer, 2001); only the male shows dominance (Packer, 2001). Female lions are more likely to have an egalitarian structure (Packer, 2001), which has an ambiguous dominance hierarchy, with no linear dominance and no unidirectional agonistic interactions (Wittemeyer and Getz, 2007). The female male, and high cooperation in interpride encounters.
and in rearing and protecting of cubs, with voluntary communal cub rearing being one of the key features (Packer, 2001). Lions rarely deny same age-sex pride mates (pride mates of similar age and sex) access to a carcass, and if they do, it will be due to a limited amount of food. The hierarchy is determined by size; first adult male, second adult females, third sub-adults, fourth yearlings and last cubs. The study by (Packer, 2001) suggesting an egalitarian hierarchy between the lionesses was a theoretical study, collecting and reviewing data from previous studies. It would be interesting to find out if an observational study would confirm this, whether the same could be found in captivity, whether injection with contraceptive changes the hierarchy between the females. One of the prides observed in our study was newly established and no aggression between the females was observed, which aligns with the theory about an egalitarian society. The females did, however, all show aggression towards the male. In nature female lions show aggression towards the male on several occasions; a lioness with cubs chases away an approaching male; when a male sniffs a female’s anal area she hits him in the head and females even join together to attack an incoming male (Schaller, 1972). These behavioural elements can be attributed to protection and revenge for previous harassment. In the newly established pride, aggression is probably protection against the male and not due to hierarchy establishment. Intra-pride aggression is not a subject that has received much attention but based on our findings, it would be very interesting to investigate it further and separate the aggression into aggression towards male and aggression towards females to see if that maybe had an influence on the egalitarian society. 

As we could not standardize, we could not elucidate the effect of the factor's enclosure design and enrichment, these factors, along with the previous mentioned factors, could be the cause of the lack of significance in the observed behaviour. In this study the groups of intact females are used as controls, but this is not optimal. The treatment should have been randomly distributed within all the groups, to eliminate the previous mentioned factors. In order to achieve more knowledge, future studies should strive after obtaining data from individuals instead of groups and try to include physiological measurements. Blood samples could also provide valuable supporting data and estimations of the hormonal level at the day of observation. The real challenge is to obtain the samples without affecting the behaviours of the animals. Alternatively, hormone levels could be estimated from feces. Estimations from feces are not a measure of a specific event but provide information about the cortisol level as a function of previous states. The advantage of this is that it gives the ability to isolate the individual of interest and collect a sample without anesthetizing the animal and without affecting the results. Collecting hormone samples from feces has been done with horses to estimate stress levels (Christensen et al., 2012) and for estimating the reproductive status of terrestrial mammals (Schwarzenberger et al., 1996). When testing for reproductive status in urine the delay is less than 5 hours, and between 12- over 48 hours when testing feces depending on the species (Schwarzenberger et al., 1996). Despite deficiencies, hopefully this study will serve as an inspiration for closer examination on the effect of contraceptive rods and the natural social structures in lion prides including female-female- and female-male aggression.

CONCLUSIONS
1. No indications of the effect of contraceptive rods on behaviour in African lionesses in captivity.
2. There is a slight indication of increased aggression, which should be studied further.
3. We need more knowledge about the effect of contraceptive rods and other strategies to avoid unwanted breeding/surplus animals on the welfare of lions.

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CONFLICT OF INTEREST
All authors confirm that there is no conflict of interest.

AUTHORS CONTRIBUTIONS
The article is made on the basis of Anne Lucia Jansen master thesis. Anne Lucia Jansen collected data. The
statistical analyses were made by Karen Thodberg. The final manuscript is made in corporation between the authors.

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