

Seasonal Variation in some Reproductive Traits of Bouscat and Giza White Rabbits

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NINETY SIX multiparous non-suckled Bouscat and Giza White rabbits were made available from the breeding stock of Faculty of Agriculture, Cairo University. Animals were divided into eight groups, 12 does each (6 does from each breed). The year was arbitrary divided into the four usual seasons. At the middle of each season, two groups were used, rabbits occurred in the first group were killed 3 days *post coitum* to provide information on ovulation, recovery and fertilization rates. Females of the second group were laparotomized 12 days *post coitum* and data on implantation sites and embryo survival were collected. Reproductive organs of does in all treatment groups were subjected to some morphological and histological studies. Data obtained revealed no marked differences between Bouscat and Giza White rabbits with respect to all traits investigated. Season, however, was shown to affect ovulation rate, number of implantation sites/doe, number of viable embryos/doe, percentage of corpora lutea associated with the formation of detectable implantation sites or viable embryos, ovarian weight and thickness of myometrium. Estimates for these parameters were obviously high during winter and spring and were markedly low during summer. Autumn was the transitional season between summer and winter. Season appeared, however, without obvious effect on fertilization rate but it did influence the diameter of blastocyst including the mucous coat. Present findings did not show with certainty the environmental factor (s) responsible for the drastic decline of the reproductive potentialities of the doe during summer, even though some evidences were available on possible involvement of ambient temperature.

Domestic rabbits (*Oryctolagus cuniculus*) of the temperate areas of the world are known to be continuous breeders (Bullough, 1961), nevertheless, they manifest seasonal variation in fecundity (Hammond and Marshall, 1925; Lush, 1925; Manresa, 1933 and Sittmann *et al.*, 1964). Evidence is rather strong indicating that late summer and fall were associated with low reproductive activity. During these periods of the year mating and conception are less frequent (Hammond, 1965 and Matassino *et al.*, 1970), postcoital ovulation failure is high (Farrel *et al.*, 1968), ovulation rate is low (Hahn and Gabler, 1971) and fertility of the doe is described to be obviously poor (Cupps, 1956).

Breeding of the domestic rabbit in this country is confined to the period from September to April. Even though, frequency of kindling is markedly high during winter months (Ragab and Wanis, 1963). Moreover, average litter size is shown to be influenced by the month of the breeding season (El-Khishin *et al.*; 1951 and Ragab and Wanis, 1963). Summer breeding is not generally practised. Reasons for that are commonly attributed to the environmental conditions of the season. No work has been done, however, to study the reproductive potentialities of the doe during summer. The involvement of the three remaining seasons in reproductive processes controlling the fertility of the female is totally unclear. Accordingly, the present work was performed to study seasonal variations in ovulation rate, fertilization rate and embryo survival in two breeds of domestic rabbits dominantly raised in this country; Bouscat and Giza White.

Material and Methods

This work was carried out at the Poultry Experimental Centre and Animal Physiology Laboratory, both of Faculty of Agriculture, Cairo University. Rabbits used in the trials were raised specifically for this purpose. During the breeding season suggested by raisers (September to April) females were allowed to complete to term two or more pre-experimental pregnancies. Does proved to be sterile or infertile were excluded. A total of 96 non-suckling does was used, and were divided according to breed into two major experimental groups of 48 rabbits each. The first group was composed of Bouscat females while the second included Giza White does. Mating was done using bucks with good fertility records. At the commencement of trials, live body weight for the Bouscat doe ranged between 2.3 and 2.6 kg and for Giza White the range was between 2.3 and 3.0 kg. During the course of the experiment, females were kept separately in wood and wire hutches located in a semi-open wooden barn. The general management of experimental animals were exactly the same as that for the breeding stock.

Rabbits were fed three times daily. Concentrates were introduced at the morning and evening meals. The concentrated ration consisted of 25% barley, 25% yellow corn, 50% wheat bran, vitamins A + D₃ (30 g/100 kg) and sodium chloride (1/2 kg/100 kg). The berseem (*Trifolium alexandrinum*) was offered at mid-day meal. During summer, green young corn-stalks or clover hay replaced berseem. The experiment was conducted in four stages equally spaced to represent the four seasons of the year as follows: winter, January and February; spring, April and May; summer, July and August; autumn, October and November. Twenty four does, -12 Bouscat and 12 Giza White, were used in each stage divided into two equal sub-groups, each composed of 6 Bouscat and 6 Giza White does. Females occurred in the first group were used for the determination of ovulation, recovery and fertilization rates. Animals of the second group were assigned for the estimation of implantation and embryo survival rates.

Rabbits of the first sub-group were autopsied 3 days *post coitum*. Day of mating was designated zero day. Their reproductive organs were exposed via a mid-ventral cut. Recent ovulated follicles were located and counted. Ovaries were removed and weighed, corpora lutea were cut apart and weighed separately. Oviducts were dissected free and flushed with 5 ml freshly prepared physiological saline. Ova were recovered and examined microscopically according to procedures described by Hafez (1961). Diameter of eggs to the outer limit of the zona pellucida as well as their diameter including the mucous coat were measured with an ocular micrometre. For the purpose of the present study, one cell eggs were considered unfertilized.

Females of the second sub-group were anaesthetised 12 days *post coitum*, day of mating was designated zero day. Anaesthesia was induced by injecting 25 mg sodium pentobarbital (Nembutal)/kg in the marginal ear vein and was maintained, if needed, with ether. Therefore, animals were laparotomized and their reproductive organs were exteriorized. The swellings in the horn were cut open and chorionic vesicles if present, were transferred into a petri dish containing normal saline, carefully opened and embryos were released. Viable embryos were those showing spontaneous or induced heart beatings or those without any detectable symptoms of disintegration, e.g., their tone, colour, proportion of the head and limbs in relation to the body and crown rump length were the same as for their counterparts with functioning hearts. Embryos otherwise were considered dead. Swellings with partial or complete disintegrated embryonic membranes revealed typical cases of embryonic mortality. Ovarian weights, number of corpora lutea, implantations and viable embryos were recorded.

Pieces from the middle part of uterine horn were removed from all experimental animals, fixed in Bouin's solution and prepared for sectioning, staining and examination. Climatic data on ambient temperature and day-light were obtained from one of the stations belonging to the Egyptian Meteorological Administration. This station is about half a mile far from the research centre where trials have been conducted. Statistical analysis of the data was carried out according to Snedecor and Cochran (1967).

Results and Discussion

Ovulation rate

Data on ovulation rate, estimated on the basis of postcoital counts of corpora lutea, appear in Table 1. Three days after copulation, ovulation rate in Bouscat and Giza White rabbits was 8.1 and 7.3, respectively. Difference between means was statistically not significant. Rates for the two respective breeds when CL counts were done 12 days *post coitum* were 8.1 and 8.7 (Table 1). Once again breed effect on ovulation rate lacked statistical significance. Ovulation rate estimated 3 days *post coitum* (7.7) was significantly lower

($P < .05$) than that determined 12 days after mating (8.4). Apparently, efficiency of CL counting at the two different postcoital intervals is responsible for such existing difference. It is understood that counting corpora lutea in rabbits slaughtered 3 days *post coitum* is much more difficult than when counts are done on laparotomized subjects with well developed corpora lutea. Ovulation rates in Bouscat and Giza White rabbits are markedly lower than estimates known for Chinchilla (Hafez and Rajakoski, 1964) and New Zealand White rabbits (Hafez, 1964 and Koditawakku and Hafez, 1969), however, higher than rates mentioned for Dutch-Belted (Maurer *et al.*; 1968 and Koditawakku and Hafez, 1969) and Polish does (Adams, 1970). It should be mentioned that in most studies cited earlier, ovulation was induced either by coitus associated with gonadotropin injection (15 to 30 i.u. HCG/ doe) or by gonadotropin administration alone (2.5 mg PLH/ doe).

TABLE 1. Seasonal variation in ovulation rate in Bouscat (B) and Giza White (G) rabbits.

Season	Ovulation rates ⁽¹⁾					
	estimated 3 days p.c.			estimated 12 days p.c.		
	B	G	B+G	B	G	B+G
Winter	10.8 ⁽²⁾	8.8 ⁽²⁾	9.8	9.3 ⁽²⁾	9.3 ⁽²⁾	9.3
Spring	8.3	9.7	9.0	8.5	12.0	10.2
Summer	7.3	6.2	6.7	7.5	7.8	7.7
Autumn	5.8	4.5	5.2	7.0	5.8	6.4
All seasons	8.1	7.3	7.7	8.1	8.7	8.4

1. Assessed by counting number of corpora lutea/ doe.

2. Mean of each season was calculated from six rabbits. p.c. = *post coitum*. Rabbits failed to ovulate were not included.

In pooled data, ovulation rate for winter, spring, summer and autumn was 9.3, 10.2, 7.7 and 6.4, respectively. ANOVA revealed that the effect of season on ovulation rate was statistically significant ($P < .01$). Reports reviewed on seasonal influence on ovulation rate are contradicting. Hammond (1921) found that ovulation rate in wild rabbits increased from 4.2 in January to 6.7 in April. His study indicated marked differences in monthly ovulation rates. Working with domestic rabbits, Hahn and Gabler (1971) came to a similar conclusion as they found that ovulation rate was high during the period from March to August and obviously low in the period from November to

February. Koefoed-Johnsen and Fulka (1966) noticed seasonal patterns in ovulation rate of domestic rabbit but observed variations were non-significant. Their experiment covered the period from October to June and ovulation was induced by HCG injection. Hafez (1964) claimed no consistent seasonal variation in ovulation rate in a group of domestic rabbits ovulated by either sterile coitus or HCG injection. Present authors believe that the administration of exogenous gonadotropin for ovulation induction may impair season effect on ovulation rate. Simply because season may affect the trait *via* its action upon rate of synthesis or/and release of endogenous ovulatory hormones.

In this study, the highest values for ovulation rate were recorded in winter and spring and the lowest estimates were those of summer and autumn (Table 1). Winter (13.4°) and spring (20.3°) are the relatively temperate seasons of the year. Thus seasons with moderate ambient temperatures appeared to be associated with high ovulation rate. This is in contrast with summer, the hottest season of the year (27.1°), where ovulation rate is obviously low. Of interest is the early work of Hammond (1921) in which he used tamed rabbits with a restricted breeding season out of it, does were in complete anestrus. Surprisingly, he succeeded to prolong the breeding season by controlling temperature and feeding. From his study it appears evident that ambient temperature has something to do with initiating the physiological readiness of the doe to ovulate if the proper stimulus is present. In the present study, the relationship between day length and ovulation rate is not that obvious as that reported for ambient temperature. If it is tentatively assumed that short day length is related to high ovulation rate, this assumption should be rejected as average day length in autumn (11.4 hr) is shorter than in spring (12.9 hr), even though ovulation rate for the latter season was comparatively high (Table 1). Apparently, seasonal variation in light hours may not be responsible for variation in ovulation rate in domestic rabbit. This conclusion was reached by Smelser *et al.* (1934). In their study, domestic rabbits were subjected to continuous light, continuous dark, increasing light and decreasing light for one month but none of these treatments had exerted any significant effect on number of ovulations.

Recovery and fertilization rates

Average number of ova recovered/doe is given in Table 2. For both combined breeds, recovery rate for winter (6.4) and spring (7.9) was higher than that for summer (5.4) and autumn (3.6). Seasonal influence on recovery rate followed the same pattern reported earlier for ovulation rate. Fertilization rate for Bouscat and Giza White rabbits was high in the four seasons investigated (Table 2). Neither breed nor season had affected the reproductive character.

Diameter of blastocyst

As there are no differences in diameters of blastocysts recovered 3 days *post coitum* from Bouscat and Giza White rabbits data were pooled and appeared in Table 3. With respect to breed effect on blastocyst diameter, Hafez and Rajokoski (1964) did not find significant difference in diameter of blastocysts

recovered 144 hr *post coitum* from New Zealand and Chinchilla rabbits. A similar conclusion was reached by Kodituwakku and Hafez (1969) who failed to detect marked differences in diameter of blastocysts recovered 144, 152 and 160 hr *post coitum* from Dutch-Belted and New Zealand White rabbits. These studies as well as the present one indicated no obvious effect of breed on blastocyst diameter.

Outer-zonal diameter of blastocysts recovered in winter, spring, summer and autumn was 169.7 μ , 169.6 μ , 175.1 μ and 179.3 μ , respectively. Season effect is not obvious, however, marked variation in blastocyst diameter was noticed in summer. Blastocyst diameter including the mucous coat was 369.3 μ , 329.7 μ , 387.6 μ and 413.5 μ in winter, spring, summer and autumn, respectively. Thus diameter in summer is obviously greater than other seasons. It appears plausible to conclude that summer and autumn stimulated the deposition of more oviductal mucin around the egg. Estimation of percentages of under- and over-sized blastocysts in different seasons was not worked out. This may be helpful in tracing, through quantitative approach, whether blastocyst size including mucous layer is related to its subsequent development or not.

Number of implantation sites

Number of implantation sites/doe is given in Table 4. Average number of implantation sites/Bouscat doe was 6.8 and for the Giza White doe it was 7.5. Difference between means lacked statistical significance. Season effect on the character was significant ($P < .01$). Mean number of implantation sites per doe was higher in spring (9.5) and winter (7.6) and low in autumn (6.2) and summer (5.2). Of interest is the percentages of corpora lutea, — counted 12 days *post coitum*, associated with the formation of implantation sites. These percentages were 81.7, 93.1, 67.5 and 96.9 for winter, spring, summer and autumn, respectively. The lowest percentage was recorded in summer. This may be due to the direct or indirect effect of high ambient temperature of the season on pre-implantation survival of blastocysts. It should be noted that fertilization rate during the season was high (97%). Thus, fertilization failures were not basically involved in the observed decrease of nidation in summer. A considerable number of fertilized eggs probably died before stimulating the uterine horn to develop swelling that could be detected 12 days *post coitum*. Previous work indicates that sperm cells can be influenced by high ambient temperatures so that fertilization rate is not affected but pre-implantation survival of embryos is decreased (Burfening and Ulberg, 1968).

Embryo survival

Data on number of viable embryos per doe followed the same pattern reported for number of implantations (Table 4). Breed had no obvious effect, meanwhile, season affected the trait markedly. Average number of viable embryos/doe in winter, spring, summer, and autumn was 6.8, 8.9, 3.0 and 5.9, respectively. Values of post-implantation survival in the four

respective seasons were 90, 94, 57 and 95 (Table 4). Deleterious effect of summer on post-implantation survival was obviously clear. As suggested by the present study, high ambient temperature of the summer may be the responsible factor for the obvious increase in embryo mortality during the season. Alliston *et al.* (1965) came to the conclusion that early rabbit embryo (one cell fertilized eggs) was directly affected by the increase in maternal body temperature that accompanied thermal stress on the doe. They added that such effects may not become apparent until late stages of embryonic development.

TABLE 2. Seasonal effects on fertilization rate in Bouscat (B) and Giza White (G) rabbits.

Season	Ova recovered/doe ⁽³⁾		Fertilization rate ⁽⁴⁾	
	B	G	B + G	B + G
Winter	8.3	4.5	6.4	99
Spring	7.2	8.7	7.9	100
Summer	6.0	4.8	5.4	97
Autumn	4.7	2.5	3.6	100
All seasons	6.5	5.1	5.8	99

3) Number of eggs recovered 3 days *post coitum* / number of does.
 4) Number of cleaved eggs 3 days *post coitum* / number of ova recovered.
 Mean for each season for each breed was calculated from six does.

TABLE 3. Seasonal influence on diameter (μ) of rabbit ova recovered 3 days *post coitum*.

Season	Number of blastocysts	Diameter of the outer limit of zona pellucida		Diameter of blastocyst with mucous coat	
		mean	range	mean	range
Winter	76	169.7	150 — 188	369.3	300 — 528
Spring	95	169.6	151 — 188	329.7	251 — 502
Summer	63	175.1	113 — 245	387.6	300 — 490
Autumn	43	179.3	170 — 188	413.5	396 — 434
All seasons	227	173.2	113 — 245	363.6	251 — 528

Ova recovered from both tested breeds were studied as one pool.

TABLE 4. Number of implantation sites and viable embryos / doe in different seasons of the year.

Season	Number of implantation sites/doe			Number of viable embryos/doe			Post-implantation survival ⁷		
	B	G	B+G	B	G	B+G	B	G	B+G
Winter	7.5	7.7	7.6	6.8	6.8	6.8	91	89	90
Spring	8.0	11.0	9.5	7.5	10.3	8.9	94	94	94
Summer	5.3	5.3	5.2	3.5	2.5	3.0	68	47	57
Autumn	6.7	5.8	6.2	6.3	5.5	5.9	95	94	95
All seasons	6.8	7.5	7.1	6.0	6.3	6.2	88	84	86

7) Number of normal embryos recovered 12 days *post coitum* / number of implantation sites counted 12 days *post coitum*.

B = Bouscat and G = Giza White rabbits.

Mean number of implantations and viable embryos / doe, for each season for each breed, was calculated from six does.

Season of the year was shown to evoke morphological and histological changes in the reproductive organs of the doe. Such changes included ovarian weight and thickness of uterine myometrium (Table 5). The rabbit ovary in winter and spring was heavier than in summer and autumn. This finding is correct, no matter ovaries were weighed 3 days or 12 days *post coitum* (Table 6). Season did not influence the total weight of lutein tissue/doe in the same pattern it affected ovarian weight. It is logic to conclude that season effect was more apparent on the weight of other ovarian compartments (follicle and interstitial tissue compartment). The present data did not outline, however, which ovarian compartment, rather than the lutein tissue, was much likely to be affected. The uterine myometrium was thinner in spring and summer than in winter and spring. Growth and activity of uterine muscularis are known to be primarily under the control of ovarian hormones.

Interpretation of data

The present study failed to show marked differences between Bouscat and Giza White rabbits with regard to all traits investigated. This is in contrast with season effect which was easily perceived. No doubt that winter and spring were the most favourable seasons of the year for rabbit reproduction in this country. During these seasons, the physiological potentialities of the doe to reproduce were obviously high as indicated by high rates of ovulation,

TABLE 5. Morphological and histological data for rabbits killed at different seasons of the year.

Observation	Winter	Spring	Summer	Autumn
<i>Imposed 3 days post coitum</i>				
Number of CL/doe	9.8	9.0	6.7	5.2
Weight of CL (mg)	3.2	2.5	4.0	4.3
Weight of lutein tissue/doe (mg)	31.6	22.7	26.9	22.2
Weight of ovary (mg)	652.0	531.7	395.4	295.9
Thickness of myometrium (μ)	944.3	1153.9	777.9	613.1
<i>Imposed 12 days post coitum</i>				
Number of CL/doe	9.3	10.2	7.7	6.4
Weight of CL (mg)	14.3	12.2	15.1	17.9
Weight of lutein tissue/doe (mg)	133.5	125.0	115.8	114.9
Weight of ovary (mg)	733.8	815.2	541.0	549.9
Thickness of myometrium (μ)	1066.8	1285.8	943.6	824.2

8) Data from both tested breeds were treated as one pool.

fertilization and post-implantation survival. The functional reproductive peak was reached in spring. In summer, the peak disappeared, this was indicated by drastic deterioration in all traits mentioned earlier. Fertilization rate was the only exception, its value persisted to be high even in this particular season. Results, however, did not indicate with certainty the environmental factor responsible for the sharp decline in reproductive capabilities of the doe during summer. Nevertheless, when comparing results of the four seasons some evidences were available implicating possible involvement of ambient temperature. No similar evidence was traced for the effect of day length. Further work is needed to elaborate the association between ambient temperature and the reproductive potentialities of the doe. Autumn was shown to be the transitional season between the valley of fecundity in summer and the onset of the reproductive peak in winter.

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التغيرات الموسمية لبعض الصفات التناسلية للأرانب البوسكات والجيزه الأبيض ،

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استعمل في الدراسة ٩٦ أرنباً كبيراً غير مرضع وبيت بمزرعة كلية الزراعة جامعة القاهرة ، قسمت الحيوانات الى ثمانية مجاميع ، ضمت كل مجموعة ١٢ أنثى (٦ اناث بوسكات ، ٦ اناث جيزه أبيض) .

قسمت السنة الى أربعة فصول ، استعمل في كل فصل مجموعتين من الأرانب . الأولى قتلت أثنائها بعد ثلاثة أيام من التلقيح لحساب معدلات التبويض ، معدل استخلاص البويضات ومعدلات الإخصاب . أجريت جراحات تجريبية في اليوم الثامن عشر من التلقيح لاثاث المجموعة الثانية وذلك لحساب معدلات الانفراس ومعدلات حياة الأجنة . درست بعض الفاييس المورفولوجية والهستولوجية لبعض الأعضاء الجنسية لأفراد المجموعتين .

تتلخص النتائج المتحصل عليها في الآتى :

١ - لم تختلف الأرانب البوسكات اختلافاً له معنوية عن الأرانب الجيزه الأبيض في جميع الصفات التي تناولتها الدراسة .

٢ - كان لفصل السنة تأثير واضح ذو دلالة احصائية على معدلات التبويض ومعدلات الانفراس ومعدلات حياة الأجنة . فكانت هذه المعدلات عالية في الشتاء والربيع ومنخفضة للغاية في الصيف وذات قيم متوسطة في الخريف . لم يؤثر فصل السنة على معدلات الإخصاب فكانت عالية في جميع فصول السنة .

٣ - كان متوسط قطر البويضة بغلافها المخاطي في فصلى الصيف والخريف أكبر منه في فصلى الشتاء والربيع .

٤ - كان لفصل السنة تأثيراً واضحاً على وزن المبيض وسمك البطانة العضلية للرحم . فكان وزن المبيض أثقل وسمك البطانة العضلية أكبر في الشتاء والربيع منه في الصيف والخريف .

٥ - تؤكد النتائج أن النشاط التناسلى للأرانب البوسكات والجيزه الأبيض كان عالياً بدرجة واضحة في فصلى الشتاء والربيع متدهوراً في فصل الصيف ومنخفضاً في الخريف .