

Ovarian Structures During Sexual Receptivity at Mating and Post Coitum Stage in Algerian Rabbits: A Comparative Study

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Abstract

The aim of the present work was a comparative study of the ovaries of local Algerian rabbit population, based on their sexual receptivity at mating. A total of 60 rabbits were divided equally into 3 groups according to the expression of their sexual receptivity at the time of presentation to a male: receptive (R^+), non-receptive + assisted mating (R^-+AM), non-receptive + AM + an injection of GnRH ($R^-+AM+GnRH$). They were weighed and sacrificed 0 to 14h *p.c.* to study ovarian structures. Receptive does were heavier than non-receptive (2.010 vs. 1.979 kg, $P < 0.05$). Females from groups R^+ and R^-+AM had a higher number of preovulatory follicles than does of group $R^-+AM+GnRH$ (60%, $P < 0.05$). The frequency of ovulation was significantly influenced by the receptivity of rabbits and the *p.c.* stage, but the ovulation intensity did not vary between groups. All receptive does ovulated at 10hp.*c*while for non-receptive, ovulations were still observed between 10 and 14h. The diameter of antral follicles of $R^-+AM+GnRH$ was significantly higher (722 vs. 567, 604 μm) due to the injection of GnRH. The number of Call Exner bodies was influenced by treatmentgroup and *p.c.*stage. We noted itwas particularly high in $R^-+AM+GnRH$ group (respectively 2.81, 3.20 vs. 3.99, + 30%, $P < 0.001$). In conclusion, sexual receptivity and GnRH influence ovarian structures and frequency of ovulation of nulliparous local Algerian rabbits.

Keywords: rabbit local, ovarian follicle, ovulation rate, GnRH, morphometry

1. Introduction

Female rabbits are induced ovulators require stimulation associated with mating to provoke GnRH release and then the surge of LH which is necessary to elicit ovulation (Bakker & Baum, 2000) or by exogenous GnRH administration to promote the development of ovarian follicles (Wei et al., 2012). Theau-Clement (2005, 2007) had proven that the productivity of receptive females is three to fourtimes higherthan that of lactating does and non-receptive ones. This result is a consequence of the superiorityinreceptive does, in the frequency and intensityof ovulation, fertilization rateandembryo viability. Receptivity, measured by a test in the presence of a male or by observation of the color and turgescence of the vulva, reflects the state of oestrus or dioestrus of does (Moret, 1980). It has been indicated, that follicles > 1.8 mm were present only in receptive females (Lefevre & Caillol, 1978). In addition, works on themorphometric follicular studiesare often ancient (Kranzfelder et al., 1984), and on genetic types generally far removed from rabbit population sencountered in the Maghreb countries.

The aim of this work was to study in three groups of nulliparous female rabbits of local population (receptive (R^+), non-receptive + assisted mating (R^-+AM), non-receptive + AM + an injection of GnRH ($R^-+AM+GnRH$)) the characteristic of ovaries (macro and microscopic) between the mating and the moment of ovulation.

2. Material and Methods

A total of 100 nulliparous females of local Algerian population, aged 4.5 to 5 months of consistent weight (1.800 kg \pm 300 g) and 5 bucks aged 7 months were used. From our basic herd, three groups of 20 rabbits are considered depending on the test result of presentation to male (receptive, non-receptive). Receptive does are mated naturally (group R^+). All non-receptive does after two consecutive days of presentation to two bucks (maximum 15 min) undergone assisted mating (group R^-+AM). Half of them receive an intramuscular injection of 0.2 ml of GnRH immediately after assisted mating.

All animals were kept under the same conditions of management at the experimental station of the University of SaadDahlab Blida, Algeria . They were fed *ad libitum* commercial pellet and water was available *ad libitum* from nipple drinkers.

2.2 Experimental Design and Measurements

2.2.1 Comportmental Tests and Sexual behavior

The females were weighted and the color of the vulva was observed. The females were then presented for the first time to bucks. Female sexual behavior was described according to Caillol et al. (1983) and Lebas (1994). Flattening throughout the test was considered a criterion of dioestrus (non-receptive doe), circling as a step towards submission to mating and mounting and lordosis as a criterion of oestrus (receptive doe). When the doe accepted mating, it arched their back in lordosis as a response to the male mounting.

The male will then approach around it, stands on the hindlegs and rests his body on the rump of the female. While holding her flanks with his four legs, the male makes pelvic thrusts to achieve penetration. In response, the female lifts the tail and exposes the perineal region thus facilitating the coincidence of the vulva and penis.

If the does refuse mating over an interval of 5 minutes they were removed from the buck's cage. Later the same day, they were introduced in the cage of a second buck for a period of 15 minutes. Does that showed behavior indicating readiness to mate, or pressed against the walls of the cage in a collapsed position with either the first or second buck, were returned back to their own cage. The next morning, the does were transferred to the cage of a third buck, if the doe continue to refuse, they were classified as non-receptive.

The assisted mated was undergone for all the non-receptive females. This method of mating is used to reproduce the position lordosis (position that the female adopts when she is naturally receptive). The tail is attached with a rope and then pulled forward to discover the perineum region. Once in the cage with a male, the female is immobilized with both hands in the lumbar region. The operator introduce the first hand between two hind limbs of the female, than the second hand is raised to clear the perineum. The does handled in this way did not fight or scream or show any other signs of stress associated with mating by the buck.

After the test, three groups (R^+ , $R^- + AM$, $R^- + AM + GnRH$) of females were selected according to their sexual receptivity and 2 females were slaughtered at each designated hour (0 h, 2 h, 6 h, 8 h, 10 h, 10 h 30, 11 h, 12 h, 13 h and 14 hours *p.c.*). Their ovaries were then removed immediately to evaluate the different macroscopic parameters. The rest of 40 does were found after slaughtering pseudopregnant because of the presence of ancient corpus luteum in the ovaries, then they were eliminated from our experiment.

2.2.2 Ovarian Macro Scopic Examination

Once the ovaries were removed, they were submerged in a flacon plastic tissue culture dishes (60 x 15 mm) containing saline solution. Ovarian measurements comprising of weight (g), and all existing preovulatory, haemorrhagic follicles more than 1 mm in diameter and fresh *corpora lutea* were recorded both in left and right ovary of each doe, as described by Lorenzo et al. (1996). Fresh *corpora lutea* were recorded thus allowing us to analyze the intensity and frequency of ovulation.

2.2.3 Ovarian Histological Examination

All ovaries were fixed in a solution of 10% formaldehyde for subsequent histological sections.

Microscopic study to measure the diameters of antral follicles (containing several small antral cavities or a single large antral cavity) were based on a technique described by Gougeon and Chainy (1987). Call Exner bodies were counted within the granulosa. They have been described in the rabbit by Motta (1965) and Gosden et al. (1989). In their most typical aspect, they consist of a crown of granulosa cells arranged around a cavity full of liquid similar to follicular fluid and their function is unknown.

2.3 Statistical Analysis

The weight of the rabbits at the time of presentation was analyzed using analysis of variance (ANOVA), taking the effect of the fixed group (3 levels: groups R^+ , $R^- + AM$, $R^- + AM + GnRH$). Ovarian weight, number of preovulatory and hemorrhagic follicles, fresh *corpora lutea*, the number of Call and Exner bodies and diameter antral follicles were studied by analysis of variance taking into account the effect of the lot (3 levels: Lots R^+ , $R^- + AM$ and $R^- + AM + GnRH$), the *postcoitum* stage (10 levels: 0 h, 2 h, 6 h, 8 h, 10 h, 10 h 30, 11 h, 12 h, 13 h, and 14 h) and the interaction point x group. The frequency of ovulation was analyzed according to the same analysis model considering that ovulation follows a Bernoulli (variable 0-1).

3. Results and Discussion

At the time of presentation, rabbits weighed an average 2.028 ± 0.125 kg. Receptive does were heavier than non-receptive ones (2.010 vs 1.979 kg, $P < 0.05$). According to Berchiche and Kadi (2002), the average weight of rabbits Kabyle local population in adult age is 2.490 kg with great variability in different regions of Algeria. In our test, it is lower due to their age (4.5-5 months).

The weight of the ovaries was 0.28 g in our study and it was near to weight recorded by Belabbas et al. (2011) in the female of the same age and parity. However, this last one does not vary significantly between the different groups studied.

The number of preovulatory follicles varies significantly according to the sexual receptivity ($P < 0.05$, Table 1).

Indeed, receptive and non-receptive does undergoing assisted mating have a higher number than the non-receptive does undergoing assisted mating followed by an injection of GnRH (respectively 68% and 27% preovulatory follicles, $P < 0.05$).

The number of preovulatory follicles varies significantly depending on the stage *p.c.* ($P < 0.05$). In fact, from 12 h *p.c.*, this number generally decreases and at 14 h *p.c.* it falls drastically, except in receptive does (interaction group* stage). At 14 h *p.c.*, the number of preovulatory follicles falls drastically, probably due to the increase in circulating levels of LH secreted by the pituitary gland inducing ovulation. All receptive does ovulated 10 hours after mating, while the non-receptive ovulate between 10 and 14 hours (respectively 6 and 8 of 12 rabbits for the group R- + AM and + R- + AM+ GnRH).

This result is original, and this time of ovulation could explain defects fertilization or total embryonic mortality of non-receptive does. Indeed, Thibault (1967) showed in the rabbit that fertilization with aged gametes (coupling too early or too late after ovulation) could be responsible for chromosomal abnormalities including early abortions. Possible explanation could be related to the lower number of preovulatory follicles on the surface of the ovary and / or the absence of LH receptors on the surface of granulosa cells could respond to the preovulatory LH surge

Table 1. Macroscopic study. Ovarian weight, number of follicles, ovulation rate and number of *corporalutea* during the 14 hours *post coitum*. Arithmetic means

	Effectif	Ovaries weight (g)	Preovulatory follicles	Haemorragic follicles	ovulation rate (%)	Fresh corpus luteum
<i>General means</i>	60	0.275	3.4	0.5	43.3	2.5
Group		NS	P<0.05	NS	P<0.05	NS
R+	20	0.280±0.076	5.1 ^a ±3.284	0.8±1.713	60.0 ^a ±0.503	3.3±3.307
R-+AM	20	0.254±0.038	3.7 ^a ±3.813	0.6±1.191	30.0 ^b ±0.470	1.6±2.644
R-+AM+GnRH	20	0.292±0.091	1.6 ^b ±2.326	0.1±0.224	40.0 ^{ab} ±0.503	2.5±3.576
Stage <i>p.c.</i>		NS	P<0.05	NS	P<0.05	P<0.05
0h	6	0.275±0.047	6.2 ^a ±4.916	0.8±1.329	0.0 ^a ±0.000	0.0 ^a ±0.000
2h	6	0.243±0.022	4.2 ^{ab} ±3.869	0.0±0.000	0.0 ^a ±0.000	0.0 ^a ±0.000
6h	6	0.292±0.044	6.3 ^a ±3.011	0.8±2.041	0.0 ^a ±0.000	0.0 ^a ±0.000
8h	6	0.250±0.042	5.3 ^{ab} ±3.830	0.0±0.000	0.0 ^a ±0.000	0.0 ^a ±0.000
10h	6	0.238±0.039	2.7 ^{ab} ±1.862	0.5±0.837	66.7 ^b ±0.516	2.2 ^{ab} ±2.401
10h30	6	0.297±0.138	3.7 ^{ab} ±2.160	0.5±0.837	50.0 ^{ab} ±0.548	3.2 ^{ab} ±3.545
11h	6	0.327±0.113	2.2 ^{ab} ±2.787	1.7±0.837	83.3 ^b ±0.408	4.7 ^{ab} ±3.933
12h	6	0.253±0.054	1.3 ^b ±1.506	0.0±0.000	83.3 ^b ±0.408	5.2 ^b ±2.714
13h	6	0.267±0.066	1.8 ^{ab} ±3.545	0.2±0.408	50.0 ^{ab} ±0.548	2.8 ^{ab} ±4.021
14h	6	0.308±0.072	0.8 ^b ±2.041	0.0±0.000	100 ^b ±0.000	6.5 ^b ±0.837
Group*stage		NS	P = 0.020	NS	NS	NS

Means with different letters are significantly different ($P < 0.05$). NS: Not significant.

The number of preovulatory follicles evaluated macroscopically was higher among females in estrus especially compared to non-receptive treated with GnRH. Interaction group* *p.c* stage results from the fall in the number of preovulatory follicles after ovulation in receptive does. Lefèvre and Caillol (1978) and Kermabon et al. (1994) showed that the number of preovulatory follicles is higher in receptive females compared to non-receptive.

The number of hemorrhagic follicles is influenced neither by the receptivity or the physiological state of the rabbits in the *p.c* stage. The ovulation rate varies according to the receptivity of does and the *p.c* stage. Receptive does ovulate more frequently than non-receptive untreated with GnRH (60 vs 30%, P <0.05).

The treatment with GnRH did not significantly improve the frequency of ovulation of non-receptive does, although there is a 10% difference in favor of the treated animals (40 and 30% respectively). However, the difference is not significant. Our results are not in agreement with the work of Theau Clement et al. (1991). Indeed, these authors injected French angora rabbits with 0.8 µg of GnRH have shown that hormone treatment had improved the frequency of ovulation in treated animals compared to a control group mated naturally (74% vs 28%). Furthermore, In other strains, Theau Clement et al. (1990) found that over 95% of receptive females ovulate after natural mating or following an injection of GnRH. Receptive rabbits of local Algerian population have therefore a low ovulation rate (60%). GnRH treatment can slightly improve the ovulation rate of non-receptive does, however, the difference was not significant (respectively 40-30%). The number of *corpora lutea* with red and turgid stigma related to recent ovulations was not influenced by the sexual receptivity of does. However, it differs significantly with the *p.c* stage because the first ovulation is observed only from 10h *p.c*. At 12h and 14h *p.c*, the number of *corpora lutea* was significantly higher than in other stages. The lack of significant effect on the number of *corpora lutea* according to the receptivity is likely related to a too small number. In our experience, in ovulated rabbits, the number of *corpora lutea* in females R + and R-untreated GnRH are similar (5.4 and 5.3). The ovulation rate is low compared to females of the same origin and the same parity (Belabbas et al., 2011: 7 *corpora lutea*; Zerrouki et al., 2009. 10.7 *corpora lutea*). This could be the result of a control system does not allow the expression of optimal potential of rabbits in our experiment. Furthermore, some studies have shown that GnRH and its analogues also exert a direct effect on gonadal function influencing rabbit oocyte maturation both *in vivo* and *in vitro* (Yoshimura et al., 1992).

Table 2. Microscopic study. Diameter antral follicles and Call and Exner bodies number Results of analysis of variance (estimated means)

	<i>Effectif</i>	Antral follicle diameter(µm)	Call Exner bodies number
<i>General mean</i>	422	640	3.32
Group		P<0.001	P<0.001
R+	156	567 ^a	2.81 ^a
R-	123	604 ^a	3.20 ^a
R- + GnRH	143	722 ^b	3.99 ^b
<i>p.c.stage</i>		NS	P<0.001
0h	50	532	4.03 ^a
2h	45	714	3.64 ^a
6h	43	608	2.64 ^{bc}
8h	43	718	3.51 ^{ac}
10h	38	595	2.68 ^c
10h30	34	630	3.70 ^a
11h	29	606	2.72 ^c
12h	57	669	3.74 ^a
13h	42	542	3.95 ^a
14h	41	700	2.67 ^c
group*stage <i>p.c</i>		NS	P<0.001

Means with different letters are significantly different (P<0.05). NS: Not significant.

The diameter of antral follicles (Table 2) differed significantly depending on the group. It is higher among non-receptive does undergoing assisted mating and treated with GnRH (respectively 722 vs 567 and 604 µm for R + and R- + AM, P < 0.001). However, it does not vary significantly according to the stage p.c. The diameter of antral follicles is high in the hours before ovulation, due to the rapid increase of follicular growth in the pre-ovulatory phase.

Non-receptive does treated with GnRH have a significantly higher number of Call Exner bodies (respectively 3.99 vs. 2.81 and 3.20 for R + and R- + AM). However, the group and the p.c. stage interact on the number of Call Exner bodies (P < 0.001). In fact we see only in rabbits treated with gonadoreline, the number of Call and Exner bodies is higher in the following mating (from 0 to 2 p.c) hours. To our knowledge no study has put in relation the number of Call Exner bodies with sexual receptivity and *post coitum* stage.

The literature does not tell us about the relationship between sexual receptivity, the diameter of antral follicles, or the number of Call Exner bodies. The diameter of antral follicles equal to 640 µm is close to that obtained by Zitny et al. (2004; 682 µm) but less than that obtained by Kranzfelder et al. (1984; 800 µm). It appears that females of group R- + AM + GnRH have antral follicles with a diameter greater than that of the other two groups. This result suggests that the secretion of FSH and LH induced by GnRH, increased follicular growth and likely proliferation of Call Exner bodies in granulosa, leading to an increase in the volume of follicular fluid.

4. Conclusion

This original work does not show a link between sexual receptivity in rabbits of local Algerian population at the time of mating with ovarian weight, the number of hemorrhagic follicles, the number of corpora lutea in 14 h pc. In contrast, receptive does are slightly heavier and ovulate more frequently and more rapidly than non-receptive. An injection of GnRH can slightly increase the frequency of ovulation, the diameter of antral follicles and the number of Call Exner bodies in non-responsive does. The role of these bodies is little known today. Their presence and increase in number and volume in the granulosa of antral follicles may contribute to the increase in follicular fluid. It would be convenient to study, the interest of injecting of GnRH on an important number of rabbit does refusing mating.

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