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Influence of Mating Time on some Serum Biochemical and Endocrine Parameters of Rabbit Does Following Estrus Synchronization

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Abstract

Studies on the influence of mating time on some serum biochemical/endocrine parameters were carried out using forty adult rabbits does, following estrus synchronization, artificial insemination, and natural service. The rabbit does use were New Zealand White, Dutch, California, Chinchilla, and Gray breeds. They were divided into four group types of 10 rabbits does with different breeds each, designated as natural service (NS), Artificial Insemination (A.I), Natural service + Gonadotropin (NS + GnRH), Artificial Insemination + Gonadotropin (A.I + GnRH). The NS + GnRH and A. I + GnRH rabbit does were synchronized using 0.5 ml Gonadotropin-Releasing Hormone (GnRH) analog (Lecirelin) i.m injection at point of insemination/Service. The mean albumin value differs significantly ($P \leq 0.05$) with 3.5 ± 0.2 mg/l before mating and 2.9 ± 0.2 mg/l after mating. Glucose mean values also differ significantly ($P \leq 0.05$); it was higher before mating with 96.4 ± 4.8 mg/dl than after mating with 68.3 ± 2.8 mg/dl. Total protein mean values differed significantly ($P \leq 0.05$) having 5.9 ± 0.1 g/l before mating, and 5.1 ± 0.1g/l after mating. Oestrogen values were 21.3 ± 1.7 pg/ml before mating and 17.8 ± 0.8 pg/ml after mating. Mean progesterone value before mating was 3.8 ± 0.41 ng/mL and 15.0 ± 2.9 ng/mL after mating. Mean serum biochemical parameters were higher before mating than after mating and were within the normal range. It was concluded that estrus synchronization, artificial insemination, and natural service had an effect on the biochemical and endocrine response of rabbit does.

Keywords: Rabbits, Estrus, Estrogens, Progesterone, Lecirelin, Gonadotropin-releasing hormone

Introduction

Rabbits are small mammals with fluffy, short tails, whiskers, and distinctive long ears, in the Family Leporidae of the order Lagomorpha. There are more than 30 species around the world. Rabbits possess a number of features that might be of advantage in the smallholder subsistence–type integrated farming in developing countries. The rabbit industry is composed of many groups each with different production goals. Their production can be on a commercial scale, with the rabbits being used for meat, as pets, and for laboratory purposes (1). Rabbit meat production has traditionally been typical of Mediterranean countries located in temperate (mainly southern European countries) and tropical, mainly North African countries (2). In addition, in recent decades rabbit production has risen in many developing countries (such as China, Mexico, Egypt, Nigeria), most of them located in temperate, subtropical, and tropical climate areas (2). In comparison to other species where environmental conditions are widely controlled (poultry), rabbit production is characterized by the heterogeneity of its farming systems. Rational rabbit production, therefore, includes farms ranging from those with a high level of environmental control to semi-open and even open-air housing systems, where animals are highly exposed to climate changes (2). It was revealed that rabbits have a poor population as compared with other livestock in Nigeria, indicating that Nigeria can boast of 13.9 million cattle, 34.5 million goats, 22.1 million sheep, 1.7 million rabbits, and 0.5 million Guinea pigs (3, 4). (5) Reported that Nigeria belongs to a list of countries with over one million does where rabbit production is described as relatively important in the local economy, and heavily dependent on backyard systems. This study was carried out to determine the influence of mating time on some serum biochemical/endocrine parameters of rabbits does following estrus synchronization, artificial insemination, and natural service.

Materials and Methods

Study design

Forty apparently healthy female rabbits (does) and twenty male rabbits (bucks) (*Oryctolagus cuniculus*) encompassing the New Zealand White, Dutch, Gray, Chinchilla, and California

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breeds, aged 8 - 12 months, weighing between 1.8 and 3.5 kg (2.8 ± 0.07 kg) belonging to the Rabbitry Section of the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University Zaria were used for the treatment They were housed individually in standard flat deck cages with internal nest boxes using standard rabbit husbandry methods. The animals were fed on an 18 % crude protein and energy feed ration of 2,700 ME/Kcal/kg, and forage (sweet potato stem and groundnut haulms) throughout the experimental period, and water was given ad libitum. The animals were allowed to acclimatize in the rabbitry facility for four weeks; during which the animals were weighed, examined for ectoparasites, and screened for common parasitic and other diseases before the commencement of the experiment. The infected animals were treated appropriately with subcutaneous injection of Ivermectin 1% (0.2ml/rabbit) and Sulfadimidine Sodium 0.3 to 0.6 ml per kg of live body weight for three days, before their inclusion in the study or removed. The animals were individually identified by ear tattoo. Approval for this study was sought and gotten from the Ahmadu Bello University committee for animal use and care.

The semen used for the experiment was collected using a specially designed artificial vagina for rabbits by IMV Technologies 2911 model North Maple Grove, U.S.A. The ejaculated semen sample was subjected to routine semen evaluations such as semen volume, pH, colour, spermatozoa concentrations, motility, live/dead ratio and morphology with slight modifications (6, 7).

The rabbit does were divided into four groups of 10 rabbit does of different breeds each, designated as natural service (NS), Artificial Insemination (A.I), Natural service + Gonadotropin (NS + GnRH), Artificial Insemination + Gonadotropin (A.I + GnRH). The NS + GnRH and A. I + GnRH rabbit does were synchronized using 0.5 ml Gonadotropin-Releasing Hormone (GnRH) i.m injection at point of insemination/Service. Blood for the hormonal assay was collected between 9 and 10.00 am on non-fasting rabbits. This was determined twice, before mating/insemination (day 0) and during pregnancy (day 14). Five (5) MLS of the blood samples was collected through the middle ear veno-puncture into sample bottles without anticoagulants, labeled appropriately, and transported to the hormone laboratory of the Biotechnology Research Programme, NAPRI where the serum was harvested, placed in serum vials, and stored at -20oC until the determination of serum levels of estradiol, Progesterone, Serum glucose, Albumin, and Total serum protein. Estradiol and progesterone values were determined using ELISA kits purchased from Agappe diagnostics Switzerland GmbH, while albumin, serum glucose, and total serum protein were determined using kits from Agappe diagnostics Switzerland GmbH.

Statistical Analysis

Data obtained were expressed as the mean ± standard error of mean (±SEM). The differences between variables were analyzed using a one-way analysis of variance (ANOVA), and Tukey's post hoc test was used to compare the mean values between the groups. Graphpad prism version 5.0 for Windows was used for all statistical analyses. Values of P were considered significant at ≤0.05.

Results

Estrus synchronization, artificial insemination, and natural service had an effect on serum biochemical response. Mean serum biochemical and endocrine parameters were higher before mating than after mating and were within the normal range. Though reasons for the high estrogen values observed even in the pregnant does during this study is not known, it is thought to be responsible for the excessive myometrial activity creating an unfavorable environment for the sustenance of pregnancy, thereby leading to maternal rejection of the fetuses and subsequent loss of the embryo, causing a low fertility rate.

The mean (± SE) effect of estrus synchronization, artificial insemination, and natural service on serum biochemistry and endocrine responses of rabbits does is illustrated in Table 1. The mean albumin value differed significantly (P≤0.05) with the highest albumin values before mating in the NS having 3.84 ± 0.44 g/l and the least was A. I + GnRH with 2.6 ± 0.57 g/l while after mating NS + GnRH had the highest value of 4.3 ± 0.39 g/l and lowest was in NS does having 2.2 ± 0.49 g/l. Glucose mean values also differed significantly (P≤0.05); NS does before mating had 109 ± 7.1 mg/dl and 72±3.6 mg/dl after mating, AI had 120 ± 9.3 mg/dl before mating and 85.0 ± 5.4 mg/dl after mating which was the highest recorded glucose value after mating, NS + GnRH does have 103.0 ± 15 mg/dl before mating and 67 ± 4.3 mg/dl after mating, A. I + GnRH does record 68 ± 10 mg/dl before mating and 67 ± 5.2 mg/dl after mating while NS + DLS had 90 ± 11 mg/dl and 74 ± 7.9 mg/dl before and after mating, then A. I + DLS does record 88±12 mg/dl before mating and 45 ± 7.6 mg/dl after mating which was the last recorded value for glucose after mating.

Total protein mean values differed significantly (P≤0.05) having the highest value of 7.1 ± 0.62 g/l before mating and 5.4 ± 0.32 g/l after mating in AI + DLS, which was followed by the NS + DLS with 6.2 ± 0.31 g/l and 5.5 ± 0.34 g/l before and after mating respectively. NS + GnRH and AI + GnRH does had 5.5 ± 0.21 g/l and 5.0 ± 0.19 g/l before mating while after mating values were 5.0 ± 0.19 g/l and 4.7 ± 0.27 g/l. The least total protein values were observed in the natural service only group with 4.9 ± 0.60 g/l before mating which further reduced after mating to 4.8 ± 0.12 g/l after mating.

Oestrogen values were 21.0 ± 1.7 pg/ml, 22 ± 2.1 pg/ml, 16 ± 2.4 pg/ml, 14 ± 0.7 pg/ml, 17 ± 1.5 pg/ml and 16 ± 1.6 pg/ml before mating and 18.0 ± 1.7 pg/ml, 16 ± 1.3 pg/ml, 21 ± 1.1 pg/ml, 26 ± 6.7 pg/ml, 26 ± 6.8 pg/ml and 21 ± 1.7 pg/ml after mating for the NS, AI, NS + GnRH, AI + GnRH, NS + DLS and AI + DLS groups respectively.

Mean progesterone values before mating for the NS, AI, NS + GnRH, AI + GnRH, NS + DLS and AI + DLS groups were 6.2 ± 1.1 ng/mL, 3.7 ± 1.0 ng/mL, 2.7 ± 0.1 ng/mL, 2.5 ± 0.1 ng/mL, 2.6 ± 0.1 ng/mL and 5.3 ± 1.7 ng/mL while after mating values were 2.6 ± 0.1 ng/mL, 15 ± 8.2 ng/mL, 17 ± 7.9 ng/mL, 17 ± 7.9 ng/mL, 19 ± 7.6 ng/mL and 17 ± 7.8 ng/mL respectively.

Discussion

The lower albumin levels observed in this study could be a result of increased alpha fetoproteins (8). The lower albumin level results agree with reports (9, 8) who reported a decreased

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albumin level during the mid and late gestation length, while the normal values observed are similar to reports (10, 11).
The observed low glucose levels obtained, may be as a result of decreased feed intake, due to the physiological status of the animal (pregnancy), this agrees with (12), who had a significant decrease during the mid and late periods of gestation, though normal values were reported (13, 9) in similar studies.

Table 1. Mean (± SE) endocrine and serum biochemical parameters of rabbit does following estrus synchronisation, artificial insemination and natural service before and after mating (n=40)

	A1	A2	B1	B2	C1	C2
Albumin (g/l)						
(BM)	3.84±0.44^{a}	3.5±0.51^{a}	3.8±0.46^{a}	2.6±0.57^{b}	3.8±0.39^{a}	3.2±0.54^{a}
(AM)	2.2±0.49^{b}	2.2±0.52^{b}	4.3±0.39^{a}	3.8±0.70^{a}	2.3±0.29^{b}	2.4±0.56^{b}
Glucose (mg/dl)						
(BM)	109±7.1^{b}	120±9.3^{a}	103±15^{b}	68±10^{d}	90±11^{c}	88±12^{c}
(AM)	85±5.4^{a}	85±5.4^{a}	67±4.3^{c}	67±5.2^{c}	74±7.9^{b}	45±7.6^{d}
Total Protein (g/l)						
(BM)	4.9±0.26^{c}	5.8±0.19^{a}	5.5±0.21^{b}	5.4±0.17^{b}	6.2±0.31^{a}	7.1±0.62^{a}
(AM)	4.8±0.12^{b}	5.3±0.15^{a}	5.0±0.19^{a}	4.7±0.27^{b}	5.5±0.34^{a}	5.4±0.32^{a}
Estrogen (pg/ml)						
(BM)	21±1.7	22±2.1	16±2.4	14±0.69	17±1.5	16±1.6
(AM)	18±1.7	16±1.3	21±1.1	26±6.7	26±6.8	21±1.7
Progesterone (ng/mL)						
(BM)	2.6±0.06	3.7±1.0	2.7±0.05	2.5±0.09	2.6±0.1	5.3±1.7
(AM)	16.2±1.1	15±8.2	17±7.9	17±7.9	19±7.6	17±7.8

a-d Different superscripts within the same row indicate a significant difference (P<0.05)**.** BM- Before mating, AM- after mating. A1- Natural service**.** A2- Artificial Insemination**.** B1- Natural Service + GnRH**.** B2- Artificial insemination + GnRH**.** C1- Natural Service + DLS (Doe litter separation)**.** C2- Artificial insemination + DLS

The lower total protein levels observed in NS, A. I + GnRH could be a result of the physiological status of the animal (pregnancy), as some of the protein available was being utilized for the growth of the fetuses, thereby reducing the quantity available for the pregnant does (10). However, the total protein level was all within the normal range and similar to reports by (9, 10).

Differences in serum biochemical and endocrine parameters for natural service that was observed may be as a result of the genetic make-up of the different breeds of rabbits used for this study. The differences may also be as a result of the nutritional status, physiological state (pregnancy) of the does, and environmental conditions. This agrees with the report of a reduction in serum metabolites (14).

The nutritional flushing of the does shortly before mating may have caused an enhancement of the hypothalamus-ovary activity, due to the increased amount of energy available for the doe. This caused the increased estrogen level observed in this study, which is similar to some reports (15). Previous works have also reported a correlation of estrogen concentration in plasma with vulva color and receptivity of female rabbits (16, 6). The increased plasma estrogen levels before mating could be a reflection of increased follicular steroidogenesis, causing a high sensitivity of the pituitary gland to exogenous GnRH, which in conjunction with the absence of suckling would cause an LH response and a high effect on fertility (16). The high estrogen levels observed are similar to the findings of a significant increase in estrogen levels (15); though differ from findings with no significant difference in estrogen values (17).

The increased progesterone values observed during this study are a result of pregnancy. In rabbits, prolonged elevation of progesterone levels is indicative of pregnancy, determining the cause of pregnancy (18). The findings of this study disagree with those who observed a decreased progesterone values in New Zealand White does during pregnancy (13) and the report who observed no significant difference amongst the groups studied (17).

Conclusion

Generally, the serum biochemical and endocrine parameters evaluated showed that albumin before mating was highest in Natural Service, Natural Service + Gonadotropin-Releasing Hormone and Natural Service + Doe Litter Separation and lowest in Artificial Insemination + Gonadotropin-Releasing Hormone with 2.6 ± 0.57 and after mating was highest in Natural Service + Gonadotropin-Releasing Hormone with 4.3 ± 0.39. Glucose before mating was highest in Artificial Insemination with 120.0 ± 9.3 and lowest in Artificial Insemination + Gonadotropin-Releasing Hormone with 68.0 ± 10 while after mating; it was highest in Artificial Insemination with 85.0 ± 5.4 and lowest in Artificial Insemination + Doe Litter Separation with 45.0 ± 7.6. Total protein was highest in Artificial Insemination + Doe Litter Separation with a mean of 7.1 ± 0.62 before mating and highest of 5.5 ± 0.34 after mating in Natural Service + Doe Litter Separation. Mean (± SE) estrogen values before mating was highest in the Artificial Insemination group of does with 22.0 ± 2.1 while after mating it was highest in Natural Service + Doe Litter Separation 26.0 ± 6.8. The highest mean progesterone values before mating were 5.3 ± 1.7 in Artificial Insemination + Doe Litter Separation and 19.0 ± 7.6 in Natural Service + Doe Litter Separation after mating.

Conflicts of interest

The authors declare that they have no competing conflicts of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Approval for this study was sought and gotten from the Ahmadu Bello University Committee for animal use and care.

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