EFFECT OF SOME THERAPIES ON FUNCTIONAL REPRODUCTIVE EFFICIENCY IN A RABBIT MODEL

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ABSTRACT

The present study aimed at evaluating the efficacy of intervention with calcium, gonadotrophic releasing hormone (GnRH) or bromocriptine (BCr) on the reproductive performance and fertility traits of lactating does. For this purpose, NZW pluriparous does (n=120) were allocated into one of four treated groups: first three groups received 100 mg/head calcium citrate tablets orally, 0.8 µg of GnRH *i.m* or 2.5 mg of BCr orally. The remaining group was left as control. GnRH and BCr significantly (*P<0.05*) affected the ovarian biometry in opposite direction i.e. while the former increased the later decreased the ovarian biometry. Microscopically, treated group showed a mild (in GnRH), moderate (in BCr) and high (in calcium) rate of follicular growth. Luteinization of stromal cells was high in calcium and GnRH groups and moderate after BCr treatment. Calcium significantly (*P<0.05*) increased the intermediate cells at the 8th hour post treatment, while no considerable changes were recorded in other groups. GnRH and BCr substantially increased progesterone level when compared to control. The pregnancy rate was lower after calcium, GnRH and anti-prolactin treatments as compared with control. Litter number and size was higher in the calcium group. Litter number was low in GnRH and moderate in BCr.

Keywords: Bromocriptine, Calcium, Fertility, GnRH, Rabbit.

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1. INTRODUCTION

Rabbits occupy a vital midway between ruminants and monogastric animals. They have been used as a model for human and other mammals in a variety of research areas like physiology, toxicology, biotechnology, etc. They are medium size, readily accessible, easy to handle and easy to maintain [1, 2]. During the postpartum period, rabbits were found to be highly receptive from the zero day of parturition and after weaning (Day 28 of lactation), where they are able to ovulate [3]. Receptivity is well known to vary depending on the number of parity (i.e., primiparous are less receptive than nulliparous) and the stage of lactation (i.e., lactating are less receptive than non-lactating), an evidence which may influence the reproductive performance of rabbits [4]. Waves of follicles continuously develop to the antral stage under the tonic action of FSH and regress at approximately 7-10 day intervals [5]. In rabbits, ovulation can also be induced by exogenous administration of hormones [6]. The occurrence of ovulation by direct examination of the ovaries was detected between 10 and 14 h after intravenous administration of LH [7] or LH-RH [8]. Ovulation was also detected after intravenous administration of hCG [9] and intramuscular administration of gonadotrophic releasing hormone [10]. In commercial rabbit production systems, GnRH has been used after insemination to stimulate ovulation [11]. Calcium plays an important role in stimulus secretion coupling in most exocrine and endocrine

glands [12]. There is evidence that calcium plays an important regulatory role in steroidogenesis in both mammalian [13] and non-mammalian ovarian cells [14]. Calcium administration to adult non-pregnant female rabbits has been found to induce a significant increase in estradiol and FSH serum levels [15].

Bromocriptine (BCr); an ergot derivative with potent dopamine receptor agonist activity; has been mainly used for the inhibition of lactation (anti-prolactin), treatment of menstrual disorders and infertility as a dopamine agonist in clinics [16], though its use in animal field is still in advance and need more investigations.

The present study aimed at evaluating the efficacy of intervention with calcium preparation, GnRH or BCr on reproductive characteristics and fertility traits of lactating rabbit does.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

The present study was conducted on 120 New Zealand White pluriparous Does (7-12 months old and weighed 2.7–3.2 kg) and five California bucks (1–1.5 years old and weighed 3.5–4.5 kg) of proven fertility during the period from April 2010 to March 2012. All animals were reared in individual elevated wire cages in rooms with controlled light (16:8 h light: dark), temperature (20 to 25 ºC) and relative humidity (55 to 60%). They received a pelleted commercial diet (IBEX®) 150-200 g per head per day and alfalfa hay *ad libitum*.

Does were allocated into four treatments groups; first group (n=30) received 100 mg/head calcium citrate tablets orally. Second group (n=30) received 0.8 µg of GnRH; buserline acetate (Receptal®, Intervet, The Netherlands) *i.m.* Third group (n=30) received 2.5 mg of anti-prolactin hormone; Bromocriptine mesylate (Parlodel®, Sandoz, Basel, Switzerland) per os. The last group was left as control (n=30). All does were naturally bred by means of well proven bucks two hours post-treatment and were check for pregnancy 15 days post-breeding by digital manipulation. Litter number and weight were determined per each Doe 4-5 hours after kidding.

2.2. Vaginal smears preparation and examination

Samples of exfoliated vaginal cells were collected from all does at 2, 4, 6 and 8 hours post-treatment with a cotton swab and stained with Giemsa stain (dilution 1:10) for 20 min [17]. Vaginal mucosa cells were classified into parabasal (Rounded or oval cells with a high nuclear–cytoplasmic ratio), intermediate (rounded, oval or polygonal cells with a structurally normal nucleus), superficial (polygonal cells with a pycnotic nucleus) and anuclear (cornified polygonal epithelial cells with no nucleus and a clear cytoplasm) according to Ypsilantis et al. [17].

2.3. Blood sampling and hormonal assay

Blood samples (2 ml) were collected through ear vein 12 hours post-treatment into sterile vacuum plain tubes (Biomedica Alex. Co.). Sera were harvested by centrifugation (3000 rpm for 5 min.) within 2-4 hours of collection and were kept at -20°C until assayed for progesterone concentration using direct ELISA kit (EiAsy ^{TM} Way progesterone kit, DBC; Diagnostics Biochem Canada Inc®; London, Ontario, Canada) as described by Check et al. [18]. The intra-assay coefficient of variation (C.V.) was 10.4% and the inter-assay C.V. was 11.4%. Assay sensitivity was 0.1ng/ml.

2.4. Ovarian sampling and histological examination:

Ovaries were collected from does (n=12/group) after scarification (3 does at 2, 4, 6 and 8 hours post-treatment) into clean sterile container and transported to

the laboratory within one hour after scarification to measure the ovarian length and width using a Vernier Caliper after cleaning off the extraneous tissue.
Small tissue specimens from each ovary were fixed in 10% neutral buffered formalin, dehydrated in ascending strength of ethyl alcohol, cleared in xylol and embedded in paraffin. 5 µm paraffin sections were prepared and stained with Haematoxylin and eosin stain for microscopic examination according to Kuehnel [19].

2.5. Statistical analysis

The results were expressed as mean (S.E). Analysis of variance (ANOVA) and Fisher's Least Square Difference (LSD) post hoc tests were used for comparing means variables between treated groups using the statistic software program SPSS according to Armitage et al. [20]. The level of significance is usually set at 0.05.

3. RESULTS

3.1. Ovarian biometry

The length of both ovaries and width of the left ovaries differed significantly ($P<0.05$) among treated groups. The ovarian length as well as the width of GnRH group was significantly ($P<0.05$) larger than other groups. In the meantime, the ovarian biometry of BCr group was the smallest among all treated groups (table 1).

3.2. Ovarian histomorphology:

Ovarian sections of treated does showed a clear difference in the follicular distribution and activity of stromal cells (Fig. 1& Table 2). Treatment with calcium preparation was associated an increase in follicular population and a high rate of stromal cell lutinization. In the meantime, rabbits treated with BCr characterized by a moderate increase in follicular number and stromal cells. GnRH treatment was characterized by a slight increase in the follicular number and high rate of lutinization of stromal cell. Cystic dilatation of some ovarian follicles was evident in all treated groups that were noticed at 8th hour in calcium and GnRH groups and at 6th hour in BCr group.

3.3. Exfoliated vaginal cells

In calcium group, although, intermediate cells (IC) showed a significant ($P<0.05$) increase at eight hours post-treatment, there was a non-significant fluctuation in parabasal cells (PBC), superficial cells (SC) and anuclear cells after treatment. In GnRH and BCr groups, there was a non-significant change in the cellular type of vaginal smear along eight hours monitoring period after treatment (Fig. 2).

3.4. Circulating progesterone levels

Analysis of serum levels of progesterone in treated rabbits revealed a significant increase in P4 level for GnRH and BCr groups when compared to control (Fig. 3).

Table 1 Effect of treatment with calcium (Calcium citrate®), gonadotrophic releasing hormone (Receptal®) and bromocriptine (Parlodel®) on ovarian biometry of lactating rabbits

	Left ovary		Right ovary	
Animal group	Length	Width	Length	Width
Calcium	1.32±0.12^{ab}	0.52±0.06^{a}	1.34±0.11^{ab}	0.50±0.08^{a}
GnRH	1.46±0.03^{a}	0.50±0.03^{a}	1.42±0.04^{a}	0.52±0.04^{a}
Bromocriptine	1.03±0.03^{b}	0.33±0.03^{b}	1.13±0.09^{bc}	0.33±0.03^{a}
Control	1.07±0.09^{b}	0.40±0.06^{ab}	1.00±0.06^{c}	0.50±0.06^{a}

Values with different superscript small letters within the same column differed significantly at $P < 0.05$.

Table 2 Summary of the changes in rabbit ovarian morphology after treatment with calcium (Calcium citrate®), gonadotrophic releasing hormone (Receptal®) and bromocriptine (Parlodel®)

	Calcium	GnRH	Bromocriptine	Control
Follicular changes	Increased number of follicles at different stages of growth	Slight increase of follicular number	Moderate increase in the follicular number	Few follicles mostly primary and secondary follicles
Stromal changes	High rate of stromal cell lutinization	High rate of stromal cell lutinization	Moderate rate of stromal cell lutinization	Excessive increase of stromal cells lutinization
Follicular degeneration	Some follicles show degeneration	Mild follicular degeneration	Moderate follicular degeneration	Absent
Follicular cyst formation	After eight hours post-treatment	After eight hours post-treatment	After six hours post-treatment	Absent

Table 3 Fertility traits of rabbits post-treatment with calcium preparation (Calcium citrate®), gonadotrophic releasing hormone (Receptal®) and bromocriptine (Parlodel®).

	Calcium	GnRH	Bromocriptine	Control
Pregnancy rate	7/30 (23%)	8/30 (27%)	8/30 (27%)	16/30 (53%)
Gestation length	30.86±0.51	30.88±0.40	31.75±0.45	30.40±0.40
(Range)	(29-32)	(29-33)	(30-34)	(28-33)
Litter number	6.4±0.4	4.5±0.8	5.6±0.6	5.8±0.5
(Range)	(5-8)	(1-7)	(3-9)	(3-10)
Litter size (g)	375.00±30.08	343.10±30.25	346.10±27.00	334.00±33.25
(Range)	(255-480)	(200-500)	(210-420)	(180-680)

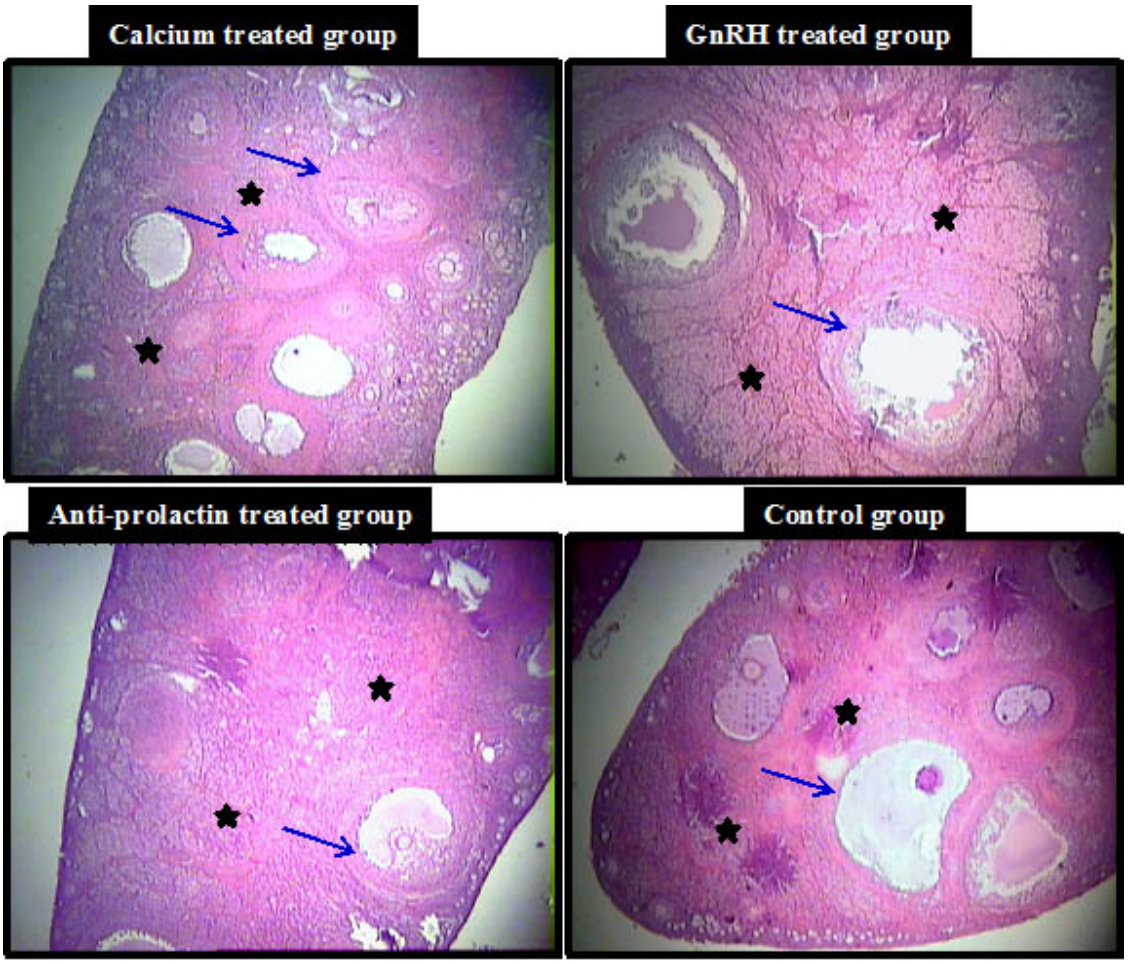

Fig. 1 Histological architecture of mature rabbit ovary stained with Haematoxylin and eosin. Notice the difference in follicular populations (arrows) and variation of interstitial cells luteinization (stars) among treated groups after treatment with

calcium, GnRH or Bromocriptine in relation to control. All microphotographs were captured at the same magnification (×10).

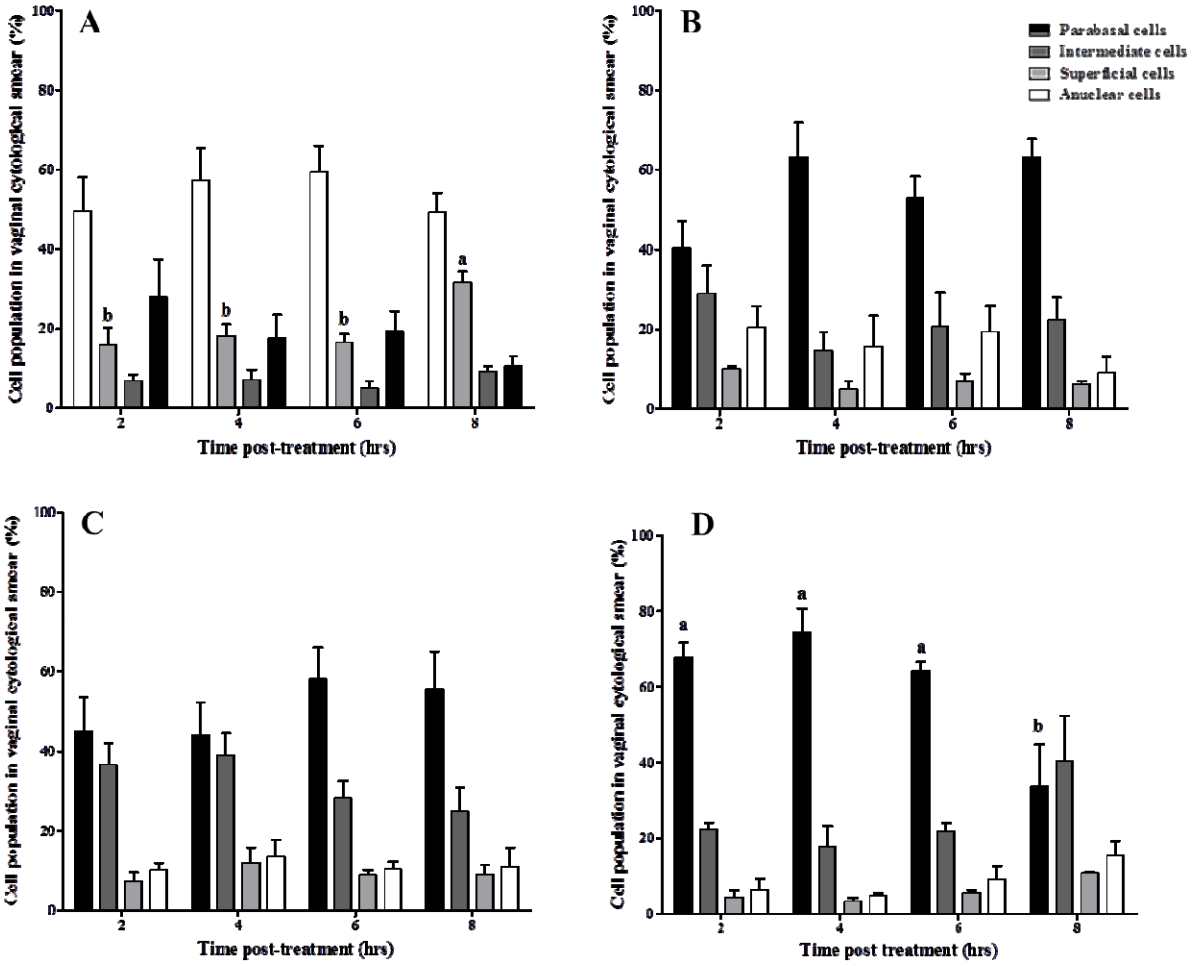

Fig. 2 Changes in the predominant vaginal cell type in treated female rabbits with calcium (A), gonadotrophic releasing hormone (B) and bromocriptine (C) and control (D) in relation to time post-treatment. Mean (±S.E.) with different letters were significantly different at $P<0.05$.

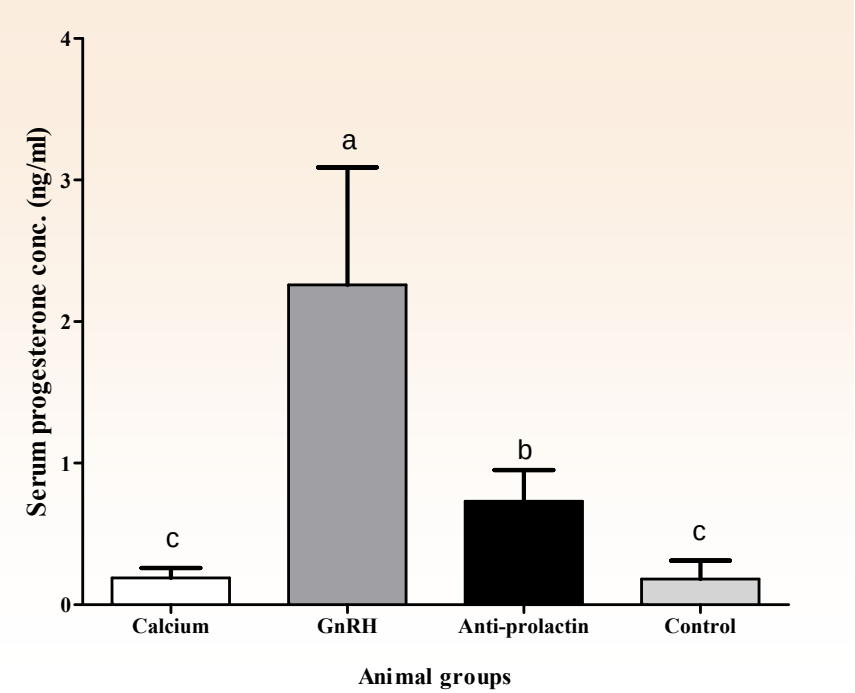

Fig. 3 Progesterone levels in the serum of rabbit after treatment with calcium, gonadotrophic releasing hormone or bromocriptine.

1.1. Fertility traits

Pregnancy rate was low in does treated with calcium, GnRH and the BCr groups compared with control. Litter number was the highest recorded in calcium group, moderate in BCr, and the lowest was in GnRH. The highest litter size appeared with calcium group, but was nearly similar in GnRH and BCr groups, but higher than control (Table 3).

2. DISCUSSION

The improvement of reproductive performance on farms is conditioned by the use of methods enabling the induction and synchronization of estrus. This concerns hormonal treatments or non-hormonal alternative methods. Hormonal treatments have been widely used in recent years. Present study evaluated the efficacy of some therapies (calcium, GnRH and BCr) on the reproductive potential of NZW rabbits and indicated that stimulated ovarian activity in rabbits prior to breeding was associated with lowered pregnancy rate, though calcium was good at improving follicular growth, increased litter number and size. In the meantime, BCr administration was concomitant with moderate ovarian stimulation and lowered pregnancy rate. In the present study, the ovarian biometry of rabbits treated with GnRH was obviously ($P<0.05$) the largest among other treated groups, though this was associated with little increased follicular number but there was high rate of stromal cell luteinization. Contrary to those, rabbits received BCr was as small as control group. Treatment of rabbits with GnRH-analogue has been found to stimulate follicular growth, evidence which might be attributed to an increased synthesis and secretion of FSH and LH from pituitary gland [22]. This finding

approved the stimulatory effect of GnRH on the ovarian follicular growth and development in the present study and others [23]. Similarly, the oocyte maturation, ovulation rate and the corpus luteum number seemed to be higher in mice treated by the GnRH-agonist [24]. Prolactin has been indicated to have many physiological processes including milk production, growth, osmoregulation and reproduction in a wide species of vertebrates [25]. The high concentration of prolactin observed during lactation was noticed to coincide with a reduction in gonadotropin secretion, a finding that, at the ovarian level, may explain inhibiting the number of LH receptors expressed [26]. It has been noticed that BCr treatment has no effect on either the number of follicles or the amount of LH receptor mRNA in does particularly on the day 11 of lactation ($P<0.05$), a finding that suggested that prolactin never plays a role on the quantity but perhaps on the activity of the follicles [26]. In the present study, the widths of left ovaries for rabbits having calcium were as wide as those of GnRH group. The increased ovarian width in calcium group probably may be due to its promotional effect on the ovarian activity. Kitai et al. [23] realized the contribution of calcium dynamics in the processes of ovulation and ovum maturation. Morphological examination of ovarian sections of does at 4 hours post-treatment of GnRH revealed a slight increase in the follicular number with mild degree of degeneration in some follicles and high rate of stromal cell luteinization. In the meantime, some cysts were developed at 8 hours post-treatment. Kobayashi et al. [27] deduced that the oocyte maturation in does appears to be gonadotropin-dependent. YoungLai [28] showed that a single injection of GnRH enhanced the ability of small and large follicles to release estradiol which was depressed 30% in the presence of LH. Yoshimura et al. [29] indicated the presence of mature oocytes initially within 2 hours of GnRH agonist exposure. Wei et al. [22] showed an increase in the number and growth of the primary and secondary follicles in rabbits having GnRH-analogue with enlargement of nucleolus and mitochondria as well as broadening and lengthening of zona pellucida and microvillus of the oocytes, a finding this suggested that GnRH-A promotes development of the ovarian follicles by increasing synthesis and secretion of FSH and LH. In the present study, the ovaries of calcium group showed a similar activity to GnRH action characterized by an increase in the number of follicles at different stages of growth (primary, secondary and tertiary). Some follicles showed degenerative changes, while others indicated the occurrence of cystic formation eight hours post-treatment. There was high rate of luteinization in the stromal cells associated with a comparative decrease in its number due to high follicular growth. The current results showed that prolactin deprivation came in association with a moderate increase in the number of follicles incorporated with moderate follicular degeneration and cystic formation at six hours post-treatment. Moreover, there was a moderate rate of luteinization of stromal cell. It has been reported that lactation negatively influences sexual receptivity at the time of insemination, ovulation, and embryo development in addition to more productivity of rabbit does [30]. Prolactin, responsible for milk

production, could participate in inducing such negative effect via its effect on the pituitary gland by depressing the release of FSH and LH particularly responsible for follicular growth and ovulation [31]. At the level of the ovary, prolactin can limit the in vitro follicular growth and positioning the LH receptors, thus decreasing the efficiency of endogenous hormones [26, 32]. Transient separation of nursing does from their litters before artificial insemination results in a decrease in plasma prolactin concentrations that could promote growth of follicular waves, and high steroidogenic activity, leading to increased estradiol concentrations and inducing higher sensitivity of the pituitary gland to exogenous GnRH [33]. Litter separation for short periods of time augmented sexual receptivity and fertility of the doe as a result of priming the massive release of GnRH, LH and FSH triggered by mating or artificial insemination in litter-separated mothers [34]. Bromocriptine has been mainly used for the inhibition of lactation, treatment of menstrual disorders, and infertility as a dopamine agonist [16].Calcium has been indicated to play an important role in stimulating the secretion coupling in most exocrine and endocrine glands [12]. Kitai et al. [23] verified the role of calcium dynamics in the processes of ovulation and ovum maturation. Calcium movement into the cell and release from intracellular sites is vital for priming events of receptor-stimulated cell surface to cellular responses as stimulus-response coupling [35]. The oral administration of calcium citrate in a dose of 100 mg/kg body weight to adult non-pregnant female rabbits was found to produce a significant increase the serum levels of estradiol and F.S.H. at a rate of 210% and 140% respectively [15]. Such effect might be the reason for the stimulated follicular growth in the present study.

In rabbits, the present study implemented the cytological examination of vaginal smears (CEVS) to predict the ovarian changes and the estrogenicity of domestic rabbit's after various treatments. The use of cytological evaluation of a vaginal smear as a bioassay for estrogen influence is even more reliable than a single plasma estradiol determination [36]. In their study to select rabbit does for superovulation, Ypsilantis et al. [17] found that the cytology of vaginal smears may help to identify does with a significantly higher likelihood of yielding low numbers of CLs, oocytes, or normal zygotes. Ola and Olatunbosun [37] verified the applicability of changes in the exfoliated cells in the vaginal lumen as a predictor to the response to male stimuli on the estrous. In the present study, cell types in the vaginal smears taken from does having either GnRH or bromocriptine showed a none-significant fluctuation along eight hours monitoring period after treatment, a finding that may indicate their failure in stimulating the aromatase enzyme activity and steroidogenesis in developing follicles during this period.

In calcium group, the intermediate cells showed a significant ($P<0.05$) increase at eight hours, while parabasal, superficial and anuclear cells none-significantly differed with time. This finding came in difference to that observed in the control which showed a highly significant ($P<0.01$) difference in the parabasal, superficial and anuclear cells during the monitored period after treatment. The former was lowest at eight hours post-treatment. While the superficial and anuclear cells were

higher at eight hours. In contrast, intermediate cells showed a none-significant variation during eight hours post-treatment. As shown in the present results, estimation of the serum level of progesterone in mature rabbits after treatment with GnRH, bromocriptine and calcium revealed a significant increase in P4 level for does having GnRH (2.26±0.83 ng/ml) and bromocriptine (0.73±0.42 ng/ml) when compared to that in the control (0.18±0.13 ng/ml). Meanwhile, no significant differences in P4 level between calcium group (0.18±0.13 ng/ml) and control. It is well known that GnRH from hypothalamus regulates the secretion of LH and FSH from the anterior pituitary gland by which the ovaries are controlled. Hilliard et al. [38] revealed that LH induces a sharp increase in the secretion of several hormones such as estradiol-17α, progesterone, 20α-Hydroxyprogesterone (20α-OHP), and testosterone in rabbits, all of which reach high values in the circulating blood only a few minutes after GnRH treatment or mating. Interestingly, among these steroids, the 20α-OHP is the main progestin produced by the ovary in quantities ten-fold higher than progesterone. Therefore, GnRH is generally used to stimulate ovarian function. Prolactin, responsible for milk production, could participate in the negative effect [31]. It could act upon the pituitary and depress gonadotropin secretion (FSH and LH, particularly responsible for follicular growth and ovulation respectively) [31]. At the level of the ovary, prolactin could limit in vitro follicular growth [32] and the positioning of LH receptors [26], thus decreasing the efficiency of endogenous hormones. Fortun et al. [39] stated that hyperprolactinaemia during lactation is responsible for the reduced progesterone concentration in pregnant lactating does. On the other hand, bromocriptine has been mainly used for the inhibition of lactation, treatment of menstrual disorders, breast tumors and infertility as a dopamine agonist. Ginther et al. [40] showed that prolactin suppression by bromocriptine treatment was associated with P4 increase and this is attributable to an increase in LH. Calcium plays an important role in stimulus secretion coupling in most exocrine and endocrine glans [12]. Calcium movement into the cell and release from intracellular sites is vital for the coupling of receptor-stimulated cell surface events to cellular responses (i.e., stimulus-response coupling) [41]. Accordingly we may predict an increase of different hormonal secretions following administration of calcium. The results obtained in the current study indicated that oral administration of calcium to adult non-pregnant female rabbits did not affect ovarian progesterone secretion, but might other reproductive hormones. A finding that came in accordance with Rabia et al. [15] found that oral administration of calcium gluconate in a dose of 100 mg/kg body weight to adult non-pregnant female rabbits produced a significant increase of estradiol and FSH serum levels with percentage increase exceeded 210% & 140% respectively.

In the present study, does having calcium treatment were assigned to have higher litter number (6.4±0.4) and size (375.00±30.08 g) but low pregnancy rate (23%). In the meantime, the litter size and pregnancy rates for does having bromocriptine came in comparable to the GnRH treated group, while the litter

number differed among the groups (5.6±0.6 vs. 4.5±0.8, respectively). Pregnancy rate, litter number and size were 53%, 5.8±0.5 and 334.00±33.25 g in control group. Fischer and Meuser-Odenkirchen [42] stated that gonadotropin treatment is efficient in increasing the number of embryos in all seasons of the year, however, insemination of non-receptive females yielded smaller numbers of embryos compared with natural mating. Zapletal and Pavlik [43] examined the effect of GnRH dosage now of insemination on the reproductive performance and found that the conception rate in nulliparous does range from 10.0% to 89.5% after GnRH treatment, though abortions have been recorded at higher doses. Bromocriptine treatment results in a transient decline in sucking by the newborn, and this in itself allows a rise in gonadotropin levels, and thus implantation. Former study by Flint and Renfree [44] showed that implantation could be induced in suckling rats by bromocriptine, though administration of bromocriptine at 2 mg/kg was associated with pregnancy loss. It has been recognized that calcium plays a critical role in offspring production, as a considerable amount of calcium is required to support the development of offspring skeleton. In mice, Schmidt and Hood [45] showed that calcium availability influences litter size and that the mothers fed on the low-calcium diet produced smaller litters.

From this study, we can conclude that calcium was good at improving follicular growth, increased litter number and size but lowered pregnancy rate. In the meantime, BCr administration was concomitant with moderate ovarian stimulation and lowered pregnancy rate.

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تأثير بعض العلاجات على الكفاءة التناسلية الوظيفية في نموذج الأرنب

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الملخص العربي

هدفت الدراسة الحالية إلى تقييم تأثير التدخل العلاجي باستخدام الهرمون الموجه للغدد التناسلية (جى ان ار اتش)، والمثبت لهرمون البرولاكتين (بروموكريبتين)، والكالسيوم على الخصائص الإنجابية وخصوبة الأرانب المرضعات. استخدم لهذا الغرض مجموعة من الارانب النيوزيلندية البيضاء (ن = 120) وخمس من الذكور الكاليفورنيا الخصبة. تم تقسيم الارانب الى واحدة من أربع مجموعات معالجة: تلقت المجموعات الثلاث الأولى 100 ملجم من سترات الكالسيوم / كجم من وزن الحيوان عن طريق الفم، 0.2 مل من "جى ان ار اتش" تحت الجلد، و2.5 ملجم من البروموكريبتين عن طريق الفم. اما المجموعة الرابعة فعولجت بمحلول ملح (المجموعة الضابطة). العلاج باستخدام هرمون "جى ان ار اتش" اظهر زيادة معنوية ($P<0.05$)فى القياسات الحيوية للمبيض بينما أحدث العلاج باستخدام البروموكريبتين انخفاضاً فى قياسات المبيض بشكل ملحوظ ($P<0.05$)مقارنة بالمجموعة المعالجة. المجموعة المعالجة باستخدام الكالسيوم اظهرت زيادة فى عدد الجريبات المبيضية فى مراحل النمو المختلفة بعد 4 ساعات من العلاج مع ظهور بعض التكيسات بعد 8 ساعات من العلاج بينما ارتبطت العلاجات باستخدام هرمون "جى ان ار اتش" بزيادة طفيفة فى الجريبات مع وجود بعض التكيسات عند 8 ساعات بعد تلقي العلاج. أظهرت النتائج ان المعالجة بالبروموكريبتين صاحبها وجود زيادة معتدلة في عدد الجريبات والتي عانت من بعض علامات الاضمحلال. الخلايا البينية فى نسيج المبيض ذات النشاط الهرموني اظهرت زيادة فى العدد في المجموعات التي تلقت الكالسيوم وهرمون "جى ان ار اتش". بينما كانت الزيادة معتدلة فى المجموعة المعالجة بالمثبت لهرمون البرولاكتين. لم يلاحظ تغيرات معنوية فى الخلايا المهبلية المبطنة بعد العلاجات باستخدام هرمون "جى ان ار اتش"، والمثبت لهرمون البرولاكتين على مدار الثمانية ساعات بعد العلاج بينما اظهرت المجموعة المعالجة بالكالسيوم زيادة معنوية ($P<0.05$) فى الخلايا المتوسطة بعد ثماني ساعات من العلاج. اظهرت النتائج وجود زيادة معنوية ($P<0.05$) فى مستوى هرمون البروجسترون فى المجموعات المعالجة باستخدام هرمون "جى ان ار اتش"، والمثبت لهرمون البرولاكتين بالمقارنة مع المجموعة الضابطة. عدد الاجنة اظهر انخفاضاً فى المجموعة المعالجة هرمون "جى ان ار اتش"مقارنة بباقي المجموعات .معدل الحمل اظهر انخفاضاً المجموعات المعالجة الكالسيوم. كان عدد الاجنة وحجمها أعلى في المجموعة المعالجة بالكالسيوم بين باقى المجموعات المعالجة. من هذه الدراسة نستخلص ان العلاج بمركبات الكالسيوم له تأثير اجابى فى تحسن وظائف المبيض والتبويض وان لم يخلوا من التأثير السلبي على الحمل فى الارانب المرضعات مقارنة بالمجموعة الضابطة وهذا يحتاج الى دراسات لمعرفة الاسباب المصاحبة لذلك.

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