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The Effect of Metformin Levels on Ovarian Activity, Embryo Quality and Reproductive Performance of (Baladi Black) Rabbit Does

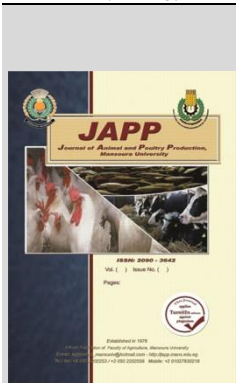
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ABSTRACT

This education was conducted toward investigate the effect of metformin on ovarian activity and embryo quality of local breed rabbit does. A total of 21 mature does were alienated into three groups (7 rabbits in each). Does in the 1st group were assumed oral 3ml sterilized saline (Control, G1). Does in the 2nd (G2) and 3rd (G3) groups were specified 20 and 40 mg metformin/mg/kg live body weight, respectively for 3 weeks before neutral coupling by fertile bucks from the matching breed. Weight and litter size at natal and weaning were documented from 4 does in each group. Embryos being composed by flushing from 3 does in each group with phosphate buffer solution from oviduct and uterine horn in each doe in all groups after 72 hrs of mating, where the ovaries structure on the ovarian site was recorded. Inconsequential contrasts between location of the ovary within the cruel number of little, medium and large follicles. Whereas, the cruel number of CL and bleeding follicles in right were altogether ($P < 0.05$) higher than cleared out (5.78 vs. 4.11) and (1.22 vs. 0.67). The rate of practical embryos was higher in G1 (95.2%, $P < 0.05$) than those in G2 (88.9%) and in G3 (75.0%). Whereas fetus recuperation rate was higher in G2 than in other bunches, but the adjustments among them were not noteworthy. These results indicated that treatment improved ovarian response and reproductive performance although it's reducing embryo recovery and viability rate for rabbit does.

Keywords: Rabbit, reproductive performance, metformin embryo recovery.

INTRODUCTION

Metformin (1,1-dimethylbiguanide hydrochloride) is a verbal antihyperglycemic sedate broadly utilized for the activity of sort 2 diabetes. Its fundamental instrument of activity is through the starting of the AMP-activated protein kinase, which acts as an vitality sensor by checking the AMP/ATP status of the cell (Corton., 1994 and Zhou, 2001). Enactment of AMP- actuated kinase produces the pleiotropic useful impacts of this medicate, such as the concealment of endogenous glucose generation and the advancement in lipid digestion system (Zhou, 2001).

Various ponders have uncovered that metformin recoups lipid profiles, diminishes body weight, and regularizes menstrual cycles in PCOS patients (Ayaz, 2013 and Velazquez., 1994). In expansion, metformin has been appeared to make strides ovulation, pregnancy rates (Jungheim and Odibo 2010 and Palomba, 2014) and live birth rates (Moll, 2007) in PCOS patients. In spite of the fact that, the significance of metformin on richness is still questionable. The accessible meta-analyses that have assessed the impact of metformin on the ovary have appeared distinctive comes about. For this point, in 2007, the European Society of Human Propagation and Embryology/American Society for Regenerative Medication bunch suggested the utilize of metformin in PCOS as it were in ladies with glucose bigotry and not for ovulation acceptance (Thessaloniki 2008). In any case, in a few thinks about, modern prove has been found almost the conceivable benefits of metformin in richness treatment of PCOS patients, since it clues to an increment in ovulation

and pregnancy rates (Jungheim and Odibo 2010, Tang *et al.*, 2012 and Palomba *et al.*, 2014). Additionally, a few ponders have found no contrasts in live birth rates in PCOS patients protected with metformin (Tang, 2012 and Siebert, 2012).

The components by which metformin acts within the ovary to upgrade richness are still hazy. At the cellular level, metformin has been experiential to control oocyte development. Amid bovine and porcine oocyte development, metformin is able to deter meiotic movement (Mayes, 2007 and Tosca, 2007). There's no accessible realities on the impact of metformin richness in rabbit does. So the pointed of this think about is to examine the impact of metformin levels (0.0, 20 and 40mg/kg LBW) on ovarian movement and fetus quality of neighborhood breed (baladi dark) rabbit does.

MATERIALS AND METHODS

This education was permitted out at private farm in Belqas Daqahlya, in collaboration with the International Livestock Management Training Center (ILMTC), Kafr El-Sheikh Governorate, going to the Animal Production Research Institute, Dokki, Giza, Egypt, during the period from November 2020 to May 2021.

Experiment:

Add up to of 21 develop 6.5 months age nearby breed (baladi dark) rabbit does expending a live body weight (LBW) of 3060-3140 g were distributed into three comparative bunches (7 Rabbits in all) and permitted to adjust for 7 days in their particular pens. Females in the 1st group were assumed oral 3ml sterile saline (Control, G1).

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Females in the 2nd and 3rd groups were given 20 and 40 mg/kg live body weight of metformin (Cidophage, Metformin HCl 500mg, CID Co.Egypt) (drugs dissolved in 3 ml sterile saline). for 3 week some time recently characteristic mating. As well as three fertile bucks from the same breed were utilized for characteristic insemination of does.

Three does from each group (G1, G2 and G3) after 72 hrs of mating were butcheries for embryos collected. As well as four does from each group (G1, G2 and G3) leave until kindling.

After kindling, the litter assess at weaning and birth (include up to and live), reasonability rate at weaning and birth, ordinary live weight at birth of rabbits, and litter weight at weaning and birth were record. All does and bucks were kept underneath the same diagram of nourishing and organization interior the private farm, being autonomously housed in metal cages (40 x 50 x 60 cm) giving with feeders and water areola for drinking in each cage. All does and bucks were reinforced on useful eat less (16% CP and 2850 Kcal/kg eat less vitality) feed chemical composition of eat less are revealed in Table 1.

Table 1. Ingredients and chemical composition of experimental diets.

Elements	%	Intended examination%	%
Barely grains	32.80	Organic matter,	89.76
Clover hay	27.41	Crude protein,%	17.26
Wheat bran	17.10	Crude fibre,%	12.92
Soybean meal (44% CP)	16.24	Ether extract%	2.16
Molasses	3.00	Nitrogen free extract %	57.42
Di calcium phosphate	2.00	Ash,%	10.24
Sodium Chloride (NaCl)	0.30	Diet energy,(kcal/kg)	2518
Vitamin and minerals*	0.30	Calcium,	1.13
Lime stone	0.40	T. phosphorus,	0.80
DL-Methionine	0.40	Methonine,	0.64
Anticoccidia(Diclazuril)	0.05	Lysine,	0.86
Molasses	3.00		
Aggregate	100		

*Every 1.5kg of premix mixture contains: Vit.A 10,000.000IU, Vit.B₁1000mg, Vit.B₂5000mg, Vit.D₃2,000.000IU, Vit.E10.000mg, Vit.K₃1000mg, Pantothenic acid 10.000mg, Nicotinic acid, 30.000g; Vit.B₆ 15000mg; Vit.B₁₂10mg, Folic acid 1.0g, Biotin 50mg, Cu 4g, choline chloride 200mg, Mn 60g, Fe 30g, Co 01g, Se 0.1g, Zn 50g, Iodine 0.3g and Antioxidant 10.000mg, permitting to Feed composition for animal and poultry feed stuff used in Egypt (2001).

Embryo recovery

Arrangement of flushing medium:

Phosphate buffer saline (PBS) medium was organized concurring to Gordon (1994) for making life flushing. Around 2 mg of bovine serum egg whites (BSA) was included to one ml of PBS (2%). The organized medium was adjusted to pH respect of 7.2-7.4 by pH-meter and to an osmolality level of 280-300 mOsm/kg utilizing osmometer. Then, the medium was drinkable by a 0.22 µm millipore channel (milieux GV, millipore, Back Bedford MOA).

Embryos were recouped from uterine horns and oviducts by flushing with phosphate buffer course of action (PBS) with 4% bovine serum egg whites (BSA) at room temperature (20-25°C). A brief time afterward, the recovered embryos were totaled and observed for standard and unordinary morphological (quality) measures utilizing an modified amplifying focal point. Embryos recuperation rates were intended as (number of collected fetus / number of CL) x 100. Where the ovaries structure of each doe counting number of expansive, medium, little and

haemorrhagic follicles and corpora lutea on the ovarian were recorded into destinations ovary.

Statistical analysis:

Data were analyzed utilizing examination of fluctuation by computer program of SAS (2004) to modification the result of level of metformin on developing life (recuperation and quality) and regenerative execution utilizing one way investigation or their interaction with site of ovary on ovarian biometrics and response of rabbit does (3 x 2 factorial design). The significant differences between group means were executed using a Duncan Run Examination (Duncan, 1955). The rates values were adjusted to arcsine changed some time recently achievement the examination of fluctuation. Infers were shown after being recalculated from mangled values to rates.

RESULTS AND DISSECTION

Ovarian biometrics:

The effect of metformin levels and site of ovary on ovarian biometrics of doe baladi rabbit are obtainable in Table 2.

The average length, wide and weight of rabbits ovary in G1 (control) were insignificant ($P < 0.05$) higher (1.46 cm, 0.55 cm and 4.52 g), than those in groups G2 and G3. Also, right ovary weight was higher in all ovarian biometrics than left ovary the differences were not significant ($P < 0.05$).

Table 2. Effect of metformin level treatments on ovarian biometrics of rabbit:

Item	Ovarian biometrics		
	Length (cm)	Widest (cm)	Weight (g)
Treatment			
G1 (Control)	1.46	0.55	0.362
G2 (20 mg)	1.36	0.537	0.334
G3 (40 mg)	1.36	0.512	0.323
±SE	0.04	0.03	0.01
Site of ovary			
Right=1	1.42	0.547	0.351
Left=2	1.37	0.519	0.328
± SE	0.03	0.02	0.01

Cruel meant inside the similar column for each impact with divergent superscripts are definitively distinctive at ($P < 0.05$).

Gharakhani *et al.* (2011) detailed that, the ovarian volume associated to body mass file BMI so they recommended a conceivable relationship between ultra-sound discoveries and anthropometric characteristics. In our conclusions, the predominance of corpulence, tall androgen, C-reactive protein CRP, Hcy levels and being of metabolic disorder inside patients with bigger ovarian volumes was created in PCOS patients with typical ovarian volumes. This revelation conceivably affirmed an interaction between ovarian morphology and volume, and anthropometric characteristics.

On the other pointer, six months of metformin organization was set up to have advantageous impacts on follicle development in ladies with PCOS, as illustrated by decrease of anti-Müllerian hormone levels, such as of follicle number and ovarian volume (Piltonen *et al.*, 2005).

No critical ($P < 0.05$) impact of communication between metformin levels and location of ovary on ovarian biometrics of baladi rabbit does (Fig. 1 and 2). However, the

highest values of length, wide and weight of rabbits ovary in G1 (control) with right ovary than other interactions.

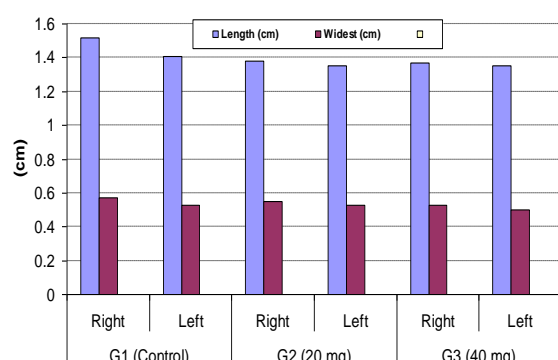


Fig. 1. Result of communication between metformin levels and site of ovary on ovarian length and widest of does rabbit, does.

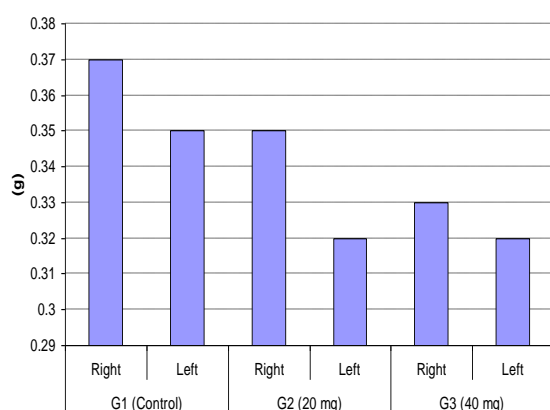


Fig. 2. Impact of interaction between metformin levels and location of ovary on ovarian weight).

The results of our think about, which are in agree-ment with Genazzani *et al.* (2004), Zeyneloglu *et al.* (2004) and Banaszewska *et al.* (2009), may convey evi-dence for the viability of metformin in balancing ovarian action. So, we conclude that met-formin in PCOS patients may cause diminishes in ovarian volume by diminishing intra-ovarian stro-mal androgens, indeed in a generally brief time, such as three months. Ladies with PCOS have numerous variations from the norm in lipid profiles.

Hyperandrogenism probably plays a role in these abnormalities, but hyperinsulinemia seems to be the more dominant influence (Lobo *et al.* (2000). In a study, PCOS patients had impaired lipid profiles as seen by increased levels of TG, cholesterol and LDL, and low levels of HDL. After three months of metformin administration, there was a beneficial effect on the lipid profile, as also confirmed by Santana *et al.* (2004) and Lord *et al.* (2006).

Ovarian responses:

The impact of metformin levels and location of ovary on ovarian activity of doe baladi rabbit are advertised in Table 3. Examination of differentiate uncovered that the cruel number of corpora lutea (CLs), dying follicles (BF) and medium follicles (MF) per ovary in gather 3 (G3) was genuinely ($P < 0.05$) higher than in bunch 2 (G2) and bunch 1 (G1) (6.17 vs. 4.83 and 3.83), (1.83 vs. 0.83 and 0.17) and (2.67 vs. 2.17 and 3.17) individually. In any case,

the cruel number of expansive follicles (LF) and little follicles (SF) appeared the inverse drift.

But the number of passing on follicles/ovary was higher in hormonal assemble than in flushing bunch without essential contrasts. Results in Table (3) uncovered immaterial contrasts between area of the ovary (right and cleared out) inside the unfeeling numeral of small, medium and expansive follicles. Whereas, the cruel number of CLs and bleeding follicles in right were essentially ($P < 0.05$) higher than left location (5.78 vs. 4.11) and (1.22 vs. 0.67).

Table 3. Effect of metformin level treatments on ovarian activity of does rabbit:

Item	Ovarian structure				
	LF	MF	SF	BF	CLs
Treatment					
G1 (Control)	5.5 ^a	3.17 ^b	7.67 ^a	0.17 ^c	3.83 ^c
G2 (20 mg)	3.67 ^b	2.17 ^b	4.67 ^b	0.83 ^b	4.83 ^b
G3 (40 mg)	3.5 ^b	2.67 ^a	5.0 ^b	1.83 ^a	6.17 ^a
±SE	0.616	0.304	0.851	0.192	0.304
Site of ovary					
Right	4.44	2.78	6.44	1.22 ^a	5.78 ^a
Left	4.0	2.56	5.11	0.67 ^b	4.11 ^b
±SE	0.503	0.248	0.703	0.157	0.248

^a and ^b

Implies implied inside the similar column for each impact by distinctive superscripts are altogether changed at ($P < 0.05$).

LF (large follicles) MF (medium follicles) SF (small follicles) BF (bleeding follicles) CL (corpora lutea)

Hyperinsulinemia stimulates the growth of antral follicles, cumulative the sensitivity of granulosa cells to FSH, thus increasing the numbers of follicles and ovarian volume (Lobo *et al.* 2000).

Pietro *et al.* (2015) too begin an advancement in follicular improvement, with a lower rate of little follicles and blisters and the next rate of antral follicles and corpora lutea after metformin organization. The enhancement in ovarian angiogenesis is likely to reestablish the aggregation of little follicles watched in PCOS rats and to diminish blister arrangement, hence moving forward follicular improvement and the rate of corpora lutea. These results open modern bits of knowledge into the ponder of metformin activity not as it were in glucose digestion system but moreover in ovarian brokenness in PCOS women.

Advancement in vascular advancement may in the long run lead to an advancement in follicular advancement. To degree this reality, we inspected the result of metformin on the follicular advancement of PCOS rats. In assention with that already labeled (Abramovich *et al.* 2012), when we compared the control vs the PCOS bunch, Our comes about found an increment in PrimFs, a diminish in PAF, preantral follicle PAFs, and no changes in antral follicles or AtF, atretic follicle; AtFs. Other than, in PCOS ovaries, we watched the nearness of cystic structures and a diminish within the rate of CL (Abramovich *et al.* 2012). Metformin treatment diminished the rate of essential and PAFs and expanded the rate of antral follicles. In addition, the rates of AtFs and cystic structures were diminished after metformin activity. Interests, the rate of CL was increased and come to values alike to those of the control bunch. These results recommend that metformin moves forward follicular growth and favoritisms the proper improvement of follicles and ovulation within the PCOS rodent demonstrate.

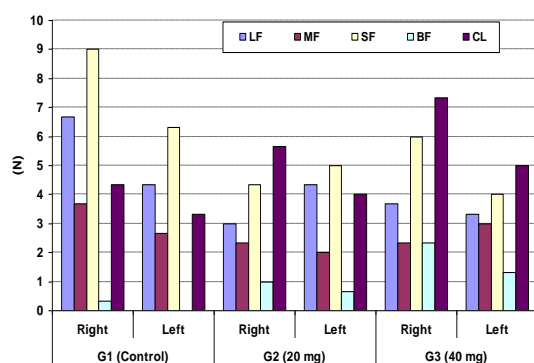


Fig. 3. Impact of communication between metformin levels and location of ovary on ovarian action

The influence of inter-action between side of the ovary and utilization on ovarian improvement is appeared in Fig. (3). Information exposed that no sizable ($P < 0.05$) impact of inter-action between location of ovary and action on ovarian constructions. While the higher values of large, medium and small follicles were (6.67, 3.62 and 9.0, respectively) in G1 with right ovary. But the higher values of corpora lutea (CLs) and bleeding follicles (BF) were (7.33 and 2.33) in G3 with right ovary. These comes about replicated the same slant impact of nourishment and hormonal on ovarian location work.

A few considers have labeled the next ovulation rate in PCOS patients protected with metformin (Ayaz *et al*, 2013, *et al*, and Morin *et al*, 2012). In any case, the components by which metformin employs this administrative activity on follicular advancement are still unidentified. Reclamation of the levels of angiogenic issues, which in turn improves vascular improvement, is likely to advance appropriate follicular improvement, driving to an upsurge in ovulation in our rodent show. The instruments by which metformin applies these impacts can be through a coordinate impact within the ovary or through a diminish within the levels of circulating affront, which may recoup follicular angiogenesis as well. Assist considers are required to clarify the inclusion of this gadget in PCOS ladies.

Metformin may execution on the control of ovarian answer to exogenous gonadotropins moving forward affront resistance. A decrease in serum testosterone and affront levels in follicular liquid was experiential after metformin treatment (Stadtmauer *et al*, 2001).

In this way, the advancement of the hyperinsulinemic and hyperandrogenic ovarian environment may be significant for a ordinary folliculogenesis, homogeneous improvement, and responsiveness of follicles and atresia of the little cohort of follicles (Palomba *et al*, 2009).

Recovery rate and viability of embryos:

Comes about advertised in Table (4) demomostarted that rate of reasonable embryos were higher in group 1 G1 (Control) (95.2%, $P < 0.05$) than those in group 2 G2 (88.9%) and in group 3 (G3) (75.0%). Whoever, embryo recovery degree was higher in group 2 (G2) than in other groups, but the changes among them remained not major.

The highest rate of practical embryos in gather 1 was related with the most reduced unviable embryos (4.8%, $P < 0.05$) as related to bunch 2 (11.1%) gather 3 (25.0%).

These comes about alluded to negative impact of metformin treatment on reasonability of spare embryos.

Table 4. Impact of metformin level medications on recuperation rate (RD) and viability of rabbit embryos

Management	Amount		Recovery degree (%)	reasonable Embryos		Unviable Embryo	
	CLs	Embryos		N	%	N	%
G1 (Control)	23	21	91.3	20	95.2 ^a	1	4.8 ^b
G2 (20 mg)	29	27	93.1	24	88.9 ^b	3	11.1 ^b
G3 (40 mg)	37	32	86.49	24	75.0 ^b	8	25.0 ^a

^{a and b} Implies meant inside the similar column for each conclusion with distinctive superscripts are essentially diverse at ($P < 0.05$).

Huang *et al.*, 2015 affirm a straight disability of intemperate androgen on oocyte quality, which can be in part spared by metformin activity. In any case, to attain the objective of getting a PCOS-like mouse demonstrate, the DHEA was given in a quitting a tall dose, which distant surpass the androgen levels in PCOS patients, makes it troublesome to extrapolate to people. Too, since of the physiological changes between mice and people, the mouse demonstrate utilized in this ponder cannot completely speak to the ture circumstance of PCOS patients. In any case, this think about giving a clue that hyperandrogenism may be a coordinate cause of destitute oocyte quality in PCOS patients amid IVF, while metformin pretreatment might inverse the disability. Assist instructions ought to be lead to affirm this result in clinical thinks about and clarify the component of the change convinced by metformin.

Palomba *et al.*, (2006) suggested that a exact effect of metformin on ovaries, presentation that PCOS patients ovulating under action had an improved ovarian artery blood flow, and an improved dominant follicle and corpus luteum vascularization.

Reproductive performance:

The most impacts of metformin level of Litter measure and litter weight of baladi rabbit females:

Data Are showed up in Table(5) litter measure (add up to and live) at birth and weaning, and litter weight at birth and weaning impressively ($P < 0.05$) extended in (G3) than in (G1), in any case no essential contrasts between (G3) and (G2) or between (G2) and (G1).

Normal pack weight at birth influenced considerably ($P < 0.05$) decreased in (G3) than in other bunches by the metformin level. Table (5).

Average kit weight total at birth, weaning and total weaning weren't affected substantially by the metformin level, being decreased by increasing the metformin level. Also, the average viability at birth and weaning the same trends Table (5).

Metformin has moreover been utilized to treat anovulatory fruitlessness in patients with polycystic ovary disorder (PCOS) (Palomba *et al.*, 2006). Metformin appears to extend the probability of ovulation and can, in combination with clomifene, upsurge the recurrence of ovulation and pregnancy in PCOS ladies (Creanga *et al*, 2008).

Metformin is the most noteworthy broadly utilized medicate for regenerative anomalies related with affront resistance additionally the eldest affront sensitizer in helpful administration. Its act diminishes hepatic glucose yield, upsurges tissue affront affectability and upgrades fringe glucose acknowledgment, coming about in second rate

concentrations of glucose without the related peril of either hypoglycemia or weight pick up (Simmons, 2010 and Hawthorne, 2006). In adding, metformin has been exposed to recover ovulation, pregnancy rates (Jungheim and Odibo 2010 and Palomba *et al* 2014) and live birth rates (Moll *et al*, 2007, 27) in PCOS patients.

Table 5. Impact of metformin level on reproductive performance in baladi rabbit does.

Parameters	Treatment		
	G1(Control)	G2 (20 mg)	G2 (40 mg)
Litter size at			
Total Birth (n.)	7.0±0.58 ^b	8.0±0.58 ^{ab}	9.33±0.33 ^a
Alive (n.)	6.33±0.33	7.0±0.58	7.67±0.33
Weaning(n.)	6.0±0.00	6.0±0.58	6.33±0.33
Litter weight at			
Birth (g)	53.67±2.73 ^a	47.33±1.2 ^b	42.67±0.67 ^b
Total Birth (g)	339.3±19.9	330.3±21.7	326.7±9.3
Weaning(g)	539.4±2.4	526.7±38	501.6±15.1
Total Weaning(g)	3236.7±14.7	3116.7±83.5	3166.7±66.7
Viability at %			
Birth	91.10±4.5	87.37±0.92	82.22±3.39
Weaning	95.24±4.76	85.52±1.21	82.74±3.9

Implies indicated inside the same row for each calculate with diverse significantly are impressively distinctive at ($P < 0.05$).

Metformin as pretreatment and co-treatment with clomiphene citrate seems to be fruitful in normalizing menstrual irregularities, maybe by sensitizing follicles to follicle-stimulating hormone. Subsequently, metformin alone or composed with clomiphene citrate has been proposed as a activity for ovulation acceptance in fruitless ladies with PCOS (Kim *et al*, 2000 and Nestler *et al*, 2002).

Metformin was in a few instructions administrated at the starting of pregnancy, since, metformin treatment all through pregnancy condensed the event of premature delivery (Glueck *et al.*, 2002; De Leo, 2011). Be that as it may over an expanded period, ponders have experiential that metformin has the capacity to cross the placenta (Glueck *et al.*, 2004; Vanky *et al.*, 2005), and its utilize amid pregnancy increments concerns approximately potential antagonistic impacts on both the ma and baby (Salomäki, 2013; Lautatzis, 2013; Harris, 1971). A few ponders have watched that in utero presentation to metformin early in pregnancy does not appear to cause any unfavorable impacts or inherent distortions (Glueck *et al*, 2002; Nawaz *et al*, 2008 and De Leo *et al*, 2011; Balani *et al*, 2006; Bolton *et al*, 2009; Kovo *et al*, 2009). In spite of this, results on gonad development of the embryo have not been clearly considered.

CONCLUSION

These results indicated that treatment with metaformin improved ovarian response, reproductive performance. Although it's reducing embryo recovery and viability rate for rabbit does (baladi black).

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تأثير مستويات من مادة الميتفورمين علي امهات الارانب البلدي ترك محمد ابراهيم درة^١، شريف مغاوري شامية* أميره محمود منصور إبراهيم^١ وأية أحمد علي عطوة^١ ^١قسم الانتاج الداجني كلية الزراعة جامعة المنصورة مصر ^٢قسم فسيولوجيا الحيوان - محطة سخا مصر

تهدف الدراسة الي تقييم تأثير مادة الميتفورمين علي النشاط المبيضي وجودة الاجنة في الارانب البلدي. عدد الامهات في الناضجة ٢١ أم تم تقسيمها الي ٣ مجموعات. المجموعة الاولى ماء شرب طبيعي والمجموعة الثانية والثالثة تم تجربتها مادة الميتفورمين بنسبة ٢٠ و ٤٠ مجم /كجم وزن حي لمدة ٣ أسابيع قبل التلقيح الطبيعي للامهات من دكور ناضجة من نفس النوع وتم تقدير مقاييس الوزن عند الميلاد وتسجيل البيانات لعدد ٤ امهات من كل مجموعة. (تم تجميع الاجنة باستخدام PBS (من الرحم وقرني الرحم بعد ٧٢ ساعة من التلقيح وتم تسجيل قياسات المبيض وموقع كل منهما. وتم فحص الاجنة المجمعة من حيث كونها طبيعية /غير طبيعية باستخدام الميكروسكوب. النتائج: متوسط الوزن والطول والعرض لقياسات المبيض في المجموعة الاولى اعلي من المجموعتين الاخرتين. المبيض الايمن في المعاملات اعلي من الايسر من حيث عدد الحويصلات الصغيرة والمتوسطة والمدممة والجسم الأصفر. النسبة المئوية للاجنة المتاحة في المجموعة الاولى عن الثانية والثالثة. بينما معدل استرداد الاجنة في الثانية اعلي من الاولى والثالثة. بينما معدل استرداد الاجنة في الثانية اعلي من الاولى والثالثة ولكن الاختلاف في الوزن للاجنة عند الميلاد ومقاييسها في المجموعة الثالثة عن المجموعتين الاولى والثانية