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Embryo Recovery, Characterization and Progesterone Level as affected by Oxytocin, Prostaglandin $F_2\alpha$ Treatments in rabbit.

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SUMMARY

This study was carried out to investigate the various effected of intrauterine cavity injection just after mating with prostaglandin $F_2\alpha$ (33.3μg/kg) and oxytocin (OT) 1.6 IU/kg on embryo recovery, characterization and progesterone concentration in rabbit. There are significant differences ($P < 0.05$) in ovulation rate between groups, the does that receiving oxytocin, $PGF_{2\alpha}$ exhibited the highest number of corpora lutea (CL) than control group 13.0, 9.0 and 8.3 respectively.

The percentage of embryos recovered was higher in $PGF_{2\alpha}$ treated group 70.4% followed by Oxytocin group 56.4%, than control group 36.0 associated with no significant variations between treatments in the percentage of visible abnormal embryos as detected by degeneration of mass cell or embryos having irregular morphology. Average measurements for embryo dimension were studded and found that, zona pellucida (ZP) thickness of embryos recovered was significantly ($P < 0.05$) thickes in oxytocin treated 26.7 μm compared with control 24.1 μm and $PGF_{2\alpha}$ 20.6 μm. Progesterone concentration at 24hr were significantly higher and sharply increased at 48hr to reach nearly 3 time higher than control group. In conclusion injection rabbit does with $PGF_{2\alpha}$ or oxytocin, increased ovulation rate and embryos recovery, which may increase does raising profitability in term of larger litter size.

Keywords: Embryo, $PGF_{2\alpha}$, Oxytocin, Ovulation, Progesterone.

INTRODUCTION

Studies have linked prostaglandins with several pathological conditions such as In cattle, elevated prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations in the uterine lumen has also been shown to lower reproductive efficiency by decreasing embryonic survival and development (Hockett et al., 2004 and Scenna et al., 2004), decreasing the ability of bovine embryos to escape from the zona pellucida (hatching; (Scenna et al., 2004)), and by decreasing pregnancy rates (Sales et al., 2004; Scenna et al., 2005). Further implications for $PGF_{2\alpha}$ as an embryotoxic agent was observed when administration of several prostaglandin synthesis inhibitors increased pregnancy rates after embryo transfer in cows (Elli et al., 2001; McNaughtan et al., 2002; Pugh et al., 2004; Purcell et al., 2004; Scenna et al., 2005). To date, the mechanisms through which $PGF_{2\alpha}$ reduces embryonic survival and development in the animal are still unclear.

Even though release of $PGF_{2\alpha}$ during embryo transfer may not result in luteolysis, embryonic survival may be compromised by the presence of small concentrations of $PGF_{2\alpha}$ in the uterine lumen, creating a "hostile environment" for embryonic development. Scenna et al. (2004) demonstrated that development of *in vitro*-produced 16- to 32-cell embryos to blastocyst stage was reduced by addition of $PGF_{2\alpha}$ in the culture media. In addition, culture of *in vivo*-derived compact morulae in medium containing $PGF_{2\alpha}$ did not affect development to blastocyst, but reduced hatching rates (Scenna et al., 2004). This is in agreement with Maurer and Beier (1976) who reported that addition of $PGF_{2\alpha}$ to culture media directly decreased the ability of 8-cell rabbit embryos to form expanded and hatched blastocysts, but had no effect on development to early blastocyst. Successful hatching of the embryo is thought to be a key event for further embryonic development and proper implantation in humans (Petersen et al., 2004). In fact, assisted hatching of human embryos (consisting in creating a complete or partial hole in the zona pellucida) has been shown to result in better implantation rate after embryo transfer in humans (Ali et al., 2003; Kung et al., 2003; Wong et al., 2003).

Embryonic losses in bovine appear to occur soon after the embryo enters the uterus (5 to 8 days after mating or insemination), when the morula stage embryo is transitioning to blastocyst (Ayalon, 1978; Maurer and Chenault,

1983; Wiebold, 1988; Dunne et al., 2000). Previous studies have shown a detrimental effect of $PGF_{2\alpha}$ on *in vitro* development of rat, rabbit and bovine embryos (Maurer and Beier, 1976; Breuel et al., 1993; Scenna et al., 2004). Furthermore, administration of $PGF_{2\alpha}$ on days 5 through 8 54

After artificial insemination or mating has also been shown to decrease embryonic development, quality and pregnancy rates in progesterone-supplemented cows (Buford et al., 1996; Hockett et al., 2004; Sales et al., 2004). In addition, administration of prostaglandin synthesis inhibitors increased pregnancy rates after transfer in cows (Elli et al., 2001; McNaughtan et al., 2002; Pugh et al., 2004; Purcell et al., 2004; Scenna et al., 2005).

Oxytocin was produced only by granulosa cells derived from preovulatory follicles, and after application of Oxytocin, only the granulosa cells cultured from preovulatory follicles elicited an increase in progesterone production (Einspanier et al., 1995 and1997). Functional Oxytocin receptors have been detected in bovine granulosa cells, suggesting that Oxytocin may be an autocrine factor during follicular growth (Okuda et al., 1997). Oxytocin also increased the rate of mouse blastocyst development and might therefore play some role in the early stage of development of fertilized oocytes (Furuya et al., 1995). Thus Oxytocin may act primarily as a local mediator and not as a circulating hormone during parturition. In an autocrine/paracrine system within the uterus, significant changes of Oxytocin, prostaglandins, and sex steroids could occur without being reflected in the maternal circulation (Mitchell et al., 1998).

In the decidua, Oxytocin has a separate action of stimulating the release of $PGF_{2\alpha}$. At the end of pregnancy, an increased secretion of $PGF_{2\alpha}$ drives luteolysis and thus leads to progesterone withdrawal and labor initiation in rodents. Furthermore, the normal decline of serum progesterone concentrations that precedes parturition did not occur. This indicates that $PGF_{2\alpha}$ acts upstream of Oxyttocin to induce luteolysis, i.e., production of progesterone (Sugimoto et al., 1997 and Gerald Gimpl and Falk Fahrenholz, 2001).

MATERIALS AND METHODS

1. Source of Zygotes

Mature Rabbits does from New Zealand White breed of 9 months and 3.0 Kg average body weight were divided randomly into three groups of five does

each. Female rabbits were mated twice with fertile bucks. Zygotes were recovered around 48 h post-coitum (hpc) by flushing oviducts with Dulbecco phosphate buffered saline (PBS, D5773, Sigma, Egypt) supplemented with 20% (v/v) Fetal Calf Serum (FCS) (hereafter: s-PBS). Embryos collected were examined quickly using an inverted microscope fitted with calibrated eyes piece micrometer reflected on video-monitor.

2. Experimental

In this experiment, the effect of intrauterine cavity injection just after mating with prostaglandin $F_2\alpha$ and oxytocin.

Three groups served as, the first group (T_1) was treated with 33.3µg/kg estrumate (a synthetic $PGF_{2\alpha}$analogue produced by Swiss Pharma SAE., Cairo under license from Sandoz Pharma Ltd. Basle, Switzer Land). The second group (T_2) was treated with 1.6 lU/kg Oxytocin (a syntocinon synthetic oxytocin, produced by Swiss Pharma SAE., Cairo under license from Sandoz Pharma Ltd. Basle, Switzer Land). While, the third group served as control.

3. Blood sampling

Heparinized blood samples were taken pre-treatment, 7 days after mating and 14 days post mating, all blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma and then stored at -20 C until radioimmunoassay performed.

4. Statistical analysis

Analyses of variance (ANOVA) were carried out using the SAS software package (1996)

Duncan's multiple range test was used to comparemean value of individual treatments, when the *F*-value was significant ($P < 0.05$).

RESULT AND DISCUSSION

Average ovulation rate, determined by counting corpora lutea (CL) at 48 hr after mating. There were significant differences in ovulation rate between groups. Generally, all treated groups had higher number of CL than the control (table 1) and the does that receiving oxytocin and $PGF_{2\alpha}$ exhibited the highest number of CL than control group (13.0, 9.0 and 8.3) respectively. It may be oxytocin may be an autocrine factor during follicular growth (Okuda et al., 1997), also increased the rate of rabbit blastocyst development and might therefore play some role in the early stage of development of fertilized oocytes (Furuya et al., 1995). besides Oxytocin and $PGF_{2\alpha}$ have a direct role in ovulation in terms of

LH release and connective tissue breakdown leading to ovulation (Gerald Gimpl and Falk Fahrenholz, 2001).

The percentage of embryos recovered was higher in $PGF_{2\alpha}$ treated group 70.4% followed by Oxytocin group 56.4%, while the decline percentage of embryos recovered from control group 36.0, (tables 1). These results agree with Torres et al., 1987.

Table1: Embryos recovered and corpora lutea as affected by prostaglandin $F_2\alpha$, and oxytocin. ($\bar{x} \pm se$).

Treatments	CL ($\bar{x}$)	Embryo recovered		Abnormal embryos	
		$\bar{x}$	%	$\bar{x}$	%
Control	$8.3^{b} \pm 1.8$	$3.0^{a} \pm 0.6$	36	0.3 ± 03	11.1
$PGF_2\alpha$	$9.0^{ab} \pm 1.2$	$6.3^{b} \pm 0.7$	70.4	1.7 ± 1.7	26.3
Oxytocin	$13.0^{ab} \pm 1.5$	$7.3^{b} \pm 0.4$	56.4	1.0 ± 0.6	13.6

a,b Different superscripts in the same columns differ statistically ($P < 0.05$).
CL corpora lutea,

Alternatively there were no significant variations between treatments in the percentage of visible abnormal embryos as detected by degeneration of mass cell or embryos having irregular morphology. In addition, culture of *in vivo*-derived compact morulae in medium containing $PGF_{2\alpha}$ did not affect development to blastocyst, but reduced hatching rates (Scenna et al., 2004). This is in agreement with Maurer and Beier (1976) who reported that addition of $PGF_{2\alpha}$ to culture media directly decreased the ability of 8-cell rabbit embryos to form expanded and hatched blastocysts, but had no effect on development to early blastocyst.

Average measurements for embryo dimension were studded and found that, zona pellucida (ZP) thickness of embryos recovered was significantly thicker in Oxytocin treated than other treatments and the ratios between the ZP thickness and embryos diameter with their coverings 1:6.1, 1:7.2 and 1:4.8 in $PGF_{2\alpha}$, Oxytocin and control group respectively (table 2). Also significant differences between treatments were found in the intrazonal diameter, the ratios between the intrazonal diameter and embryos diameter with their coverings 1:31.0, 1:28.6 and 1:29.9 in $PGF_{2\alpha}$, Oxytocin and control group respectively, similar results were obtained by El-Keraby et al., 1991.

Table 2: Measurements of recovered embryos as affected by prostaglandin $F_2\alpha$, and Oxytocin. ($\bar{x} \pm se$).

Treatments	Embryo No.	Measurements (μm) of embryos $\bar{x}$			
		Mucin coat	Z.P	Interzonal	T.Diametre
Control	12	103.0 ± 3.2 (28.3%)	17.5^{b} ± 2.98 (4.8%)	113.7^{b} ± 4.5 (31.0%)	356.3 ± 13.3
$PGF_2\alpha$	14	114.7 ± 4.6 (29.5%)	24.1^{b} ± 1.04 (6.1%)	111.3^{ab} ± 4.46 (28.6%)	388.8 ± 11.2
Oxytocin	19	109.0 ± 6.6 (29.7%)	26.7^{a} ± 1.2 (7.2%)	94.9^{a} ± 3.98 (25.9%)	366.3 ± 16.2

a,b Different superscripts in the same columns differ statistically ($P < 0.05$).
Z.P, Zona pelocida, T Diametar Total Diameter,

Progesterone concentration at 24hr were significantly higher and sharply increased at 48hr to reach nearly 3 time higher than control group in all treatments (table 3). In human corpus luteum, progesterone concentrations reached peak levels of ~25 μg/g tissue shortly after ovulation and in the early luteal phase (Swanston et al., 1977). These values are within the range of the progesterone concentrations that were effective in our study (Burger et al., 1999). The Oxytocin system may not be fully operative during the continuous presence of high progesterone concentrations. In each case, the ratio of estrogen to progesterone increases in the maternal plasma concomitantly with increase production of prostaglandins from intrauterine tissues, and an upregulation of Oxytocin receptors (Fuchs et al., 1995, Mitchell et al., 1995). Finally, the uterine quiescence that was maintained by the high progesterone level ceases, and parturition can occur.

Table3: Progesterone concentration (ng/ml) of does as affected by prostaglandin $F_2\alpha$, and oxytocin. ($\bar{x}$ ± se).

Treatments	Progesterone Concentration		
	Time of blood collection ($\bar{x} \pm se$)		
	0hr	*24hr*	*48hr*
Control	0.36 ± 0.06	1.54^{b} ± 0.21	2.80^{b} ± 0.2
$PGF_2\alpha$	0.39 ± 0.03	2.88^{a} ± 0.10	6.26^{a} ± 0.3
Oxytocin	0.35 ± 0.06	3.08^{a} ± 0.10	7.00^{a} ± 0.4
Overall mean	0.38 ± 0.05	2.64 ± 0.20	5.90^{a} ± 0.6

a,b Different superscripts in the same columns differ statistically ($P < 0.05$).

We concluded that injection rabbit does with Prostaglandin $F_{2\alpha}$ or Oxytocin increased ovulation rate and embryos recovery, which may increase does raising profitability in term of larger litter size. The plasma progesterone concentration in superovulated rabbits was higher by 5 to 9 times as much as that in normally rabbits at 24 to 120 hr post mating.

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معدلات جمع الأجنة و خصائصها و مستوي هرمون البوجستيرون كنتيجة للمعاملة بهرمون الأوكسي توسن و البروستجلاندين في الأرانب.

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أجري هذا البحث لدراسة التأثيرات الناتجة عن الحقن ببعض الهرمونات المنشطة للتبويض في الأرانب لزيادة معدلات جمع الأجنة منها حتى يمكن الحفاظ علي السلالات المعرضة للتناقص و إمكانية الإكثار من أعدادها. تم أستخدم ثلاث مجاميع من الأرانب قسمت الي المجموعة الأولي تم معاملتها ب (33.3 ميكرو جرام/كحم) برو ستجلاندين و المجموعة الثانية تم معاملتها بـ (1.6 وحدة دولية) من هرمون الأوكسي توسين و الجموعة الثالثة لم يتم معاملتها لتصبح كنترول. كلا المجموعتين الأولي و الثانية تم معاملتها بالحقن قبل التلقيح مباشرا و بعد 48 ساعة من التلقيح تم ذبح الأمهات و عد الأجسام الصفراء علي المبيض و جمع الجنة و دراستها. و لقد أوضحت النتائج ما يلي:

1- المعاملة الهرمونية كان لة تأثير معنوي علي معدل التبويض و بالتالي علي عدد الأجسام الصفراء المشاهد علي المبيض و كان أعلي معدل للمعاملة بهرمون الأكسي توسن (13 جسم أصفر) ثم البروستجلاندين (9 جسم أصفر) و أقل عدد كان للكنترول (8.3 جسم أصفر).
2- لوحظ اختلاف معنوي في متوسط عدد الأجنة المجمعة كان اعلي (7.3) في مجموعة الأكسي توسن ثم (6.3) لمجموعة البروستجلاندين وأقلهم كان (3) لجموعة الكنترول.
3- لم يلاحظ تأثيرات معنوية للمعاملات علي كلا من طبقة اليوسين و القطر الكلي للجنين و الكتلة الخلوية بينما لوحظ أختلاف معنوي في سمك طبقة الزونا بلوسيدا حيث سجلت القياسات 26.7 و 24.1 و 17.5 الكلا من مجموعة الأكسي توسن ثم البروستجلاندين ثم الكنترول و كما لوحظ أختلاف معنوي لمنطقة الأنترازونا كان اعلاهم للكنترول 113.7 و أقلهم للأكسي توسين 94.9 ميكروملليمتر.
4- أثرت المعاملات الهرمونية المختلفة تأثيرا معنويا علي تركيز هرمون البروجستيرون في بلازما دم الأمهات المحقونة في 24 و 48 ساعة من الحقن و التلقيح بالمعاملات الهرمونية حيث كانت اعلي في الإناث المعاملة عنها في الكنترول كما لوحظ زيادة سريعة في تركيز هرمون

البروجستيرون بعد 48 ساعة من الحقن و التلقيح حيث سجل ارتفاع ملحوظ قدر بحوالي 3 أضعاف المجموعة الكنترول.

و علي ذلك يمكن استخدام المعاملة الهرمونية لزيادة معدل التبويض و بالتالي زيادة عدد الأجنة المجمعة من الأمهات و التي قد تؤدي في النهاية لزيادة معدلات الأنتاج من هذه الحيوانات و خاصة إذا ما وضع في الاعتبار الأهمية الأستراتيجية للسلالات المحلية و تعرضها لمشكلة تناقص الأعداد و أهمية المصادر الوراثية الحيوانية.